The Pathogenesis of Mesothelioma

Michele Carbone, Robert A. Kratzke, and Joseph R. Testa

About 80% of malignant mesotheliomas (MM) in the Western World develop in individuals with higher than background exposure to asbestos. Only a fraction of those exposed to asbestos develop mesothelioma, indicating that additional factors play a role. Simian virus 40 (SV40), a DNA tumor virus that preferentially causes mesothelioma in hamsters, has been detected in several human mesotheliomas. The expression of the SV40 large tumor antigen in mesothelioma cells, and not in nearby stromal cells, and the capacity of antisense T-antigen treatment to arrest mesothelioma cell growth in vitro suggest that SV40 contributes to tumor development. The capacity of T-antigen to bind and inhibit cellular p53 and retinoblastoma (Rb)-family proteins in mesothelioma, together with the very high susceptibility of human mesothelial cells to SV40-mediated transformation in vitro, supports a causative role of SV40 in the pathogenesis of mesothelioma. Asbestos appears to increase SV40-mediated transformation of human mesothelial cells in vitro, suggesting that asbestos and SV40 may be cocarcinogens. p53 mutations are rarely found in mesothelioma; p16, p14ARF, and NF2 mutations/losses are frequent. Recent studies revealed the existence of a genetic factor that predisposes affected individuals to mesothelioma in the villages of Karain and Tuzkoy, in Anatolia, Turkey. Erionite, a type of zeolite, may be a cofactor in these same villages, where 50% of deaths are caused by mesothelioma. Mesothelioma appears to have a complex etiology in which environmental carcinogens (asbestos and erionite), ionizing radiation, viruses, and genetic factors act alone or in concert to cause malignancy.
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MESOTHELIAL CELLS form the serosal lining of the pleural, pericardial, and peritoneal cavities.[1] Among the most undifferentiated cells of our body, mesothelial cells are capable of differentiating morphologically into epithelial-like cells or fibroblast-like cells. Mesothelial cells can be distinguished from epithelial cells and fibroblasts because of their ultramicroscopic characteristics and their immunophenotype.[1] Mesothelial cells are the adult equivalent of mesoderm, which covers the celomic cavity. In the adult, the mesoderm is called mesothelium, but to the best of our knowledge, mesoderm and mesothelium are equivalent. Another characteristic of these cells is their high level of wild-type p53. The levels are four to five times higher than in fibroblasts, and p53 is easily detectable by immunostaining (normally only mutated p53 is detectable by immunohistochemistry because wild-type p53 does not accumulate to sufficient amounts to be detectable by this technique). Malignant mesotheliomas (MM) are very aggressive tumors that originate from mesothelial cells. Due to the tumor's aggressive nature and resistance to chemotherapy, the median survival from the time of diagnosis is about 1 year. The term "malignant" is often added to that of mesothelioma, because, unfortunately, the latter is also used to describe a number of rare and mostly benign tumors that have little, if anything, to do with MM.[1] We will briefly describe these entities and thereafter use the term "mesothelioma" to indicate the classic malignant tumors of the pleura associated with asbestos exposure and recently to Simian virus 40 (SV40) infection and genetic abnormalities.

BENIGN MESOTHELIOMAS

Multicystic mesothelioma, also called multilocular peritoneal inclusion cyst, is a benign mesothelial lesion, characteristically formed by multiple cysts arranged in grape-like clusters. Adenomatoid mesotheliomas are benign mesothelial lesions of the genital system. Mesothelioma of the atrioventricular node is neither a mesothelioma nor a tumor. This lesion represents congenital heterotopia of the endodermal sinus in the atrioventricular node. Well-differentiated papillary mesothelioma is found more often in the abdominal cavity of young women. Histologically, it is formed by multiple papillary structures covered by cytologically benign mesothelial cells. The lesion is benign, but there have been occasional cases in which several years after diagnosis the patient developed a true mesothelioma. Localized mesothelioma, better re-

From the Cancer Immunology Program, Cardinal Bernardin Cancer Center, Department of Pathology, Loyola University Chicago; Department of Medicine, Veterans Affairs Medical Center, Section of Hematology and Oncology, Minneapolis MN; and the Fox Chase Cancer Center, Philadelphia, PA.

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Address reprint requests to M. Carbone, MD, PhD, Cardinal Bernardin Cancer Center, Room 205, 2160 S First Ave, Maywood, IL 60153.

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ferred to as localized fibrous tumor of the pleura, is thought to originate from the submesothelial cells, but it is unclear what submesothelial cells are. The tumor cells have a benign fibrous appearance and are characteristically negative for cytokeratin and positive for CD34, which suggests that these cells are not of mesothelial origin. Occasionally, localized fibrous tumors of the pleura are histologically and cytologically malignant and clinically these tumors are characterized by multiple recurrences after resection, and a poor prognosis. As stated above, these benign mesotheliomas should not be confused with the classic MM, which are among the most aggressive human tumors. However, to further complicate the matter, histologically malignant mesotheliomas have occasionally been associated with long survival durations of years or even decades. Whether the latter should be called MM is debatable.

ASBESTOS

Prior to the 1950s, MM were extremely rare. The first documented case of mesothelioma, according to current diagnostic criteria, was published in 1947.[2] In fact, some authors even questioned whether mesotheliomas existed at all because using current methodologies and reviewing 47,000 autopsies performed at the Massachusetts General Hospital from 1896 until 1947 they did not find any evidence of mesothelioma.[3] From 1947 until 1990, about 100 mesotheliomas were recorded at autopsy at the Massachusetts General Hospital.[3] In the last half century, the incidence of mesothelioma has significantly increased, and, currently, 2,000 to 3,000 cases are diagnosed per year in the United States alone.[4] The exact number of mesotheliomas in the United States is, however, unknown, because these numbers are projections from the Surveillance, Epidemiology and End Results (SEER) program. The SEER provides population-based, tumor-specific data on all histologically proven cancers occurring in selected geographic sites in the United States, and includes approximately 12% of the US population. Projections of these data are used to predict the total number of different tumor types. However, the total number of mesotheliomas appears to be very difficult to predict because the incidence of mesothelioma is higher in states with asbestos industries. The population that provides the SEER data was not chosen according to the presence or absence of asbestos sources. Furthermore, the *Atlas of Cancer Mortality in the US 1950-1994*, compiled by the National Cancer Institute (NCI), combines cancers of the lung, trachea, bronchus and pleura, making it impossible to study the incidence of a relatively rare cancer such as mesothelioma.[5] Therefore, the incidence of mesothelioma in the United States cannot be estimated precisely.

The increase in mesothelioma has paralleled a much more widespread use of asbestos in this century.[6-10] Approximately 80% of mesotheliomas are associated with asbestos exposure, and about 5% of asbestos workers develop mesothelioma.[4,10] These observations establish an indisputable link between asbestos and mesothelioma.[4,6-10] At the same time, these findings indicate that most people exposed to asbestos do not develop mesothelioma, and that there are about 400 to 600 mesothelioma cases per year in the United States alone, which are not associated with asbestos. Rare cases of mesotheliomas in children and infants also suggest that other factors may be involved in the etiology of the tumor.[4] Mesotheliomas develop in adults after a very long latency. Unless asbestos acts differently in children than in adults, cases of mesothelioma in children are not the result of asbestos. Thus, it is arguable that other factors, alone or in conjunction with asbestos, play a role in the development of mesothelioma.

In addition to mesothelioma, asbestos causes two other lethal diseases: lung cancer and asbestosis,[7] and two benign diseases: pleural plaques and pleural effusions.[1,7] There is also some evidence that asbestos may increase the risk of laryngeal cancer.[4] Asbestos and cigarette smoking have a synergistic carcinogenic effect in the pathogenesis of lung cancer.[7] Smoking also increases the risk of asbestosis, because smoke inhibits the mucociliary activity and increases fiber retention. Asbestosis develops in individuals exposed to very high amounts of asbestos (Table 1). There is a threshold level below which asbestosis is not seen, and the lower the exposure, the longer it takes to develop the disease. However, only a fraction of individuals exposed to fibrogenic doses of asbestos develop asbestosis, indicating that additional and presently unknown factors play a major role in determining individual susceptibility.[7]

The relationship between asbestos and mesothelioma is unique. In contrast to lung cancer and asbestosis, smoking does not increase the risk

Table 1. Asbestos Exposure and Human Diseases Based on Lung Content Analyses

	Uncoated Fibers[79]*	Crocidolite[76]†	Amosite[76]†	Amosite[7]‡
Controls	0-20	0.14-1.00	0.09-0.93	0.7
Mesothelioma	67	53 (30PTL-MM)	103 (100PTL-MM)	0.9
Asbestosis	690	1,100	450	10

* Fibers/g × 10^3 (uncoated fibers, mostly amosite).
† Million fibers/dry lung.
‡ Fibers × 10^6/g dry lung.
Abbreviation: PTL-MM, peritoneal malignant mesothelioma.

of mesothelioma.[11] In contrast to asbestosis, in which there is a dose-response relationship (the higher the exposure the higher the risk), this association does not exist for mesothelioma (Table 1), except for chrysotile asbestos, which may be oncogenic only at high doses.[7]

Asbestos, in fact, is a generic name for a family of naturally occurring silicate minerals with different carcinogenicities.[8] The various types of asbestos are divided into two major groups: serpentine represented by crysotile, the most common and economically important form of asbestos in the Western World; and the amphiboles, which include crocidolite, the most oncogenic type of asbestos, amosite, anthophyllite, and tremolite.[6-10] The role of crysotile in the pathogenesis of mesothelioma is controversial, whereas the capacity of amphibole asbestos to cause mesothelioma is well established epidemiologically.[8-10]

There are several reason for the confusion about crysotile carcinogenicity.[8,9] First, many patients are exposed to various types of asbestos. For example, Canadian crysotile is contaminated with about 1% of tremolite. Some authors attribute the development of mesothelioma to this contaminant,[8] others disagree.[9] Second, crysotile miners have more amphibole than crysotile fibers in their lungs.[7-9] This is because crysotile can be partially digested, and it is removed from the lung. Amphibole asbestos is more resistant to digestion by cellular enzymes, and accumulates in the lungs.[7] In conclusion, many patients have been exposed to both crysotile and amphibole asbestos, and it is difficult to establish the amount of exposure to the different types of asbestos.

When asbestos fibers reach the alveolar region, the body's initial response is an attempt to phagocytose and remove them from the lung. If the fiber is short and easily engulfed by alveolar macrophages, it is efficiently removed from the lung via transport to the ciliated epithelium of the smaller airways and eventually to the trachea and throat where it is swallowed. However, if the fiber is too long to be phagocytosed, it cannot be removed unless it is solubilized, which is not possible with amphibole asbestos. Shorter fibers can also be retained if the dose is so large that it *overwhelms* the pulmonary defense mechanism. Asbestos fibers have the capacity to reach the pleura either through the lymphatics, or by direct penetration, and to cause fibrosis, pleural plaques, and eventually mesothelioma.[12] In the pleura, amphibole fibers are distributed heterogeneously, and are concentrated in the "black spots" of the parietal pleura.[12] These black spots are the anthracotic deposits observed on the parietal pleura of smokers and of people exposed to coal dust, and correspond to areas of deposit along the lymphatics. It has been suggested that the concentration of asbestos in the black spots accounts for the similar location of pleural plaques and for the observation that mesothelioma usually originates on the parietal and diaphragmatic pleura rather than on the visceral pleura.[12] Whether mesothelioma originates from or close to these black spots is unknown.

What happens when asbestos reaches the parietal pleura that ultimately leads to mesothelioma is unclear. It is known that asbestos fibers cause mutagenic changes through the production of hydroxyl radicals and superoxide anions, leading to DNA strand breaks and deletions.[4,6,7,13,14] However, the same oxygen radicals are made in response to crystallin silica[7] (which does not cause mesothelioma), suggesting that additional factors

contribute to asbestos carcinogenicity and to the development of mesothelioma. Alternatively, silica, in contrast to asbestos, does not reach the pleura, but this is unclear. Through mechanically interfering with mitotic segregation, asbestos can also alter chromosomal morphology and ploidy of Syrian hamster embryo cells and cause malignant transformation.[15] However, human mesothelial cells are much more susceptible to the toxicity of asbestos than hamster embryo cells, and mesothelial cells that phagocytose crocidolite asbestos usually die and do not become transformed.[16] In any event, asbestos may induce DNA damage either by direct physical interaction,[15] or by the indirect action of reactive oxygen species produced by inflammatory cells in response to asbestos.[4,6,7,13,14] Asbestos also induces DNA double-strand breaks in cultured cells exposed to chrysotile for 24 hours, and DNA repair mechanisms are critical to permit cell survival following asbestos exposure.[17] Asbestos has also been found to influence the normal activity of genes that regulate the cell cycle and that are altered in certain human cancers. Crocidolite asbestos was found to stimulate the autophosphorylation of the epidermal growth factor (EGF) receptor in mesothelial cells, which in turn triggers the extracellular-regulated kinase (ERK) cascade and leads to increases in AP-1 activity and either cell mitosis if the DNA damage can be repaired, or apoptosis if it cannot be repaired[6,7] (Fig 1). Recent experiments using cDNA expression arrays to screen differentially expressed genes in human bronchial epithelial cells immortalized by human papillomavirus and subsequently transformed by a single 7-day exposure to chrysotile asbestos demonstrated that activation of the insulin receptor, and inactivation of DCC (deleted in colon cancer) and Ku70 may cooperate in malignant transformation.[18] It will be important to test if similar results are obtained in mesothelial cells exposed to asbestos.

An additional mechanism by which asbestos may favor tumor development is mediated through the immune system, because macrophages produce DNA-damaging oxyradicals and lymphokines that depress immune function upon phagocytosis of asbestos fibers.[13,14,19,20] Thus, asbestos is considered both a local and systemic immunosuppressant[19].

SIMIAN VIRUS 40

(SV40) is a DNA tumor virus capable of inducing mesotheliomas, as well as other tumors, in hamsters and of transforming human cells in vitro.[4,21,22] The SV40 genome is a double-stranded circular DNA molecule containing 5243 base pairs that can be divided into two regions, early and late, according to the order in which they are transcribed. The early region encodes three proteins, large T-antigen (Tag), small t-antigen (tag), and 17kT, and it is responsible for the transforming ability of the virus. The late region encodes viral coat proteins. Tag binds and inhibits cellular p53, pRb, and several other tumor-suppressor gene (TSG) products.[23-25] Tag is also mutagenic and causes numerous chromosomal alterations.[23-25] The SV40 tag contributes to malignancy by inhibiting the activity of phosphatase 2A (PP2A) and thus altering the phosphorylation state of several cell cycle–regulatory proteins.[26] These effects are highly oncogenic, and Tag is considered one of the most potent carcinogens.[27]

Although SV40 is endogenous to the rhesus monkey, the virus infected the human population through contaminated polio vaccines, both attenuated and killed, between 1954 and 1963.[27,28] Polio vaccines were prepared in cell cultures grown on monolayers of infected rhesus monkey kidney cells. Since the virus produces no cytopathic effects in these cells, their infection went unrecognized until 1960, when infectious SV40 was isolated from some lots of the vaccine. As a result of this contamination, it is estimated that 96 million adults and children in the United States alone were injected with polio vaccines potentially contaminated with SV40. It was estimated that about one third of those vaccines contained various amounts of infectious SV40, which means that some 32 million people in the United States were injected with infectious SV40.[4,21,22,27,28] Aside from contaminated polio vaccines, transmission of SV40 to humans may have also occurred between 1957 and 1967, when parenteral adenovirus vaccines contaminated with SV40 were used in the military and, to a limited extent, administered to the general population.[21,22] In addition, a high incidence of SV40-neutralizing antibodies has been demonstrated in persons handling rhesus monkeys, as well as laboratory workers handling rhesus monkey kidney cell cultures.[21,22] Aside

from these known exposures, other routes of SV40 transmission to the human population are likely. Individuals born after 1962 with limited risk of exposure to contaminated polio vaccines had an approximately 5% to 10% positive rate for SV40-neutralizing antibodies.[22] Since this percentage cannot be accounted for by the aforementioned exposure events, human to human transmission may be the source. While little is known about human to human transmission of the virus, horizontal and/or vertical transmission cannot be ruled out. In addition, children excreted infectious virus in their stools 3 to 5 weeks after ingestion of the contaminated oral polio vaccine, providing another possible route for person to person transmission. Finally, a recent report presented documents indicating that SV40 may have contaminated polio vaccines even after 1963.[27] Thus, whether the use of contaminated polio vaccines introduced a new virus into the human population or simply broadened the infected population is not known.[21,22,27]

The actions of SV40 in different cell types are diverse.[16] Monkey cells are permissive, meaning they allow SV40 replication which causes cell lysis. In contrast, rodent cells are nonpermissive, since SV40 does not replicate in these cells and SV40 injection often leads to tumor development because the viral DNA becomes integrated into the host cell genome. Human cells are semipermissive, as they allow SV40 multiplication, and on occasion, following viral integration in the cellular genome also cellular transformation. Recently, it was found that human mesothelial cells (HM) are unique regarding their susceptibility to SV40.[16] HM are infected much more efficiently than human fibroblasts and epithelial cells.[16] However, following infection, the high levels of wild-type p53 normally present in mesothelial cells bind and inhibit Tag-mediated SV40 replication; fewer viral particles are formed, and the cells are not lysed. Therefore, in mesothelial cells, SV40 can persist in an episomal state.[16] The accumulation of Tag in mesothelial cells causes a rate of malignant transformation in tissue culture which is 1,000 to 100,000 times higher than that observed in other human cell types infected with SV40.[16] Thus, mesothelial cells are very susceptible to SV40 infection and transformation.[16]

EXPERIMENTAL MODELS

Asbestos

Experimental models demonstrating asbestos carcinogenicity have been established in several animals.[29] In 1969, Wagner and Berry injected 96 rats intrapleurally with amosite, crocidolite, and chrysotile asbestos and found that mesotheliomas developed in 38, 61, and 55 rats, respectively.[30] Therefore, in contrast to the data in humans, in animals, all types of asbestos can induce tumors without notable differences. It was determined that the likelihood of mesothelioma development in rats was directly proportional to the quantity of asbestos injected.[30] Hamsters, similar to rats, developed mesotheliomas upon receiving intrapleural injections of asbestos, and the incidence of the malignancy was also demonstrated to be directly proportional to the dose administered: 20% and 4% of two groups of 50 hamsters each developed mesothelioma when injected with 10 and 1 mg of crocidolite asbestos, respectively.[30] In humans, a dose-response relationship between asbestos exposure and mesothelioma was not found (Table 1). It should also be noted that 10 mg of crocidolite occupies a volume of about 1 cm^3, which is a very large volume considering the chest volume of a rat. Mesotheliomas in these animals occurred after 18 months, were often small, and were rarely (<10%) the cause of death.[31] The adjacent pleura typically showed evidence of fibrosis and local invasion was rarely observed.[31]

Inhalation studies are considered by some authors to be a more realistic model of human mesothelioma. When rats were exposed to inhalation of equal weight concentrations of different asbestos types (10.1 to 14.7 mg/L), the incidence of mesothelioma was one in 146 of those exposed to amosite, two in 145 of those exposed to anthophyllite, four in 141 of those exposed to crocidolite, four in 137 rats exposed to Canadian crysotile, zero in 144 rats exposed to Rhodesian crysotile, and zero in 126 of the control, nonexposed group.[32] After the conclusion of the 2-year experiment, Wagner et al measured the amount of asbestos retained in the lungs. They found that the weight of amphibole asbestos was about 15 times higher than that of crysotile even though the air the rats breathed contained similar amounts by weight of both asbestos types.[32] These results indicated that crysotile fibers are less persistent in

the lung, and that in animals all types of asbestos are equally oncogenic.

SV40

The oncogenicity of SV40 has been studied mostly in hamsters. Sixty percent of hamsters injected intracardially with SV40 developed pleural mesotheliomas within 6 months, and when SV40 was injected into the pleural spaces of these animals, 100% developed pleural mesotheliomas in 3 to 6 months.[33] Mesotheliomas, however, did not develop in hamsters when SV40 was injected intracerebrally, subcutaneously, or in the femoral vein. These observations suggest that the route of SV40 inoculation influenced the induction of specific tumor types. It is possible that in the case of intracardial injection of SV40, small amounts of virus reached the pleura through the external surface of the needle. Hamster mesotheliomas were large, multicentric, and histologically very aggressive, with both epithelioid and sarcomatoid areas, and invasion of adjacent tissues.[31,33] No fibrosis of the adjacent pleura was observed. Mesotheliomas were uniformly fatal in hamsters within 3 to 6 months. Thus, in hamsters, there are striking morphological and clinical differences among mesotheliomas caused by SV40 and those caused by asbestos.[31]

ASBESTOS EXPOSURE

It is generally agreed that background exposure (ie, most people who live in industrial areas or in large cities contain some asbestos fibers in their lungs) does not constitute a health risk, and that mesothelioma and other diseases are associated with higher than background levels of exposure. However, measuring higher than background levels of exposure is a difficult task prone to errors. Some studies rely on history of exposure, while others look to the amount of asbestos fibers found in the lungs (lung content analyses).

Studies based on history of exposure suffer from the inherent imprecision of this methodology. Some history studies are based on patient interviews, others on interviews of relatives or friends, others on death certificates or medical records. Not surprisingly, these studies produce quite different results, and the proportion of asbestos-associated mesothelioma in the literature varies from 16% to 90%.[34] Some patients (or friends and relatives of the patients) may not be aware that they were exposed; others may erroneously think that they were exposed to asbestos, when in fact they were exposed to dust that did not contain asbestos. Histories are often taken decades after exposure and it is often impossible to confirm the patient's recollection of the events. Furthermore, at least for the studies conducted in the United States, there is a strong incentive to report some type of exposure because the US Courts of Law award substantial compensatory damages to patients with asbestos-associated mesotheliomas. Even when these histories are based on cohorts of asbestos workers, it is often very difficult to quantify exposure.[10] Different methodologies have been used to measure asbestos fiber concentrations in the work place air. Different workers may be exposed to different amounts of asbestos because of different jobs within the same company. These variables make precise estimates of exposure problematic.[10]

The studies based on lung content analyses may be more reliable because there is an objective amount of asbestos to measure. However, these studies also face difficulties because the amount of asbestos varies in different areas of the same lung, and because the results obtained are so dependent on the instrumentation used, and other unknown factors, that interlaboratory comparisons are considered unreliable.[7] Therefore, the results of lung content analyses can be compared only with the standards of the laboratory that has conducted the analyses (Table 1). Within these limitations, studies of lung content analyses offer an objective measure of asbestos exposure. However, relatively few of such studies have been conducted because lung tissue is seldom available. A review of some of the published lung content studies seems to indicate that the incidence of mesothelioma is not directly related to the amount of exposure. Surprisingly, a higher incidence of mesothelioma was observed in individuals with above background exposure but with exposures considerably lower than those observed in patients with asbestosis (Table 1).

The mean latency for asbestosis was 12.6 to 20.2 years following exposure,[7] compared to 32 years for mesothelioma[35]; thus, some individuals may die of asbestosis before they can develop mesothelioma. However, only a fraction of individuals exposed to high levels of asbestos develop asbestosis,[7] and most mesothelioma patients do not appear to have very high levels of asbestos fibers in their lungs

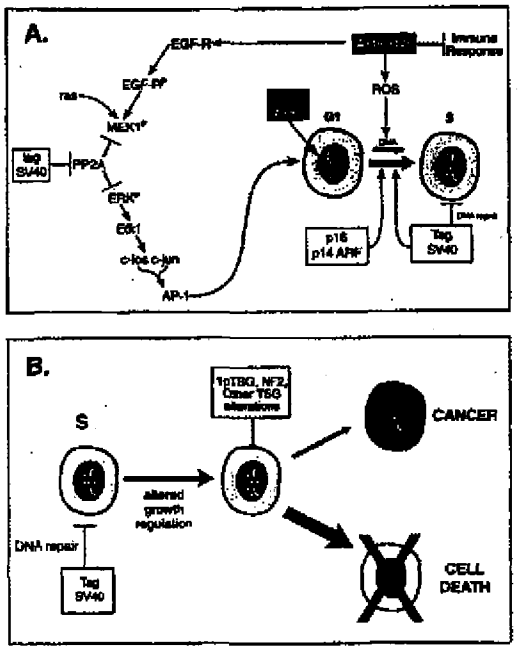

Fig 1. Mesothelioma pathogenesis: possible pathogenic mechanisms of asbestos and SV40. Arrowheads indicate a stimulatory effect; crossed bars indicate an inhibitory effect. Tag, SV40 large T-antigen; tag, SV40 small t-antigen; ROS, reactive oxygen species; TSG, tumor-suppressor genes; PP2A, phosphatase 2A. The circle indicates nonintegrated SV40; however, in some tumors SV40 has been found integrated in the host cell DNA; NF2, neurofibromatosis type 2 gene (often deleted in mesothelioma[4,72]). (A) Asbestos induces autophosphorylation of the EGF receptor, which activates the mitogen-activated protein (MAP) kinases, and through a set of intermediaries this process induces AP-1 activity, which leads to cell mitosis and apoptosis.[4,6] If the mesothelial cell contains alterations of p16, a CDK inhibitor, or of the related p14 ARF, which inactivates MDM2 and thus interferes with the p53 and pRB cellular pathways, cell division rather than apoptosis may ensue. Similarly, if the mesothelial cell is infected with SV40, the Tag-mediated inhibition of the cellular damage repair pathway and the anti-apoptotic activity of low amounts of Tag may favor cell division. Furthermore, tag-mediated inhibition of cellular PP2A, which normally dephosphorylates and thus inactivates the MAP kinases, may increase the effects of asbestos on AP-1. Mesotheliomas often contain alterations of p16 and p14 ARF, and of several other cell regulatory genes.[4] The precise mechanisms that cause these alterations are unknown. (B) The figure proposes that the mutagenic ROS produced by mononuclear phagocytes following ingestion of asbestos fibers, and the mutagenic activity of Tag, may both contribute to genetic damage. Damaged cells will most often execute apoptosis, but rarely they may divide, accumulate additional genetic damage, such as 1p and NF2 deletions which are often found in mesothelioma,[4] and become malignant.

(Table 1). It is also possible that very high levels of asbestos are so toxic for mesothelial cells that few, if any, cells are left alive near conspicuous asbestos deposits. This is supported by tissue culture experiments which demonstrate that relatively high levels of crocidolite asbestos (>2.5 $\mu g/cm^2$) cause 100% mesothelial cell death within 1 week.[16] This hypothesis, summarized in Fig 2, surmises that individuals with higher than background levels of asbestos exposure have an increased risk of developing mesothelioma. When the levels of exposure are very high, most mesothelial cells near amphibole asbestos deposits may be dead and, therefore, there may be fewer potential targets for malignant transformation. This may account for the unexpected findings presented in Table 1.

The possible lack of a direct correlation between amount of exposure and mesothelioma (Table 1) further suggests that individual susceptibility[36] and/or additional carcinogens[4] play a major role in determining, among exposed individuals, those who will develop mesothelioma. For example, several studies have reported mesothelioma in the wives of asbestos workers (indirect exposure), especially asbestos miners.[37] Apparently these women were exposed by washing their husband's

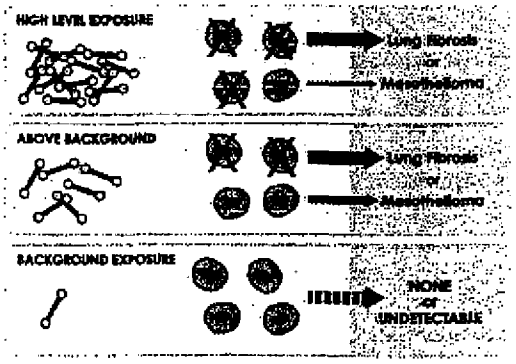

Fig 2. Working hypothesis to account for the findings reported in Table 1 (thick arrow indicates a higher frequency compared to the thin arrow). Top: High levels of crocidolite or amosite asbestos induce a high percentage of mesothelial cell death; few mesothelial cells remain. Because the number of potential targets for malignancy (ie, the mesothelial cells) is reduced, asbestosis is more frequent than mesothelioma. Middle: Moderate exposure above background induces some mesothelial cell death; however, many mesothelial cells survive and an occasional cell may become transformed. Bottom: At background levels, a higher risk for mesothelioma or asbestosis is relatively rare because the amount of exposure is insufficient to cause disease.

work clothes, which were contaminated with asbestos. One would surmise that these women were exposed to amounts of asbestos considerably lower than their husbands. The minimum length of time of indirect exposure was 5 years,[37] but most mesotheliomas occurred in women whose husbands had worked for 10 or more years in the asbestos industry.[37] It has been suggested that for a given amount of asbestos exposure, prolonged exposure to moderate amounts is much more dangerous that intense exposure to the same amount within a brief interval of time.[10]

A high incidence of mesothelioma in certain families[36,38-40] suggests the existence of a putative mesothelioma susceptibility gene. The limited size of these families, however, hampers the possibility of isolating this putative gene (see below). An alternative explanation is that the entire family was exposed through the contaminated clothes of a family member,[36,38,39] or worked with a contaminated asbestos oven.[40] Even if the entire family was exposed to asbestos, the incidence in several family members is too high to be attributed solely to asbestos, because the incidence of mesothelioma in asbestos miners is 5% or less.[10] Thus, exposure to asbestos alone should not account for mesothelioma in several family members, unless additional factors, such as a genetic defect or viral infection, made the members more susceptible to asbestos carcinogenicity.

ASBESTOS AND HUMAN MESOTHELIOMA

In a mortality study of 1,225 deaths among 7,317 men who worked in crocidolite or amosite mines in South Africa, there were 30 mesotheliomas.[10] Twenty of these were in crocidolite miners (in 423 deaths, 4.7%), four were in amosite miners (in 648 deaths, 0.6%), and six in miners with mixed exposure (in 154 deaths, 3.9%).[10] Among crocidolite-associated mesotheliomas, six developed in individuals exposed from 12 to 95 months, six in miners exposed for 96 to 191 months, and eight in miners exposed for more than 192 months. Among the 10 amosite or mixed exposure mesotheliomas, four developed after exposure of 3 to 11 months, two in miners exposed from 12 to 95 months, and four in miners exposed for more than 192 months. The mean latency of mesothelioma from exposure was 44.9 years for crocidolite miners, 51 years for amosite miners, and 12.3 years for miners exposed to both crocidolite and amosite. This study indicated that crocidolite is more dangerous than amosite in humans.[10] It also suggested that there is a threshold level below which the increase in mesothelioma risk cannot be detected.[10] In fact, no mesotheliomas were observed in miners with a period of exposure below 3 months, and the higher risk of mesothelioma was in miners exposed for more than 16 years.

Lanphear and Buncher[35] reviewed 21 reports of a total of 1,690 mesotheliomas and found that 99% of mesotheliomas occurred 15 years or more after asbestos exposure, 96% had a latent period of at least 20 years, and none occurred less than 10 years from exposure. The mean latency was 32 years. What happens during this considerable latent period remains unclear, but two major theories can be suggested. The first postulates that malignant transformation occurs relatively soon after asbestos exposure, but that it takes a long time (years) for the tumor to grow. If true, this scenario would have important clinical implications, because it implies that, similar to carcinoma in situ of the cervix and to colon adenomas, we should have many years to detect mesothelioma in its early stages when it can be cured by surgical resection. Unfortunately, mesothelioma, in contrast to cervical carcinomas or colon adenomas, does not have a long premalignant noninvasive phase. If such phase exists, it lasts for a short period of time, because it is not clinically detectable by current methods.[1] It should be noted that other benign lesions of the pleura, such as fibrous tumors, are detected clinically, because these lesions cause pain and accumulation of fluid in the pleural cavity.

The second theory argues that genetic alterations induced by asbestos accumulate over time and eventually after about 30 years lead to a malignant cell. This theory requires that either the same cell or cell lineage accumulates damage over more than 30 years. It may appear unlikely that, given the high toxicity of asbestos for mesothelial cells,[16] a damaged cell or its daughters could survive for 30 or more years in the presence of asbestos while accumulating sufficient genetic damage to become malignant. However, mesothelial cell transformation may be more likely if a key regulatory gene, such as the INK4a/ARF locus on chromosome 9p21 is deleted or silenced by an epigenetic mechanism, ie, promoter methylation. In this event, additional mutations may accumulate

relatively rapidly. INK4a/ARF codes for p16, a cyclin-dependent kinase inhibitor, and for p14ARF (the murine homolog is p19ARF), which promotes MDM2 degradation and thus prevents MDM2 neutralization of p53. Both p16 and p14ARF are often deleted in mesothelioma.[4,25] Most mutations may be incompatible with cell survival, but occasionally this process may lead to a malignant phenotype. The likelihood of this process may be increased if mutations accumulate rapidly. Once the mesothelial cell has reached the malignant transformed phenotype, cell growth will be very rapid and the tumor will soon become clinically detectable. This scenario would account for the clinical observation that malignant mesotheliomas are very aggressive and fast growing malignancies which develop 20 to 50 years after asbestos exposure. Figure 3 summarizes these concepts.

SV40 AND HUMAN MESOTHELIOMA

The discovery that SV40 caused the development of mesotheliomas in hamsters led to polymerase chain reaction (PCR) analysis of human mesothelioma specimens for the presence of the virus. Altogether, 29 of 48 (60%) mesothelioma samples contained SV40 sequences. SV40 Tag expression was also detected by both immunohistochemistry and immunoprecipitation experiments.[41] These results were confirmed by an independent multilaboratory study organized by the International Mesothelioma Interest Group,[42] and by more than 26 other laboratories worldwide.[43,44] Two studies did not detect SV40 in mesotheliomas.[43] Furthermore, Hirvonen et al, at the Finnish Institute of Occupational Health, were unable to find SV40 in Finnish mesotheliomas, but in the same study US mesotheliomas tested positive.[45] This finding suggests that there may be marked geographical differences regarding the presence of the virus in human tumors. These differences may be related to the fact that SV40-contaminated vaccines were not administered in Finland,[45] to the markedly different amounts of contamination among different batches of poliovaccines distributed in the United States and Europe,[21,22,27,28] or to other unknown factors. Aside from mesotheliomas, SV40 has also been detected in human tumors of the same types that it induces in experimental animals, including ependymomas, choroid plexus tumors, and osteosarcomas.[43] Several techniques, including PCR, in situ hybridization, microdissection, and immunostaining, have shown that SV40 sequences or antigens are present in tumor cells but not in normal adjacent tissue.[46-49] Caution should be used, however, when using anti-Tag monoclonal antibodies, because some preparations may be contaminated with a protein that causes false-positive reactions.[50] These false-positive reactions cause a cytoplasmic staining, and thus can be distinguished from true Tag staining, which is nuclear. Furthermore, contaminated Tag monoclonals stain 100% of the cells, regardless of cell type.[50] In contrast, pure Tag monoclonals only stain the nuclei of mesothelioma cells and not the nearby stromal cells,[41] and stained only two of 38 human mesothelial cell cultures: approximately 96% of the cells of these two cultures and none of the cells of the other 36 SV40-negative mesothelial cell cultures.[51]

Experiments to investigate if Tag was biologically active in human mesotheliomas found that Tag and p53 were often coexpressed.[49] Immunoprecipitation reactions demonstrated that Tag binds and stabilizes p53 in mesotheliomas, allowing its detection.[49] Furthermore, tumor cells expressing Tag failed to induce p21, indicating that p53 was inactivated by its interaction with Tag.[49] Treatment of SV40-positive mesothelioma cell lines with Tag antisense restored the p53 pathway and induced p21 expression and growth arrest, which suggests that Tag expression was required for malignant transformation.[52] Aside from p53, Tag was also shown to bind, stabilize, and inactivate members of the retinoblastoma (Rb) tumor-suppressor family—pRb, p107, and pRb2/p130 in human mesotheliomas,[25] and to cause the inactivation of RassF1A genes.[53]

SV40 particles were detected by electron microscopy in human mesothelioma cells, and in these cells SV40 caused hepatocyte growth factor production and the activation of the MET oncogene, which in turn promoted mesothelial cell growth.[54] These findings were recently presented at a consensus meeting at the University of Chicago; the consensus was that (1) it has been convincingly demonstrated that SV40 is present in about 40% to 50% of human mesotheliomas in the United States; and (2) that the role of SV40 in the pathogenesis of mesothelioma has been considerably strengthened in the past 4 years.[44]

RADIATION AND HUMAN MESOTHELIOMA

There have been several case reports in which mesothelioma developed in patients who had received radiation to the thorax or to the abdomen, or who had received Thorotrast intravascularly.[55] Mesothelioma in these patients developed after an interval of 7 to 36 years. In many of these cases, the possibility that asbestos or other factors had contributed to mesothelioma could not be entirely ruled out. However, at least for those mesotheliomas which developed in young adults who had received intensive radiotherapy in their childhood because of Wilm's tumor, radiation appeared as the only possible causative factor. In these cases, a portion of the lung, and thus of the pleura, was included in the radiation fields of the abdomen. Lung content analyses studies were performed in one of these cases and revealed background levels of asbestos only, supporting radiation as the causative factor of that particular mesothelioma.[56] Studies in rats support the notion that radiation exposure can cause mesothelioma: 27% of rats exposed by intraperitoneal injection to $^{239}PuO_2$ developed epithelial mesothelioma, and 38% developed sarcomas, possibly sarcomatous mesotheliomas.[57] It seems safe to state that intensive radiation exposure can cause mesothelioma; however, the overall number of mesotheliomas caused by radiation is probably small.

GENETICS AND HUMAN MESOTHELIOMA

In the villages of Karain (population ~600) and Tuzkoy (population ~1,400) in Cappadocia, a region in Central Anatolia, Turkey, characterized by volcanic tuffs and natural caves, 50% or more of deaths are caused by malignant mesothelioma. These two villages, like most others in the region, were built with stones mined from the nearby natural caves. When Dr Y.I. Barish found a very high incidence of mesothelioma in Karain,[58] scientists looked for asbestos, which in the 1970s was the only known causative factor for mesothelioma. Some asbestos was found,[59] but subsequent studies demonstrated that in Cappadocia, asbestos is almost everywhere because it is a natural component of that volcanic terrain and because asbestos-based stucco (containing tremolite asbestos) has been widely used in building construction.[60] It appeared that asbestos could not account for the unique high incidence of mesotheliomas in these two villages.[61] Another type of mineral fiber, erionite, which had been detected in the lungs of several villagers, was suspected to be the causative agent.[58,61] Erionite is a type of fibrous zeolite commonly found in the stones of the houses of Karain and Tuzkoy. When injected intrapleurally into animals, erionite causes mesothelioma, and it was concluded that erionite was the cause of mesothelioma in these villages.[61] Erionite therefore appeared much more potent than asbestos in causing mesothelioma because more than half of the villagers died of this disease. Actually, erionite was claimed as the most potent chemical human carcinogen.[61] Studies tried to link erionite to other human tumors, but except for mesotheliomas, there is no significant difference in the incidence of any other tumor types in these two villages compared with the rest of Turkey.[62] Why such a potent carcinogen would specifically cause mesothelioma was unknown. Why about 50% of villagers appeared to suffer no consequence from exposure to such a potent carcinogen was also unknown. During repeated visits to these villages one of us (Carbone) suspected that erionite was not the only cause of mesothelioma. In the villages of Karain and Tuzkoy, mesothelioma developed mostly in certain houses, called "the houses of death" because all the residents had died of mesothelioma. Residents of nearby houses had not developed mesothelioma. It was stated that the amount of erionite was higher in the houses of death, but the only proof for this statement was that residents of those houses had died of mesothelioma. If this hypothesis was correct there had to be an extremely high amount of erionite in the houses of death, and proportionally a very low amount of erionite in the other houses next to the houses of death. Yet, both types of houses appeared to have been built at about the same time and with the same type of stones. In Karain and Tuzkoy, families live together for multiple generations; therefore, people living in the same house are often related. It appeared possible that susceptibility to mesothelioma was genetically transmitted and that the presumed higher amount of erionite in the houses of death was a misleading hypothesis. The genetic hypothesis was strengthened by the observation that only one mesothelioma had been observed in Karlik, 1.5 miles south of Karain, with a population of about 1,500. This mesothelioma

had occurred in a woman from Karain who had moved to Karlik because of marriage. The houses of Karlik appeared identical to those of Karain and had been built with stones mined from the same caves which are located midway between Karain and Karlik on the same side of the mountain on which these two villages were built. Again, the argument was made that there was more erionite in the houses of Karain, but during a local inspection of Karlik and Karain, one of the highest amounts of what appeared to be erionite (erionite has a white color and a soft consistency and looks like an area of decay within the zeolite stones used to build the villages) was found in the stones of the fountain of Karlik, which provides water to the whole village. The marriage of the woman of Karain with a man of Karlik was unique. Residents of Cappadocia consider people from the two mesothelioma villages "weak" and they are afraid that mesothelioma will spread in their families if they marry with villagers of Karain and Tuzkoy. In fact, people from Karain and Tuzkoy have problems even selling their products to the market because residents of nearby villages are afraid to buy anything that comes from these two villages[61] (M. Carbone, personal observations). The consequence of this incredible situation is that most marriages in Karain and Tuzkoy are between villagers and marriages outside the villages are infrequent. Since there was reason to suspect that mesothelioma in Karain and Tuzkoy had a genetic cause, Dr Carbone organized a research team to study this possibility. Iman Roushdy-Hammady, a PhD student in Medical Anthropology at Harvard University, with extensive experience with the Muslim culture and who is fluent in Turkish, agreed to live among the villagers for 2 years 8 months to collect information. She constructed preliminary pedigrees from several families. When these pedigrees were further analyzed by a geneticist, it was apparent that mesothelioma was genetically transmitted, possibly as an autosomal-dominant disease.[63] About 50% of the descendents of affected parents developed mesothelioma, whereas mesothelioma was absent in other families. When members of unaffected families married into affected families, mesothelioma appeared in their descendents.[63] Mineralogical analyses revealed no differences in the amount or type of erionite among the houses of death and other houses, including those of Karlik (Umran Dogan, University of Ankara, personal communication). These same analyses confirmed the presence of a very high amount of erionite in the fountain of Karlik. Thus, it appears that in these villages susceptibility to mesothelioma is genetically transmitted. The possibility that erionite is a cofactor that contributes to mesothelioma in genetically predisposed individuals is presently being investigated in our laboratories. Furthermore, we will try to isolate the putative mesothelioma susceptibility gene that predisposes some families of Karain and Tuzkoy to this malignancy. This should lead to the development of therapeutic approaches for members of these families. It is possible that the same gene that is genetically mutated in Cappadocia may be the target of asbestos and SV40 carcinogenesis, and thus it is hoped that the eventual isolation of this putative gene might clarify the molecular pathogenesis of mesothelioma, and also benefit all mesothelioma patients.

GENETIC ALTERATIONS IN MESOTHELIOMA

Whatever the cause, genetics, asbestos, SV40, or radiation, mesothelial cells eventually accumulate a number of genetic alterations and become malignant. Human mesothelioma cells are characterized by many different genetic abnormalities and separating those that play a causative role during the establishment of the transformed phenotype and tumor progression from those that do not has not been possible to date, particularly since data on early lesions are not available.

Karyotypic studies and comparative genomic hybridization (CGH) analyses have revealed multiple chromosome alterations in most human mesotheliomas.[4,25,64] Although a specific chromosomal change is not shared by all mesotheliomas, several prominent sites of chromosomal loss have been identified. Deletions of specific regions in the short (p) arms of chromosomes 1, 3, and 9 and long (q) arm of 6, 13, and 15 are repeatedly observed in these tumors. Loss of a copy of chromosome 22 is the single most consistent numerical change seen in mesotheliomas. Monosomy 4 and monosomy 14 are also common. These recurrent losses frequently occur in combination in a given tumor. Chromosomal gains appear to be less common than losses in this disease, although recurrent gains of 5p and 7 have been reported. It is not possible to determine a temporal sequence of these

pathogenetic events due to the lack of data for early stages of this disease. The high frequency of genomic losses in mesothelioma is consistent with a recessive mechanism of oncogenesis. Loss and/or inactivation of TSGs residing in recurrent sites of chromosomal deletion is thought to contribute to the development and progression of mesothelioma. As a prelude to the isolation of these genes, investigators have begun to molecularly map these regions by performing loss of heterozygosity (LOH) analysis with polymorphic DNA markers. Such molecular studies have demonstrated high frequencies of allelic loss from 1p22, 3p21, 4q, 6q, 9q21, 13q13-14, 15q11-15, and 22q12. To date, TSG loci within two of these regions have been shown to be frequently altered in mesotheliomas.

Tumor-Suppressor Genes

The TSGs in mesothelioma have been reviewed extensively.[4,25,64] A high frequency of mesothelioma cell lines exhibit homozygous deletion of the 9p21 region. The *CDKN2A* locus, which encodes the alternative TSG products $p16^{INK4a}$ and $p14^{ARF}$, is located in this region. The p16 protein is capable of binding to the cyclin-dependent kinase CDK4, thereby inhibiting the catalytic activity of the CDK4/cyclin D enzymes. Therefore, loss/inactivation of $p16^{INK4a}$ would lead to cell cycle deregulation through the loss of a key inhibitor of G1/S progression. More than 80% of mesothelioma cell lines have homozygous deletions of one or more $p16^{INK4a}$ exons, and most of the remainder have greatly downregulated expression of $p16^{INK4a}$, due to promoter hypermethylation. PCR analyses have documented homozygous deletions of $p16^{INK4a}$ at a much lower frequency in mesothelioma tissues than in cell lines. This discrepancy may be an in vitro phenomenon resulting from the selective advantage provided by $p16^{INK4a}$ deletion during the culturing process. On the other hand, mesothelioma samples contain a significant amount of contaminating normal stroma, which can mask the existence of a homozygous deletion in the malignant cell population. Fluorescence in situ hybridization analysis has revealed reduced copy numbers of the $p16^{INK4a}$ gene in most mesothelioma specimens, particularly in sarcomatous and biphasic tumors. The majority of these tumors show homozygous loss of the INK4a locus in either all tumor cells or in a significant subset (>10%) of the tumor cell population. Immunohistochemistry studies suggest that loss of $p16^{INK4a}$ expression is a universal finding in mesothelioma tissues.[65] Taken collectively, the available data suggest that loss of one copy of $p16^{INK4a}$ occurs in many of these tumors, with the remaining allele silenced by promoter hypermethylation. In a given tumor, a subset of cells may contain homozygous loss of $p16^{INK4a}$, with these cells presumably having a proliferative advantage when placed into long-term culture. Importantly, alteration of $p16^{INK4a}$ appears to play a critical role in mesothelial cell tumorigenesis, because re-expression of $p16^{INK4a}$ in mesothelioma cells has been shown to result in cell cycle arrest and apoptosis, as well as inhibition of tumor formation and diminished tumor size.[66]

Homozygous deletions of $p16^{INK4a}$ would in many cases also lead to the inactivation of $p14^{ARF}$, because these two genes share exons 2 and 3, although their reading frames differ. The product of the $p14^{ARF}$ TSG is required for activation of p53 in response to the action of oncogenes such as *RAS*. Since the product of the $p16^{INK4a}$ gene induces a G1 cell cycle arrest by inhibiting the phosphorylation of pRb, homozygous loss of $p14^{ARF}$ and $p16^{INK4a}$ would collectively affect both the p53- and pRb-dependent growth-regulatory pathways, respectively. Recent experiments have demonstrated that adenovirus-mediated transfer of $p14^{ARF}$ in mesothelioma cell lines induces G1 phase arrest and apoptosis.[67] Together, the available data suggest that alteration of either product of the *CDKN2A* locus, ie, $p14^{ARF}$ or $p16^{INK4a}$, contributes to the pathogenesis of mesothelioma. The success of the experiments with $p14^{ARF}$ and $p16^{INK4a}$ adenoviral constructs have led investigators to propose that such gene therapy-based approaches may prove useful in the treatment of mesothelioma patients.[68] Loss of p16 and $p14^{ARF}$ is also associated with loss of p15, although the importance of this loss to mesothelioma is unknown.[69] Loss of p16 is usually associated with retention of wild-type pRB.[25,70] p53, instead, is rarely mutated in mesothelioma,[71,72] possibly because it is inactivated by the interaction with the SV40 Tag.[49] Genetic and epigenetic inactivation of p16 and $p14^{ARF}$, together with the inhibitory binding of Tag with p53 and pRb-family proteins, may lead to the complete inactivation of the normal cellular pathways of these cellular genes and

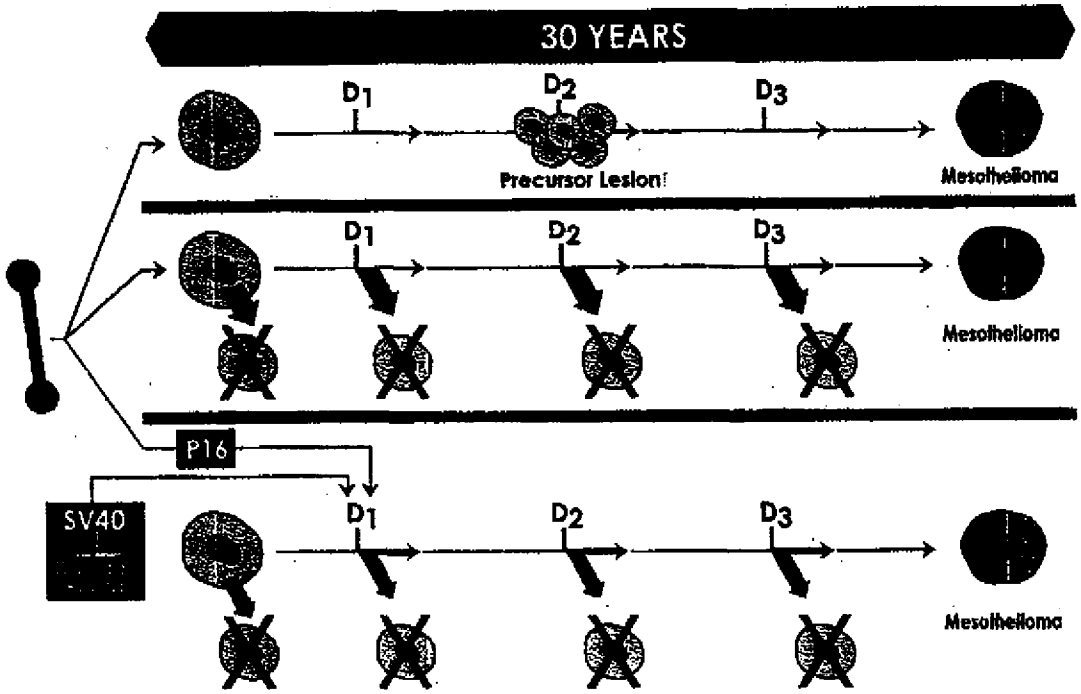

Fig 3. Working hypothesis to account for mesothelioma latency. D, DNA damage. According to recent research, at least 3 distinct genetic damages are required to cause malignant transformation of human cells. Top: Asbestos, represented on the left as a ferruginous body with a dumbbell configuration, induces DNA damage, which leads to mesothelial cell proliferation and to a precursor lesion. Additional DNA damage over the years leads to mesothelioma. Middle: Asbestos induces DNA damage which leads to apoptosis[16] (thick arrow). Rarely, the cell survives and eventually continued exposure to asbestos over the years will cause a second DNA damage. Again, most cells will die, but a rare cell may survive. A third DNA damage will cause apoptosis in most cells, but a rare cell may exceptionally survive all 3 genetic changes and become malignant. Bottom: The first genetic damage induced by asbestos involves a cell-cycle regulatory gene such as p16 or the cells are infected with SV40, and therefore, the damage/repair and apoptotic pathways are inhibited. In either circumstance, a greater number of cells may escape apoptosis and complete mitosis. When cell cycle regulatory genes and/or DNA damage repair genes are altered, additional mutations may accumulate more easily, the apoptotic pathway will be less efficient, and the cell may have an increased possibility of surviving and of becoming malignant.

contribute to malignancy. Finally, in contrast to lung cancer, ras mutations are rare in mesothelioma.[71,72]

Extensive LOH analysis of chromosome 22 losses in mesothelioma has not been performed, because an entire copy of chromosome 22 is lost in most cases. Although the neurofibromatosis type 2 TSGe, *NF2*, predisposes affected individuals primarily to tumors of neuroectodermal origin, so-

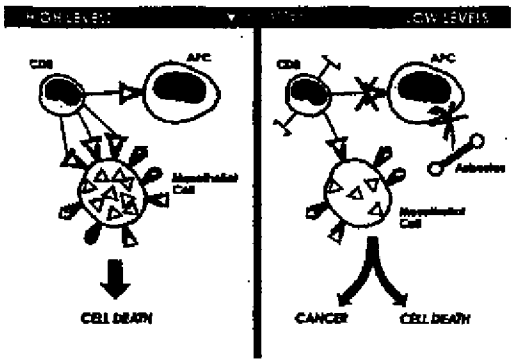

Fig 4. Working hypothesis illustrating that asbestos by interfering with the host immune system[19] may favor the growth of cells expressing SV40 antigens. Left: SV40-infected mesothelial cells express high levels of viral antigens. CD8 T lymphocytes see and kill these cells with the help of antigen-presenting cells (APC). Right: Asbestos directly and indirectly (ie, by inducing the release of various cytokines by macrophages which phagocytose asbestos fibers[20]) interferes with the normal activity of both CD8 T lymphocytes and APC. The immunosuppressive effects of asbestos, together with low levels of expression of SV40 antigens often observed in SV40-positive mesothelioma cells,[32] may allow SV40-positive mesothelial cells to escape immune detection and become malignant.

matic mutations of *NF2* have occasionally been identified in seemingly unrelated malignancies. *NF2* somatic mutations predicting either interstitial in-frame deletions or truncation of the *NF2* gene product (*merlin*) have been reported in 40% to 55% of mesothelioma cell lines. In many cases, it was possible to confirm the mutation in matched primary tumor DNA.[73] In some samples that showed *NF2* gene transcript alterations, no genomic DNA mutations were detected, suggesting that aberrant splicing may constitute an additional mechanism for *merlin* inactivation.[73] Western blot analyses revealed complete absence of *merlin* expression in cell lines that exhibited alterations of the *NF2* gene, suggesting that truncated forms of the protein are unstable. LOH analyses documented allelic losses at the *NF2* locus in more than 70% of mesothelioma cases. All cases exhibiting mutation and/or aberrant expression of *NF2* showed allelic losses, implying that inactivation of *NF2* in mesothelioma occurs via a "two-hit" mechanism.[4,73]

Crocidolite asbestos has been shown to induce the proto-oncogenes *c-fos* and *c-jun*, which encode transcription factors that activate various genes critical in the initiation of DNA synthesis.[6] The persistent induction of these transcription activators following asbestos exposure may enhance cell division. Mesothelioma may begin with stem cell proliferation via such an epigenetic mechanism, followed by the progressive accumulation, over several decades, of spontaneously occurring mutations in TSGs and asbestos-induced missegregation of chromosomes. Thus, such activation of proto-oncogenes and inactivation of TSGs may cooperate in a multistep process to regulate critical events intrinsic to the pathogenesis of mesothelioma. The presence of SV40 may accelerate malignant transformation, because SV40 induces DNA alterations. SV40 may give the cell more time to accumulate sufficient genetic alterations, because SV40 Tag, through its inhibitory binding of p53 and its BCL-2 like domain, inhibits apoptosis (very high levels of Tag, however, can induce apoptosis). Furthermore, the very recent finding that SV40 immortalizes infected human mesothelial cells by inducing telomerase activity, indicates that the presence of SV40 induces a background of cells prone to develop into malignancies when genetic damage is induced.[74] At the same time, asbestos by interfering with the immune system may favor the emergence of malignant cells that express SV40 antigens. According to this hypothesis, exposure to both asbestos and SV40 may increase the risk of mesothelioma in some individuals. Figures 1 and 4 summarize these concepts.

CONCLUSIONS

Most mesotheliomas in the Western World are caused by exposure to asbestos. Certain types of asbestos, especially crocidolite asbestos, appear more carcinogenic than others (crysotile). SV40 in the United States and in parts of Europe, and genetic predisposition in the villages of Karain and Tuzkoy, have recently been linked to mesothelioma. Rare cases of mesothelioma have been associated with radiation exposure. Whether these factors act independently or interact in causing mesothelioma is currently under investigation. Both scenarios appear possible. There is some evidence that SV40 and asbestos might be cocarcinogens in human mesothelial cells in tissue culture. Genetics and erionite may cooperate in causing mesothelioma in Karain and Tuzkoy. NF2, p16, and p14ARF mutation/loss are the most common acquired genetic lesions detected in mesothelioma. Mesothelioma may be a cancer in which genetics, radiation, viruses, and environmental carcinogens such as asbestos and erionite interact to cause malignancy. The eventual future isolation of the putative mesothelioma susceptibility gene that causes mesothelioma in Cappadocia may represent the key to understanding the precise pathogenesis of mesothelioma and to developing effective therapeutic approaches.

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