Inflammation-Related IL1β/IL1R Signaling Promotes the Development of Asbestos-Induced Malignant Mesothelioma

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Abstract

Exposure to asbestos is causally associated with the development of malignant mesothelioma, a cancer of cells lining the internal body cavities. Malignant mesothelioma is an aggressive cancer resistant to all current therapies. Once inhaled or ingested, asbestos causes inflammation in and around tissues that come in contact with these carcinogenic fibers. Recent studies suggest that inflammation is a major contributing factor in the development of many types of cancer, including malignant mesothelioma. The NALP3/NLRP3 inflammasome, including the component ASC, is thought to be an important mediator of inflammation in cells that sense extracellular insults, such as asbestos, and activate a signaling cascade resulting in release of mature IL1β and recruitment of inflammatory cells. To determine if inflammasome-mediated inflammation contributes to asbestos-induced malignant mesothelioma, we chronically exposed Asc-deficient mice and wild-type littermates to asbestos and evaluated differences in tumor incidence and latency. The Asc-deficient mice showed significantly delayed tumor onset and reduced malignant mesothelioma incidence compared with wild-type animals. We also tested whether inflammation-related release of IL1β contributes to tumor development in an accelerated mouse model of asbestos-induced malignant mesothelioma. NF2+/Cdkn2a+/− mice exposed to asbestos in the presence of anakinra, an IL1 receptor (IL1R) antagonist, showed a marked delay in the median time of malignant mesothelioma onset compared with similarly exposed mice given vehicle control (33.1 weeks vs. 22.6 weeks, respectively). Collectively, these studies provide evidence for a link between inflammation-related IL1β/IL1R signaling and the development of asbestos-induced malignant mesothelioma. Furthermore, these findings provide rationale for chemoprevention strategies targeting IL1β/IL1R signaling in high-risk, asbestos-exposed populations. Cancer Prev Res; 9(5): 406–14. ©2016 AACR.

Introduction

Malignant mesothelioma is an aggressive cancer that arises primarily from the mesothelial lining of the pleural and peritoneal cavities. Patients with malignant mesothelioma usually present with advanced-stage disease that is often surgically inoperable and refractory to standard chemotherapeutic regimens. Epidemiological studies have established that exposure to asbestos fibers is the primary cause of malignant mesothelioma (1). Despite asbestos abatement efforts, the incidence of malignant mesothelioma in the United States has remained stable since 1994, causing approximately 3,200 deaths annually (2), but will increase by 5% to 10% per year in Europe over the next 25 years (3). Furthermore, a marked increase in malignant mesothelioma incidence is predicted in developing countries, where usage of asbestos is increasing at an alarming rate (4). With estimates of >20 million individuals at risk worldwide, new approaches for the prevention and management of this disease are urgently needed.

Malignant mesothelioma is a cancer characterized by recurrent genomic losses that typically occur in combination (5). Molecular genetic studies of human malignant mesotheliomas have uncovered recurrent somatic mutations of several tumor-suppressor genes, particularly CDKN2A (encoding p16INK4A and p14ARF, refs. 6, 7), NF2 (8, 9), and BAP1 (10, 11). Genetically engineered mouse (GEM) models of malignant mesothelioma have demonstrated that haploinsufficiency for Cdkn2a, NF2, or Bap1 accelerates asbestos-induced malignant mesothelioma onset and progression (12–16), thereby providing both experimental evidence in support of the pivotal role of these tumor-suppressor genes in malignant mesothelioma pathogenesis and relevant murine models to facilitate preclinical studies of novel chemopreventive and chemotherapeutic agents.

More than a century ago, Virchow demonstrated a link between cancer and inflammation when he observed macrophages and other inflammatory cells in tumor biopsies (17). Upon exposure to asbestos, macrophages are recruited to sites of asbestos deposition, indicating that one physiologic response to mineral fiber exposure is inflammation. Mice exposed to asbestos exhibit...
recruitment of activated macrophages to mesothelium that interacts with asbestos fibers (18). As a result of increased macrophage accumulation, the nearby mesothelial cells are thought to be exposed to proinflammatory cytokines, such as IL1β and TNFα (18). Interestingly, IL1β and TNFα can act in concert with asbestos fibers to transform normal mesothelial cells in vitro, suggesting that inflammation may directly contribute to malignant mesothelioma development (19, 20). IL1β, as opposed to TNFα, has been shown to increase the proliferative capacity of mesothelial cells in vitro (19, 20). Interestingly, depletion of activated macrophages in an orthotopic mouse model of malignant mesothelioma development (19, 20). IL1β may contribute to mesothelial proliferation and transformation through release of the cytokines IL1β and TNFα.

Macrophages are also thought to directly interact with and phagocytize asbestos fibers (18). Interestingly, it has been hypothesized that macrophages and mesothelial cells exposed to asbestos undergo frustrated phagocytosis of elongated fibers; this process is thought to cause chronic production of reactive oxygen species (ROS) and cytokines, which contribute to transformation of mesothelial cells (22). Thus, chronic inflammation associated with exposure to asbestos may represent a form of extrinsic cancer-related inflammation fundamentally important in malignant mesothelioma development, a hypothesis that is addressed herein.

The NALP3/NLRP3 inflammasome is a protein complex made up of NALP3, ASC (apoptosis-associated speck-like protein containing a caspase-associated recruitment domain; designated Asc in the mouse), and caspase-1. The IL1β/NFκB inflammasome complex plays an important role in recognizing external stimuli and mediating inflammation in response to certain environmental insults (23–25). Upon activation, the IL1β/NFκB inflammasome facilitates the processing of IL1β from its immediate precursor form to a cleaved mature form via caspase–1-mediated activity. The processed IL1β is then released from macrophages to help stimulate inflammation. The inflammasome is also activated upon exposure to bleomycin and is required for lung inflammation and fibrosis (26). Bleomycin-mediated fibrosis and inflammation can be blocked by an IL1 receptor (IL1R) antagonist, which suggests that inflammasome-mediated production of IL1β is a required process in the inflammatory response (26).

The NALP3 inflammasome has also been shown to mediate inflammation and IL1β production from macrophages in response to asbestos and silica (27, 28). Importantly, macrophages deficient for the inflammasome components ASC or NALP3 did not produce increased levels of IL1β in response to asbestos, suggesting that macrophages produce IL1β in response to mineral fibers specifically through the NALP3 inflammasome (27). Moreover, macrophages require endocytosis and ROS production for inflammasome activation to occur in response to asbestos. In a model of asbestos inhalation, mice deficient for ASC or NALP3 exhibited reduced inflammatory cell recruitment and decreased cytokine production in the lung (27), suggesting that the NALP3 inflammasome mediates inflammation caused by exposure to asbestos.

In this investigation, we have used in vivo approaches with GEM models and a clinically relevant IL1R antagonist, anakinra, to formally test whether asbestos-induced inflammation is required for malignant mesothelioma pathogenesis. We used both an inflammasome-deficient Asc knockout model and a small-molecule inhibitor of IL1R signaling to test whether a defective inflammasome or pharmacologic inhibition of IL1β/IL1R signaling could delay malignant mesothelioma onset and progression. Asc-deficient mice do not mount a proinflammatory response to asbestos and silica (27, 28), and, thus, provide an important resource to directly test the importance of asbestos-induced inflammation in malignant mesothelioma development/progression. We provide the first in vivo evidence with GEM models that inflammation directly contributes to the development of asbestos-induced malignant mesothelioma and show that inflammasome-mediated IL1β/IL1R signaling contributes to cancer-related inflammation and may represent a promising potential target for the prevention of malignant mesothelioma.

Materials and Methods

Experimental animals

Asc-null mice were kindly provided by V.M. Dixit (Genentech; ref. 23). The Asc−/− mice were backcrossed with C57BL/6 mice to generate the various Asc genotypes (−/−, −/+ , +/+ ) needed for the asbestos carcinogenicity experiments. Nf2−/−;Cdtn2a−/− mice (29) were generated by crossing Nf2−/− and Cdtn2a−/− mice, each backcrossed six to seven generations into the same FVB background. The Cdtn2a−/+ (CD8129-Cdtn2a+/−) mice (30), which are haploinsufficient for both p16(Ink4a) and p19(Arf), were obtained from the Mouse Models of Human Cancers Consortium. Nf2−/− mice were obtained from T. Jacks (MIT, Cambridge, MA) and A.I. McClatchey (MCH, Charleston, MA) (31).

Treatment with asbestos

For studies assessing the requirement of inflammation in malignant mesothelioma development, Asc−/−, Asc−/+ , and Asc+/+ mice were injected i.p. with crocidolite asbestos (UIICC grade, SPI Supplies), employing a modified method previously used to induce malignant mesothelioma in heterozygous Tp53 mice (32). Male mice of 6 to 8 weeks of age were anesthetized and injected with 400 μg of crocidolite in 500 μl PBS every 21 days for a total of eight injections (total = 3.2 mg; refs. 13, 29). The 21-day interval between injections was selected based on previous work showing that 20 μg to 1 mg of crocidolite injected i.p. stimulates mesothelial cell proliferation for at least 21 days (33). Altogether, 84 animals (26+/−, 29−/−, and 29−/+ ) were chronically injected with asbestos and closely followed until evidence of disease (see below). Six Asc−/− mice were excluded in the percent disease-free comparisons due to the development of peritonitis in association with the chronic injections of asbestos. Peritonitis was not found in any of the wild-type (WT) or Asc−/+ mice.

To investigate the efficacy of an IL1R antagonist as a chemopreventive agent, Nf2−/−;Cdtn2a−/+ mice were first weighed at 8 to 10 weeks of age prior to the start of the experiment. In this experiment, mice were injected i.p. with 800 μg of crocidolite in 500 μl PBS every 21 days for a total of four injections (total = 3.2 mg). Although the total dose of asbestos fibers was identical to that used in the experiments with Asc−/− mice, only four injections of asbestos were used in the studies with Nf2−/−;Cdtn2a−/+ mice for two reasons: (i) so that the full dose of asbestos could be administered before animals began to show signs of malignant mesothelioma, which occurs relatively rapidly in the Nf2−/−;Cdtn2a−/+ model; and (ii) to minimize trauma, as...
Detection of tumors

All in vivo studies were performed according to NIH’s Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Fox Chase Cancer Center (protocol 00-26). Specifically, all mice were examined daily and sacrificed upon detection of abdominal bloating, weight loss of >10%, labored breathing, severe lethargy, or when tumor burden was otherwise obvious. At that point, mice were euthanized and all internal organs and fluids were collected and sent to our Histopathology Facility for assessment. Detailed analysis of the peritoneal, pleural, and pericardial tissues to detect malignant mesothelioma was our highest priority, although complete necropsies were performed on all mice. For the experiment involving Asc-deficient mice, we performed ascitic taps on three animals from each experimental arm at the beginning of the study in order to develop malignant mesothelioma cell lines and to relieve bloating (up to a maximum of three times or until hemorrhagic fluid was observed). Disease onset in animals that received an ascitic tap(s) was considered to be the time of the initial ascitic tap.

Tissue preparation, histology, and immunohistochemistry

All organs, including lymph nodes, were collected for examination, and histopathology was carried out with the assistance of the Histopathology Facility of Fox Chase Cancer Center. Tissues were collected and fixed in neutral buffered formalin for 24 to 48 hours, dehydrated, and then embedded in paraffin. Hematoxylin and eosin (H&E)–stained sections were used for histopathologic evaluation, and unstained sections were used for immunohistochemistry (IHC). Immunohistochemical staining was performed on 5-μm formalin-fixed, paraffin-embedded (FFPE) sections. After deparaffinization and rehydration, FFPE sections were subjected to heat-induced epitope retrieval by steaming in 0.01 mol/L citrate buffer (pH 6.0) for 20 minutes. After endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 30 minutes, sections were blocked with alcohol, cleared in xylene, and mounted. Murine samples from the Histopathology Facility of Fox Chase Cancer Center. Tissues received an ascitic tap(s) was considered to be the time of the initial ascitic tap.

Evaluation of levels of IL1β, IL6, and TNFα in asbestos-exposed Asc mice

Asbestos-exposed Asc+/− and Asc−/− mice were analyzed for plasma and peritoneal lavage fluid (PLF) levels of IL1β, IL6, and TNFα using ELISA kits purchased from BD Biosciences (BD OptEIA Mouse TNFα and IL-1β ELISA Kits) and R&D systems (mouse IL-6 Quantikine ELISA Kit). For both Asc+/− and Asc−/− mice, blood and PLF samples were collected from 5 mice that were injected 3 days earlier with asbestos, as well as from 5 mice that were not exposed previously. Two independent experiments were performed (total = 40 mice). Immediately following CO2 euthanization of animals, blood was drawn by heart puncture, collected
in sterile tubes containing heparin, and placed on ice. PLF samples were also collected in sterile tubes and initially placed on ice and then frozen at -80°C. Plasma and PLF samples were run undiluted in duplicate and assays were performed according to the manufacturer’s instructions. Cytokine levels are reported as picograms per milliliter (pg/mL) of PLF and plasma. Briefly, peritoneal lavage was performed with 5 mL of sterile PBS using an 18-gauge needle.

Statistical analysis
The Fisher exact test was used to assess the statistical significance of differences among experimental groups with respect to time from the initial asbestos injection to obvious signs of asbestos-related disease. The failure time for these analyses was defined as the age at disease detection. The median percent disease-free calculations were determined between asbestos-exposed Asc−/−, Asc+/−, and Asc+/+ mice, as well as between asbestos-exposed Nf2+/−:Cdhn2a+/− mice treated either with IL1R antagonist (anakinra) or vehicle control. For statistical analysis of malignant mesothelioma incidence, a χ2 test was used. In the experiment with Asc-deficient mice, animals dying before evidence of asbestos-associated disease were omitted from the percent disease-free computations. The excluded animals included 6 Asc−/− mice with peritonitis, as well as 3 Asc+/− and 2 Asc+/+ mice for which cause of death could not be determined.

Results

Asbestos-exposed Asc-deficient mice show delayed malignant mesothelioma onset, reduced tumor incidence, and decreased IL1β release when compared with asbestos-exposed WT mice

Mice with haploinsufficiency or with complete loss of a component (Asc) of the Nalp3 inflammasome, i.e., Asc−/− and Asc+/− mice, respectively, each showed a statistically significant delay in malignant mesothelioma onset, when compared with asbestos-exposed WT (Asc+/+) littermates. The median of percent disease-free, asbestos-exposed Asc−/− and Asc+/− mice that developed malignant mesothelioma was 69.4 and 66.2 weeks, respectively, which was significantly longer than that of asbestos-exposed WT mice (61.6 weeks), as summarized in Fig. 1 and Supplementary Fig. S1. In addition, the incidence of malignant mesothelioma in Asc−/− and Asc+/− mice (65% and 55%, respectively) was reduced compared with that of WT mice (80%). The difference in malignant mesothelioma incidence was statistically significant between WT mice and Asc−/− mice (P < 0.05), but not between WT and Asc+/− mice (Supplementary Figs. S1 and S2). Note that all tumors that were diagnosed as malignant mesothelioma histologically also stained positively for mesothelin and WT1, well-accepted markers for murine malignant mesotheliomas. H&E staining and IHC for mesothelin and WT1 in a representative malignant mesothelioma from an Asc−/− mouse are shown in Fig. 2, along with staining for Ki67, the latter indicative of active cellular proliferation in tumor cells. Histologically, most malignant mesotheliomas observed were sarcomatoid, i.e., 14 of 21 (67%) from WT mice, 13 of 19 (68%) from Asc−/− mice, and 12 of 16 malignant mesotheliomas (75%) in Asc−/− mice. Most of the remaining malignant mesotheliomas were biphasic. We next assessed the levels of IL1β, the downstream target of the Asc–inflammasome complex, in the plasma and PLF of WT and Asc+/− mice collected 3 days after injection of asbestos or vehicle. As shown in Fig. 3A, the relative levels of IL1β were significantly decreased in plasma from unexposed and asbestos-exposed Asc−/− mice when compared with that of corresponding WT animals, consistent with a previous report (37). A similar decrease in IL1β levels was found in PLF from unexposed and asbestos-exposed Asc−/− mice compared with that of WT mice (Fig. 3B). We also evaluated the levels of IL6 and TNFα, two inflammatory cytokines associated with cancer, in the plasma and PLF of unexposed and asbestos-exposed WT and Asc+/− mice. IL6 levels were significantly downregulated in both the plasma and PLF of unexposed and asbestos-injected Asc−/− mice when compared with corresponding WT animals (Fig. 3A and B). The levels of TNFα were not significantly different in the plasma and PLF of unexposed WT and Asc−/− mice; however, they were lower in both plasma and PLF of asbestos-exposed Asc−/− mice compared with WT controls (Fig. 3A and B). Together, these data demonstrate a decrease in inflammatory cytokine release in unexposed and asbestos-exposed Asc-deficient mice compared with WT mice, consistent with a central role of the inflammasome in asbestos-mediated inflammation.

The IL1β receptor antagonist anakinra delays asbestos-induced tumorigenesis in an accelerated mouse model of malignant mesothelioma

We next addressed whether IL1β release mediated by the inflammasome could be targeted as a chemoprevention strategy for asbestos-induced malignant mesothelioma. We used a Nf2−/−;Cdhn2a−/− double knockout mouse model, which we previously reported develop malignant mesothelioma in an accelerated manner upon exposure to asbestos, i.e., approximately 23 weeks in Nf2−/−;Cdhn2a−/− mice versus 52 weeks in WT littermates (29). Thus, this accelerated model has advantages for more rapid...
assessment of chemoprevention agents. Male Nf2+/−;Cdkn2a−/− mice were chronically exposed to asbestos in the presence or absence of the IL1R antagonist anakinra, and median of percent disease-free animals and tumor development was assessed. All mice in both experimental arms eventually developed malignant mesothelioma, although onset of disease symptoms was significantly delayed in the anakinra-treated animals. As shown in Fig. 4A, Nf2+/−;Cdkn2a−/− mice treated with anakinra had an approximately 50% longer median percent disease-free rate (33.1 weeks) than in vehicle-treated mice (22.6 weeks). Malignant mesotheliomas from mice treated with anakinra showed decreased IHC staining for Ki67 when compared with malignant mesotheliomas from mice treated with vehicle (Fig. 4B). Together, these findings suggest that asbestos exposure leads to IL1β/IL1R signaling that contributes to malignant mesothelioma cell proliferation and downstream oncogenic signaling, and that inhibition of IL1β/IL1R signaling with an IL1R antagonist can delay malignant mesothelioma tumorigenesis.

Discussion

The role of inflammation in cancer pathogenesis is an exciting, growing field, with many solid tumors of various origins being influenced by the tumor microenvironment and cells of the immune system. Exposure to asbestos fibers, both in humans and animal models, causes an inflammatory response primarily mediated by macrophages that attempt to phagocytose these fibers in and around the mesothelial lining. Hillegass and colleagues have shown that human mesothelial cells also have inflammasomes and can perpetuate inflammation in the absence of immune cells (38).

With longer pathogenic fibers, “frustrated” phagocytosis has been hypothesized to cause mesothelial cell proliferation due to the release of cytokines such as IL1β and TNFα (22). Moreover, the NALP3 inflammasome is now known to mediate inflammation and the release of mature IL1β from macrophages in response to asbestos and silica to cause local inflammation (27, 28). Similarly, asbestos and erionite fibers are able to prime and activate the NALP3 inflammasome, which triggers an autocrine feedback loop modulated via the IL1R in mesothelial cells targeted in pleural infection, fibrosis, and carcinogenesis (38). Thus, the
inflammation caused by exposure to asbestos requires the inflammmasome, and targeting this pathway would be expected to prevent or diminish inflammation and delay tumor development.

In this report, we have formally tested whether inflammmasome-mediated inflammation is required for asbestos-induced malignant mesothelioma development. Our findings indicate that deficiency for the inflammmasome component Asc is not sufficient to prevent asbestos-induced malignant mesothelioma development, although a decrease in malignant mesothelioma incidence and a significantly longer tumor latency were observed in Asc−/− mice when compared with similarly exposed WT littermates (Supplementary Figs. S1 and S2). Although a statistically significant difference in median percent disease-free animals was observed between WT and Asc−/− mice, the difference in malignant mesothelioma frequency was not (Fig. 1 and Supplementary Fig. S2), indicating that haploinsufficiency for Asc is, as expected, less effective than total loss of Asc with regard to asbestos-induced malignant mesothelioma incidence.

We did not observe a statistically significant difference in the median percent disease-free animals between asbestos-exposed Asc+/− and Asc−/− mice. However, this finding was complicated by the fact that 6 Asc−/− mice had to be euthanized due to peritonitis, which was confirmed by cytological assessment of peritoneal fluid. Although the infected Asc−/− animals were omitted in the tumor onset and percent disease-free calculations, it is noteworthy that none of the Asc+/− or WT mice showed any evidence of infection. Thus, the findings suggest that the immune system of Asc−/− mice was compromised, which likely played a role in their overall health and survival. This is consistent with other studies that have shown that inflammmasome-deficient mice are predisposed to infection due to a weakened inflammatory response (39).

We demonstrated that at 3 days after asbestos exposure, IL1β levels were markedly lower in plasma and PLF of asbestos-exposed Asc+/− mice compared with that of WT animals (Fig. 3A and B). Interestingly, however, at 3 months after exposure, there was no detectable difference in the levels of IL1β or IL6, and TNFα between WT and Asc−/− mice compared with that of WT (Asc+/−) mice. A, IL1β, IL6, and TNFα levels in serum of Asc−/− and Asc+/− mice exposed or not (unexposed) to asbestos. B, IL1β, IL6, and TNFα levels in PLF of Asc−/− and Asc+/− mice exposed or unexposed to asbestos. P values for differences between the indicated samples from Asc−/− (black bars) and Asc+/− (white bars) mice are provided for each cytokine and experimental condition tested.

Figure 3.
Decreased levels of IL1β and other inflammatory cytokines in serum and PLF from Asc−/− mice compared with that of WT (Asc+/−) mice. A, IL1β, IL6, and TNFα levels in serum of Asc−/− and Asc+/− mice exposed or not (unexposed) to asbestos. B, IL1β, IL6, and TNFα levels in PLF of Asc−/− and Asc+/− mice exposed or unexposed to asbestos. P values for differences between the indicated samples from Asc−/− (black bars) and Asc+/− (white bars) mice are provided for each cytokine and experimental condition tested.

IL1β/IL1R Signaling Promotes Mesothelioma Development

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Anakinra treatment delays tumor onset and inhibits cell proliferation in a mouse model of accelerated asbestos-induced malignant mesothelioma. A, Kaplan-Meier curves of asbestos-exposed N2\textsuperscript{fl/fl}:Cdkn2a\textsuperscript{−/−} mice treated with anakinra or vehicle (citrate buffer). Treatment of asbestos-exposed N2\textsuperscript{fl/fl}:Cdkn2a\textsuperscript{−/−} mice with anakinra resulted in significantly prolonged survival compared with that observed in vehicle-treated littermates. B, Ki67 nuclear staining (brown) of malignant mesotheliomas from vehicle- and anakinra-treated mice. Note that adjacent normal liver in vehicle-treated mice (40, 41). Although the experiments with Asc-deficient mice implicated IL1\textbeta-mediated inflammation, which has been shown to accelerate mouse model of malignant mesothelioma pathogenesis, we wanted to determine if targeting this pathway could be exploited as a chemoprevention strategy in asbestos-exposed mice. To this end, we demonstrated that the IL1R antagonist anakinra could delay asbestos-induced tumor formation in an accelerated mouse model of malignant mesothelioma. Treatment of mice with anakinra during and after asbestos exposure delayed time of death due to malignant mesothelioma by about 50% (Fig. 4A). In addition, the proliferative capacity of malignant mesotheliomas from anakinra-treated mice was markedly decreased when compared with malignant mesotheliomas from vehicle-treated mice (Fig. 4B). These data suggest that IL1\textbeta signaling contributes to the proliferative capacity of tumors in asbestos-exposed mice. We further demonstrated, both in vitro and in vivo, that anakinra is capable of blocking oncogenic signaling nodes downstream of the IL1R (Fig. 5A and B), which have previously been shown to be important to malignant mesothelioma cell proliferation, survival, and invasiveness. However, as noted above, all anakinra-treated mice eventually developed malignant mesothelioma, although with a prolonged latency compared with vehicle-treated mice. Thus, we presume that other cytokines or immune components of the microenvironment also play a significant role(s) in malignant mesothelioma pathogenesis and can compensate for diminished IL1\textbeta signaling resulting from treatment with anakinra. Drawing parallels to the human disease counterpart, these data demonstrate in a preclinical proof-of-principle model that high-risk individuals chronically exposed to asbestos or other carcinogenic mineral fibers, such as erionite, might benefit from chemoprevention strategies targeting inflammation-related IL1\textbeta/IL1R signaling.

These data are the first to our knowledge directly linking asbestos and IL1\textbeta signaling to malignant mesothelioma pathogenesis in GEM models of the disease. Although the pharmacologic evidence from the studies with anakinra provides a strong link between inflammation-related IL1\textbeta/IL1R signaling and malignant mesothelioma development, the genetic evidence generated by studies with Asc-deficient mice is less striking. Although Asc-deficient mice exhibited a statistically significant delay in tumor onset, the mice showed a relatively modest decrease in overall incidence of malignant mesothelioma. A study performed by Australian investigators showed that although IL1\textbeta release was diminished in inflammasome-deficient mice, malignant mesothelioma tumor incidence did not differ significantly between inflammasome-deficient NLRP3\textsuperscript{−/−} mice (BALB/c background) and WT controls (37). Asbestos (3.0 mg/mouse, similar to the 3.2 mg/mouse used in our experiments) was given as a single bolus injection in that investigation, whereas we used serial injections of lower doses of asbestos given over approximately 5 months. A single bolus injection of a large quantity of asbestos into the peritoneum of mice often results in large granulomas and a low incidence and longer latency of malignant mesothelioma (42). Thus, we chose to administer eight injections of 400 μg of crocidolite in Asc\textsuperscript{−/−} mice, given every 3 weeks, in an attempt to induce a repeated state of inflammation, which has been shown to faithfully cause malignant mesothelioma in mice of various backgrounds and genotypes (12–15, 32). In addition to differences in the injection protocols used in the two studies, another difference in the studies is that Chow and colleagues (37) used Nlrp3-deficient mice, whereas we used Asc-deficient mice. Significantly, our conclusions about the importance of inflammasome-related IL1\textbeta/IL1R signaling are bolstered by our anakinra administration studies.

In conclusion, this report provides experimental evidence implicating inflammation in a cancer whose etiology is connected with environmental exposure to a known carcinogen: asbestos. Our findings indicate that the inflammasome contributes to asbestos-induced malignant mesothelioma development, in part through the production and release of IL1\textbeta to promote mesothelial cell proliferation and transformation into a deadly cancer, malignant mesothelioma. In addition, the findings of our studies with anakinra provide rationale for targeting inflammation and IL1\textbeta/IL1R signaling as a potential chemoprevention strategy for cohorts chronically exposed to asbestos or other carcinogenic mineral fibers.
Anakinra inhibits IL1β/IL1R signaling both in vitro and in vivo. A, immunoblot analysis of normal human mesothelial cells (HM3) starved overnight and then stimulated with IL1β in the presence or absence of anakinra. Anakinra-treated cells show markedly decreased activity of AKT, ERK, and JNK, as indicated by decreased expression using phospho (P)-specific antibodies. B, immunohistochemical staining (reddish brown) for P-AKT, P-JNK, and P-ERK in malignant mesotheliomas derived from asbestos-exposed mice treated or not with anakinra. Original images, ×200 magnification. Scale bar, 100 μm.

Disclosure of Potential Conflicts of Interest
M. Christofidou-Solomidou has ownership interest (including patents) in LignaMed, LLC. No potential conflicts of interest were disclosed by the other authors.

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