Allergic Pulmonary Inflammation Promotes the Recruitment of Circulating Tumor Cells to the Lung

Anna G. Taranova,1 David Maldonado III,3 Celine M. Vachon,4 Elizabeth A. Jacobsen,1 Hiam Abdala-Valencia,5 Michael P. McGarry,1 Serge I. Ochkur,1 Cheryl A. Protheroe,1,2 Alfred Doyle,1,2 Clive S. Grant,5 Joan Cook-Mills,6 Lutz Birnbaumer,7 Nancy A. Lee,7 and James J. Lee1

Divisions of Pulmonary Medicine and Hematology and Oncology, Department of Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, Arizona; 1Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Mayo Clinic Comprehensive Cancer Center, Genetic Epidemiology and Risk Assessment, and 2Mayo Clinic Comprehensive Cancer Center, Division of Surgical Oncology, Mayo Clinic Rochester, Rochester, Minnesota; 5Allergy-Immunology Division, Northwestern University Feinberg School of Medicine, Chicago, Illinois; and 7National Institute of Environmental Health Sciences, Laboratory of Signal Transduction, Research Triangle Park, North Carolina

Abstract

Allergen-induced respiratory inflammation facilitates and/or elicits the extravasation of proinflammatory leukocytes by well-understood mechanisms that mediate the movement of multiple cell types. The nonspecific character of these pathways led us to hypothesize that circulating cancer cells use similar mechanisms, promoting secondary tumor formation at distal sites. To test this hypothesis, the frequency of metastasis to the lung as a function of allergic pulmonary inflammation was assessed following the i.v. injection of B16-F10 melanoma cells in mice. These studies showed that allergen-induced pulmonary inflammation resulted in a >3-fold increase in lung metastases. This increase was dependent on CD4+ T-cell activities; however, it occurred independent of the induced eosinophilia associated with allergen provocation. Interventional strategies showed that existing therapeutic modalities for asthma, such as inhaled corticosteroids, were sufficient to block the enhanced pulmonary recruitment of cancer cells from circulation. Additional mechanistic studies further suggested that the ability of circulating cancer cells to extravasate to surrounding lung tissues was linked to the activation of the vascular endothelium via one or more Gc coupled receptors. Interestingly, a survey of a clinical breast cancer surgical database showed that the incidence of asthma was higher among patients with lung metastases. Thus, our data show that allergic respiratory inflammation may represent a risk factor for the development of lung metastases and suggest that amelioration of the pulmonary inflammation associated with asthma will have a direct and immediate benefit to the 7% to 8% of breast cancer patients with this lung disease. [Cancer Res 2008;68(20):8582–9]

Introduction

Disseminated metastases remain the main cause of malignancy-related death in cancer patients (1) and as such are the focus of intensive investigations. Nonetheless, despite these efforts, the mechanisms that facilitate metastasis remain the subject of debate (2, 3). It seems likely that cells shed from primary tumors enter the peripheral and/or lymphatic circulation and subsequent cell-cell interactions with the vascular endothelium lead to adhesion and extravasation from circulation (see refs. 4, 5 for example). In many respects, hypotheses describing these processes share a great deal of similarity with the well-characterized recruitment of leukocytes in response to inflammation (i.e., the rolling of circulating leukocytes, tethering of these cells, and their firm adherence to activated vascular endothelium; reviewed in ref. 6). Moreover, adherent leukocytes subsequently elicit signaling events within the vascular endothelial cells that are necessary for the enhanced permeability of the endothelium and diapedesis of these leukocytes into adjacent tissues (7, 8). Although it is now well accepted that the tumor microenvironment is an important component in the neoplastic process (9), the specific mechanisms mediating seeding and development of metastases remain unresolved. Inflammation and tumor onset/growth have been linked in various animal models of cancer and human neoplastic conditions (10, 11). Interestingly, several studies have also shown that sites of chronic inflammation are often associated with the establishment and growth of malignancies (e.g., mesothelioma, melanoma, and oral squamous cell carcinoma); these observations suggest that inflammation may be supportive of tumor onset/growth (12, 13).

Similar to leukocyte recruitment, circulating cancer cells also seem to respond to the same rich milieu of localized chemokines and cell adhesion molecules engaged during inflammatory processes (see ref. 14 for example). Moreover, proinflammatory leukocytes themselves have also been shown to be a significant source of these same inflammatory mediators, suggesting that the presence of these leukocytes alone may facilitate tumor cell extravasation (14, 15). Thus, although localized tissue inflammation is necessary for the appropriate recruitment of proinflammatory leukocytes, this response itself may also create a favorable environment facilitating the recruitment and growth of circulating tumor cells.

Our recent studies using mouse models of pulmonary inflammation mediated by either endotoxin or allergen have shown the importance of signaling events occurring within endothelial cells for leukocyte extravasation (16). In addition, other studies suggest that changes in vascular permeability, cell adhesion, and the creation of a tumor-favorable microenvironment are all likely events contributing to metastasis (12, 17). Collectively, these observations suggest a link between inflammation and metastasis highlighted by common mechanisms by which both leukocytes and circulating cancer cells are recruited to tissue compartments. That
is, the cadre of inflammatory mediators (i.e., cytokines, growth factors, adhesion receptor-ligand interactions, and molecular/cellular signaling events) that lead to directed leukocyte recruitment may, in turn, be exploited by circulating cancer cells to facilitate distant organ metastasis and the spread of cancer.

This study uses mouse models of allergic pulmonary inflammation and a well-established model of metastasis in mice to show that allergic respiratory inflammation not only promotes proinflammatory leukocyte recruitment to the lung but also facilitates the recruitment of circulating cancer cells leading to enhanced metastasis. Significantly, the activation of the pulmonary vascular endothelium and the concomitant extravasation of cancer cells were shown to be required events for the increased rate of metastasis observed in mice following allergen provocation. More importantly, this relationship between allergic respiratory inflammation and elevated levels of metastasis may also exist in humans. In particular, the frequency of asthma seems to be higher than expected among breast cancer patients who have experienced a recurrence of their malignancy in the lung, suggesting that treatments targeting lung inflammation may have value as therapeutic strategies suppressing/preventing metastasis.

Materials and Methods

Animals. C57BL/6J mice were either purchased from The Jackson Laboratory or bred within the Mayo Clinic Arizona animal facility. Transgenic mice devoid of eosinophils (line: PHIL [backcrossed on a C57BL/6J background >10 generations]) were developed in our laboratory as previously described (18). GαQ−/− mice were generated as previously described (19). Briefly, mice (20–30 g) were sensitized by i.p. injections (100 μL) of 20 μg chicken OVA (Sigma) emulsified in 2 mg of Igepal Alum [Al(OH)3/Mg(OH)2; Pierce] on days −29 and −15 of a protocol in which the day of cancer cell administration is day 0 (Fig. 1). Sensitized mice were challenged with an aerosol, derived by nebulizing a 1% OVA solution in saline (OVA), on protocol days −5, −4, and −3 and then provoked with a 5% OVA nebulant on protocol days −1, +4, and +9; control animals (OVA sensitized) were challenged/provoked with saline alone.

Generation of lung metastasis using B16F10 melanoma and MC38 colon carcinoma cell lines. B16-F10 melanoma cells (American Type Culture Collection) and MC38 colon sarcoma (a kind gift from Dr. Lotze, University of Pittsburgh, Pittsburgh, PA), both of which are derived from C57BL/6J mice, were cultured (humidified atmosphere containing 5% CO2 at 37°C) in DMEM supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin. Tryptsinized cells were recovered and washed with 1× PBS before inoculation into recipient animals as a cell suspension in 1× PBS (pH 7.4). All tissue culture reagents were purchased from Invitrogen.

C57BL/6J wild-type, PHIL, or GαQ−/− mice undergoing the OVA sensitization/aerosol challenge protocol outlined above were injected (400 μL total volume) with 2 × 10^6 B16-F10 melanoma cells (in some studies, MC38 colon sarcoma cells were used) into a lateral tail vein (protocol day 0). Injected animals were euthanized on day 12 for assessment of the number of lung metastases. Specifically, harvested lungs were inflated after fixing the animals with a fixed volume (1 mL) of 10% formalin and the numbers of metastatic colonies on the surface were assessed using a low-power microscope. All evaluations of metastases were performed in duplicate as independent observer-blinded assessments.

Budesonide treatment. Corticosteroid (i.e., budesonide) treatment of mice was performed as previously described (20). Briefly, 0.5 mg/kg body weight of budesonide (Sigma) in 0.9% saline with 1% carboxymethylcellulose (Sigma) and 0.1% polyoxyethylene sorbitan monooleate (Tween 80, C0/C0) challenges of mice (0.5 mg/kg of body weight) during the OVA challenge and provocation phases of the protocol (days −6, −5, −4, −3, −1, +4, and +9).

Induction of allergic pulmonary inflammation. Allergic pulmonary inflammation was induced in mice using an established ovalbumin (OVA) sensitization/aerosol challenge model of asthma as described earlier (19). Briefly, mice (20–30 g) were sensitized by i.p. injections (100 μL) of 20 μg chicken OVA (Sigma) emulsified in 2 mg of Igepal Alum [Al(OH)3/Mg(OH)2; Pierce] on days −29 and −15 of a protocol in which the day of cancer cell administration is day 0 (Fig. 1). Sensitized mice were challenged with an aerosol, derived by nebulizing a 1% OVA solution in saline (OVA), on protocol days −5, −4, and −3 and then provoked with a 5% OVA nebulant on protocol days −1, +4, and +9; control animals (OVA sensitized) were challenged/provoked with saline alone.

Figure 1. Schematic time line representing the merger of an established model of allergic respiratory inflammation and the metastasis of circulating cancer cells to the lung. Experimental animals were sensitized to OVA by i.p. injections on days −29 and −15 of this protocol followed by a series of aerosol challenges (protocol days −5, −4, and −3) derived from a 1% OVA solution in saline (control animals received saline alone) focusing allergic immune responses to the lung. These inflammatory responses in the lung were maintained throughout the remaining 12 d of the protocol by provoking the mice on days 0, +4, and +9 with a nebulant derived from a 5% OVA solution in saline (again, control animals received saline alone). During the aerosol provocation phase of this protocol, the mice received an i.v. injection of B16-F10 melanoma cells (2 × 10^5) on day 0 of the time line and lung metastases were assessed in these animals 12 d later (tumor harvest). Variations of this protocol representing the described experiments of this report are shown below the main time line. Depletion of CD4+ T cells (T-cell depletion) was achieved via i.p. administration of the depleting mAb GK1.5 on protocol days −7, −1, and +5. Additional groups of mice were treated with the corticosteroid budesonide through intranasal (i.n.) challenges of mice (0.5 mg/kg of body weight) during the OVA challenge and provocation phases of the protocol (days −6, −5, −4, −3, −1, +4, and +9).
Antibody-mediated depletion of CD4+ T cells. The anti-CD4 monoclonal antibody (mAb) GK1.5 was used to deplete CD4+ T cells using a modification of a previously described protocol (21). Specifically, CD4+ T cells were depleted from mice by administration (i.p.) of GK1.5 mAb to mice (0.5 mg/100 g) on protocol days −8, −1, and 6 in which the day of cancer cell administration is day 0 (Fig. 1); control groups of mice were administered nonspecific rat IgG.

Histology and immunohistochemical staining. Lung tissue for histologic analyses was obtained by instilling 1 mL of 10% neutral-buffered formalin (30 cm H2O constant pressure) through a cannula inserted into the trachea. The excised lung was immersed in 10% formalin for 24 h (at 4°C) and then paraffin embedded. Sagittal sections (4 μm) were stained with H&E and analyzed by bright-field microscopy (n ≥ 5 animals per group). Eosinophil recruitment to lung tissue was determined by immunohistochemistry using a rabbit polyclonal anti-mouse major basic protein (MBP) antiserum (22).

Ex vivo assessments of melanoma cell adhesion and migration. Direct measures of cell adhesion between B16-F10 melanoma cells and an endothelial cell monolayer (i.e., mHEVa endothelial cells) were assessed in static cultures using a “cell association assay” (23). Rat anti-mouse VCAM-1 (clone MVCAMA, BD PharMingen) and anti-α4 integrin (clone PS/2, BioDesign International) were used as blocking agents in the “cell association” assays to show the requirement of these cell adhesion molecules for B16-F10 melanoma cell transendothelial cell migration. Endothelial cell viability after pertussis toxin (Sigma) treatment was determined either by assessment of glyceraldehyde-3-phosphate dehydrogenase release into the medium using the Molecular Probes Vybrant Cytotoxicity Assay (V23111) or by microscopic assessment of cells following disassociation with 0.3% EDTA using trypan blue exclusion.

A parallel plate flow chamber was used to examine migration under conditions of laminar flow (24). B16-F10 melanoma cell migration across an endothelial cell monolayer (i.e., mHEVa cells) was shown earlier to occur as a consequence of endothelial cell constitutive production of the chemoattractants/growth factors (25). The mHEVa cells have been spontaneously immortalized but not transformed (26). mHEVa cell monolayers were grown to 95% confluence on slides and treated overnight with medium only or medium containing pertussis toxin (endothelial cell monolayer (i.e., mHEVa cells) was shown earlier to occur as a consequence of endothelial cell constitutive production of the chemoattractants/growth factors (25)). The mHEVa cells have been spontaneously immortalized but not transformed (26).

Figure 2. Allergen sensitization/aerosol challenge promotes a CD4+ T-cell–dependent recruitment of eosinophils to the lungs of mice. A, lung sections from wild-type saline control (WT Saline) and OVA-treated (WT OVA) mice were stained with H&E (left) for generalized histopathologic assessments. Immunohistochemistry using a rabbit polyclonal antibody specific for mouse MBP showed the spatial localization of the pulmonary eosinophils (grayish/black staining cells) uniquely occurring in OVA-sensitized/aerosol-challenged wild-type mice. B, the tissue eosinophils associated with OVA sensitization/aerosol challenge is eliminated in mice depleted of CD4+ T cells using the GK1.5 mAb (GK1.5 OVA) and is absent in mice congenitally devoid of eosinophils (PHIL OVA). Scale bars, 100 μm.
The increase in lung metastasis was associated with allergic pulmonary inflammation but occurred independent of the allergen-induced accumulation of pulmonary eosinophils. Previous studies have shown that allergen-induced pulmonary pathologies (including the recruitment/accumulation of pulmonary eosinophils) are mediated by CD4+ T-cell activities (29). We investigated whether the allergen-induced increase in lung metastasis was similarly dependent on CD4+ T-cell activities through the administration of a cell-depleting antibody (GK1.5; ref. 21) using an administration protocol and time line described in Materials and Methods and Fig. 1, respectively. Flow cytometric assessment of splenocytes derived from mice at the time of tumor harvest (protocol day +12) showed that this cell depletion strategy is both effective (i.e., no CD4+ T cells are detectable in GK1.5-treated mice) and specific (i.e., no effects were observed on any other T-cell population). Immunohistochemical assessment of lung sections using anti-MBP antibodies showed the absolute dependence of the allergen-induced eosinophilia on CD4+ T cells with the complete absence of these granulocytes in GK1.5-treated mice (Fig. 2B). This phenomenon also extended to circulating cancer cells as the depletion of CD4+ T cells resulted in a concomitant decrease in the number of lung metastases following administration of melanoma cells (Fig. 3A). That is, similar to the complete abrogation of allergen-induced pulmonary pathologies occurring in GK1.5-treated mice, the loss of CD4+ T cells reduced the number of lung metastases in OVA-treated mice to levels observed in allergen-naive control animals (Fig. 3B).

In contrast to the apparent requirement of CD4+ T-cell activities, the singular absence of eosinophils had no effect on the increased metastasis observed in OVA-treated mice. Specifically, these studies exploited the availability of our transgenic mice (line: PHIL) that are congenitally devoid of eosinophils (18). PHIL mice subjected to the OVA provocation/metastasis protocol outlined in Fig. 1 failed to recruit any eosinophils following OVA challenges/provocations (Fig. 2B). However, unlike the depletion of CD4+ T cells, the loss of
OVA-mediated recruitment/accumulation of pulmonary eosinophils in PHIL mice did not prevent an increase in lung metastases following administration of melanoma cells (Fig. 3A). Significantly, the lack of an effect on lung metastasis in OVA-treated PHIL mice was unambiguous as no quantitative difference was observed in the number of lung metastases relative to OVA-treated wild-type control mice (Fig. 3B).

**Attenuation of allergic pulmonary pathologies by administration of corticosteroid blocks the induced increase in the number of lung metastases.** Administration of inhaled corticosteroids, a therapeutic regimen commonly used to curtail the pulmonary inflammation occurring in asthma patients, was assessed in our mouse model of asthma/metastasis for its ability not only to block the allergen-induced inflammation but also to attenuate the observed increase in metastasis to the lung. In these studies cohorts of OVA-treated mice were administered the corticosteroid budesonide (control animals received PBS vehicle alone) during the OVA challenge/provocation phase of the protocol outlined in Fig. 1. Administration of budesonide significantly decreased the level of lung histopathologies (e.g., extracellular matrix collagen deposition, airway goblet cell metaplasia, and mucin accumulation) and airway Th2 [e.g., interleukin (IL)-4, IL-5, and IL-13] cytokine expression (20, 30), as well as the recruitment and tissue accumulation of both T cells (31) and proinflammatory eosinophils (Fig. 4A). More importantly, administration of budesonide to OVA-treated wild-type mice also resulted in a significant decrease in metastasis to the lung (Fig. 4B). Interestingly, no difference was observed in the number of metastases occurring between saline control groups receiving budesonide or vehicle controls, suggesting that nonspecific effects of steroid administration were not responsible for the observed decrease in metastasis.

**Blockade of endothelial cell activation prevents allergen-induced recruitment of both proinflammatory leukocytes and circulating cancer cells.** The trafficking of leukocytes from the blood to sites of inflammation is the cumulative result of receptor ligand–mediated signaling events associated with the leukocytes themselves as well as with the underlying vascular endothelium. Our recently published studies have shown that receptors coupled to intracellular heterotrimeric G proteins containing a Go<sub>i2</sub> subfamily member elicit signaling pathway(s) in the vascular endothelium that regulate(s) a critical step required for transmigration (i.e., diapedesis) of leukocytes through the endothelium and into surrounding lung tissues (16). Specifically, these data showed that the loss of a Go<sub>i2</sub> signaling event(s) had no effects on allergen-mediated Th2 immune responses. However, the loss of Go<sub>i2</sub> signaling in the vascular endothelium of the lung prevented the accumulation of proinflammatory leukocytes in both an allergic model of asthma and an endotoxin-mediated model of pulmonary inflammation. The generalized mechanisms described by these data also suggested that other invasive cells may use a similar trafficking pathway. We subjected Go<sub>i2</sub> knockout mice to the concurrent asthma and metastasis model to define the role of endothelial cell activation in the firm adhesion and diapedesis of circulating cancer cells. Significantly, the loss of these signaling events in knockout mice prevented the accumulation of eosinophils in the perivascular/peribronchial regions (Fig. 5A). We have previously shown that this blockade is at the level of transmigration across the vascular endothelium (16), and significantly, our current studies show that metastasis formation in OVA-treated Go<sub>i2</sub> knockout mice displays a similar decrease relative to OVA-treated wild-type animals (Fig. 5B).

**Ex vivo** studies of adhesion and transmigration were performed to show and ultimately define a potential mechanism of tissue invasion by cancer cells. Specifically, **ex vivo** laminar flow assays (Fig. 6) were used to assess VCAM-1/α<sub>4</sub> integrin–dependent melanoma cell
adherence and migration through an endothelial cell monolayer (32, 33). In these studies, Goi signaling events were abrogated in the endothelial monolayer by pretreatment with pertussis toxin (16). This pretreatment of the endothelial cells had no effect on cytoxicity/viability or surface expression levels of the adhesion ligand VCAM-1 (16). The laminar flow study showed that Goi integrin/VCAM-1–dependent firm adhesion of melanoma cells was unaffected in the absence of endothelial cell Goi signaling (Fig. 6B), suggesting that effects on adhesion mediated by endothelial Goi signaling were not responsible for the blockade of metastasis in OVA-treated knockout mice. However, the subsequent migration of firmly adherent melanoma cells through the endothelial cell monolayer (i.e., endothelial transmigration) in the laminar flow assay showed that this process was a Goi-dependent event and was thus significantly inhibited following pertussis toxin treatment of the monolayer (Fig. 6C). This result suggests that a signaling event(s), absent in the venule endothelium of Goi mice, is required specifically for efficient transmigration/diapedesis.

The prevalence of asthma is disproportionately higher among human breast cancer patients with recurrence of the disease as pulmonary metastasis. In an attempt to provide initial insights about the translatability of our mouse model studies to human subjects, we examined patient records from MCSBCD assessing the frequency of asthma in patients whose disease recurrence was evidenced by pulmonary metastases. That is, we determined the prevalence of an asthma diagnosis among the 176 breast cancer patients in the MCSBCD with recurrence of disease as lung metastases. A review of these 176 breast cancer cases revealed that 30 patients had a diagnosis of asthma, although it could not be determined in one case when asthma was diagnosed. Thus, this survey of the MCSBCD revealed 29 patients with a diagnosis of asthma of which 23 were identified at least 1 year before the diagnosis of distant metastases. It is noteworthy that further examination of the patient medical records showed that none of these 23 patients had received p.o. and/or i.v. administration of corticosteroids. Indeed, these records showed that only two of these patients had any indication of inhaled corticosteroid use. Remarkably, these 23 patients alone represent >13% of the breast cancer patients who developed distant metastases in the lung (23 of 176). That is, despite being a conservative assessment, this percentage is nearly twice the predicted frequency of asthma among a random population of women in the United States [i.e., 7–8% (Surveillance for Asthma, National Center for Health Statistics, U.S. Centers for Disease Control and Prevention, 2003)], suggesting that the asthma of these cancer patients may have contributed to the appearance of lung metastases.

Discussion

The lung is the second most common site for metastasis of various tumors, including breast, colorectal, and skin (34–36). The progression of disease by metastasis to this largely vascularized organ reduces the chance for long-term survival of these patients and presents a significant clinical obstacle to their successful treatment. The present study identifies allergic pulmonary inflammation as a potential contributing factor in the process by which the lung is a selected target of circulating cancer cells. Our underlying hypothesis is that the nonspecific character of mechanisms leading to proinflammatory cell recruitment to the lung following allergen provocation represents pathways that circulating cancer cells may exploit to extravasate from peripheral/lymphatic circulation. These pathways include common cell adhesion molecule interactions as well as the expression of inflammatory cytokines/chemokines leading to cancer cell

Figure 5. Allergen treatment of Goi/- mice showed that Goi signaling was required for both the allergen-induced pulmonary inflammation leading to eosinophil accumulation and the increased number of pulmonary metastases. A, in the absence of Goi signaling, immunohistochemistry identifying MBP+ eosinophils infiltrating the lung (grayish/black staining cells) revealed that the loss of these signaling events effectively abolished the accumulation of eosinophils in the lungs of OVA-treated knockout mice (Goi/−) relative to wild-type animals (Goi/+/). B, representative photographs of lungs resected from Goi/− versus Goi/+ mice following the OVA/metastasis protocol showed that OVA treatment of Goi/− mice fails to elicit the significant elevation in the number of metastases observed in OVA-treated Goi/+ animals. Scale bar, 100 μm.
recruitment, localized proliferation, and/or the increased survival of accumulating cells.

The parallels between allergen-induced proinflammatory cell recruitment to the lung and the selective recruitment of circulating cancer cells to this organ were explored through the unique strategy of combining established mouse models of asthma and lung metastasis. Collectively, these studies showed that allergen-induced pulmonary inflammation is linked to a significant increase in the appearance of lung metastases following i.v. administration of cancer cells. It is noteworthy that allergen treatment of eosinophil-less PHIL mice showed that the recruitment of eosinophils to the lungs is not required for enhanced metastasis. Moreover, enhanced metastasis occurred in OVA-treated PHIL mice despite the fact that the loss of eosinophils resulted in a >50% reduction in allergen-induced end point histopathologies (18, 37). These results suggest that the enhanced metastasis was not a linear function of either leukocyte recruitment/accumulation or a downstream consequence of induced inflammation to structural components of the lung.

Similar to the ablation of CD4+ T cells, administration of an immunosuppressing corticosteroid (budesonide) to the lungs of allergen-treated mice both abolished the induced pulmonary pathologies (including lung-specific cytokine/chemokine expression and the allergen-induced eosinophil infiltrate; ref. 20) and, more importantly, eliminated the increased lung metastasis associated with allergen provocation. Although budesonide was shown to modulate gene expression of lung tumors and, in turn, primary tumorigenesis in the lung (38), the lack of any effect on the number of lung metastases in baseline saline control animals with and without budesonide treatment suggests that the corticosteroid treatment itself was not a regulator of pulmonary metastasis. The importance of this observation has implications beyond studies of mouse models as inhaled corticosteroids represent the prominent therapeutic modality used in the treatment of the inflammation associated with asthma patients.

Our earlier studies of proinflammatory cell recruitment in mouse models of allergen challenge showed that T-cell–mediated activation of the vascular endothelium via one or more Goi-dependent coupled receptors, and not Goi-mediated elaboration of Th2 immune responses, was critical to the extravasation of proinflammatory leukocytes from circulation (16). The data presented in this report show that circulating cancer cells use this same mechanism as both allergen-induced eosinophil recruitment and enhanced lung metastasis were lost in OVA-treated Goi/−/− mice. Moreover, our ex vivo studies using a parallel plate flow chamber system showed that whereas adhesion of melanoma cells to endothelial cells was not Goi dependent, the ability of these cancer cells to invade and transmigrate through the endothelial cell monolayer was a Goi-dependent phenomenon. In addition, our ex vivo adhesion data showed that adhesion of the melanoma cells to endothelial cells was nonetheless dependent on interactions with endothelial cell–expressed VCAM-1 (39, 40). This observation is particularly important because allergen provocation of the lung is associated with a T-cell–dependent activation of the vascular endothelium, including the enhanced endothelial cell expression of VCAM-1 (41, 42). Thus, the increased metastasis observed in mice following allergen provocation may be a consequence of both a Goi-dependent ability to extravasate from circulation and the availability of increased VCAM-1/Lot integrin interactions. That is, cancer cell avidity to the vascular endothelium of the pulmonary bed is increased following allergen provocation and, together with immune responses that lead to endothelial cell activation, these events promote transmigration in the lung parenchyma.

The significance of these mouse model studies was greatly enhanced by our preliminary examination of breast cancer patients from a surgical database showing that the frequency of asthma among patients with reoccurrence of their disease as lung metastases is elevated relative to that expected from cross-sectional assessments of the general female population. Clearly,
the provocative character of these observations will require additional and more detailed studies of the patients in this database. In particular, future studies are necessary to evaluate the importance of patient age and ethnicity, the time of asthma diagnosis as well as disease severity, and whether the apparent effects of allergic respiratory inflammation on pulmonary metastasis are lung specific versus a phenomenon affecting metastasis to other distal sites. Moreover, what effects on lung metastasis, if any, occur as a consequence of specific asthma therapies. As an example, the medical records of the patients in our breast cancer database indicated that only two were receiving corticosteroids in any form; however, will larger studies reveal that guideline use of inhaled corticosteroids for asthma control reduces the occurrence of pulmonary metastases? These are but a few questions that will be answerable from continued studies attempting to link asthma and lung metastasis. The importance of establishing a link between increased metastasis to the lung among breast cancer patients who have asthma would be difficult to overestimate given that 7% to 8% of adult women in the United States are diagnosed with this respiratory disease and one in eight women (by age 90) are diagnosed with breast cancer. Our demonstration of such a link has the potential to affect significantly the way cancer patients are treated. In particular, aggressive treatment of the underlying inflammation associated with asthma may have a considerable effect on the quality of life and/or survival of breast cancer patients with this respiratory disease.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
Received 5/2/2008; revised 7/23/2008; accepted 8/13/2008.
Grant support: Mayo Foundation; Intramural Research Program of the NIH (L. Rimbaum) and NIH grants R01CA112342 and K32-RE019709 (J. J. Lee), HL085723 (N.A. Lee), and E22HL83718 (A.G. Taranova).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Drs. Grzegorz Baran and Mark Inman as well as Katie O’Neill, Dana Colbert, and Ralph Pero for their invaluable contributions and Linda Mardel and Shirley “Charlie” Kern for their excellent administrative support.

References
Allergic Pulmonary Inflammation Promotes the Recruitment of Circulating Tumor Cells to the Lung

Anna G. Taranova, David Maldonado III, Celine M. Vachon, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/68/20/8582

Cited articles
This article cites 42 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/68/20/8582.full#ref-list-1

Citing articles
This article has been cited by 10 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/68/20/8582.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.