Guest Editorial

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Speculations on the Role of Ultraviolet Radiation in the Development of Malignant Melanoma

Interest in the etiology of human malignant melanoma has increased considerably in recent years. The basis for this heightened concern can be attributed to two seemingly unrelated issues. The first is that the incidence of malignant melanoma is increasing at an alarming rate, not only in the United States (1) but in other countries as well (1-3). At the present time, both the incidence of melanoma (2) and the mortality rate (4) are doubling every 10-17 years. A recent estimate projects that within 10-15 years malignant melanoma could comprise as many as 10% of all cancers in Norway (Magnus K: Unpublished observations). Thus identification of causal factors in the development of melanoma is an urgent necessity if this upward trend is to be curbed.

The second issue that has directed attention to the identification of etiologic factors in malignant melanoma is ozone depletion. The layer of ozone surrounding the earth shields it from solar UV radiation. Anticipated decreases in stratospheric ozone, resulting from high-altitude aircraft, nuclear explosions, and the release into the atmosphere of chlorofluoromethanes from refrigerants and aerosol sprays, raised concerns about the effects of an increased exposure of all life forms to UV light. One anticipated effect of increased exposure of humans to UV light is a corresponding increase in some types of skin cancer in humans (5). If malignant melanoma is included among the types of skin cancer at risk of increasing, then the impact of ozone depletion on humans is serious indeed. In contrast to nonmelanoma skin cancers, which grow slowly, rarely metastasize, and are usually curable by surgery, the prognosis for melanoma is less favorable, particularly in cases in which the tumor is already invasive at the time of diagnosis (6). Thus the threat of an increased level of exposure to UV light has stimulated a careful reevaluation of the role of UV radiation in the induction of melanoma (5).

EVIDENCE FOR THE PARTICIPATION OF SUNLIGHT IN HUMAN MELANOMA

Evidence implicating UV light as a causative or contributing factor in malignant melanoma comes from two main sources. Epidemiologic studies show that in susceptible populations the incidence of melanoma is highest in locations receiving the greatest solar exposure (4, 5). Further, recent reports suggest that cyclic increases in UV exposure resulting from sunspot activity might be responsible for the cyclic fluctuations observed in melanoma incidence (7, 8). In addition, the persons most likely to develop melanoma are those that are least protected against sunlight-induced damage by skin pigmentation and by their ability to suntan (9, 10). Second, clinical observations show that melanomas occur on sites of the body in a nonrandom fashion, appearing rarely on areas that normally would be covered by bathing suits (11). Although these lines of evidence suggest some relationship between UV light and skin cancer, the precise nature of this relationship is not at all clear. The arguments that were so convincing in establishing UV light as the cause of most forms of nonmelanoma skin cancer do not seem to apply to melanoma. For example, squamous cell carcinomas occur on the parts of the body that receive the maximum amount of solar exposure, i.e., head, neck, arms, and hands (12). Most melanomas, however, do not exhibit this preference for highly exposed sites, and the most frequent sites of occurrence are the upper back in males and the lower extremity in females (11). Analysis of skin cancer incidence by age and geographic location in the United States suggests that, although both melanoma and nonmelanoma skin cancers are more prevalent in locations receiving high levels of UV light exposure, nonmelanoma skin cancer is related to cumulative lifetime exposure to UV radiation, whereas melanoma may not be (13). This has been interpreted as indicat-

ABBREVIATIONS USED: DMBA = 7,12-dimethylbenz[a]anthracene; H & E = hematoxylin and eosin; MTV = lacking mammary tumor virus.

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2 I am indebted to Ms. Dolores Bubnis whose careful observation led to the discovery of our primary murine melanoma; to Ms. Eileen Gruys for her painstaking efforts in culturing and cloning this tumor; to Dr. Cora Bucana for electron microscopic analyses and photography of the tumor specimens; to Mr. Frederick Argilan for special staining of the tissue sections; and to Dr. Michael Hanna for his aid, advice, and interest in the diagnosis of this melanoma.
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Editor’s note: Periodically, the Journal publishes solicited guest editorials as a means of transmitting to investigators in cancer research the essence of current work in a special field of study. The Board of Editors welcomes suggestions for future editorials that succinctly summarize current work toward a clearly defined hypothesis regarding the causes or cure of cancer.
ing that melanoma and nonmelanoma skin cancer are related to UV exposure in different ways. Thus repeated exposures of the skin to UV radiation alone probably does not cause most melanomas.

POSSIBLE CAUSES OF MELANOMA

What then does cause melanoma? Several hypotheses have been proposed in an attempt to account for the incomplete correlation between UV exposure and melanoma. Lee and Merrill (14) suggested that a systemic effect of UV radiation might be responsible for melanoma induction and that this would account for the occurrence of melanomas on parts of the body that receive occasional, rather than regular, exposure to sunlight. They proposed that a "solar circulating factor" is produced by the action of sunlight on skin and that this factor stimulates or enhances a malignant change in melanocytes. Fears et al. (13) have attempted to account for the discrepancies by suggesting that the incidence of melanoma might be attributed to very short exposures to high intensities of UV light, such as would be encountered during recreational exposure to sunlight. Genetic factors associated with a high incidence of melanoma also exist. Patients with xeroderma pigmentosum, a rare, inherited disease characterized by an inability to repair UV light-damaged DNA, have a high incidence of both melanoma and nonmelanoma skin cancer (15). In addition, several families with a high incidence of melanoma have been identified (16, 17). In several families (17), melanoma was associated with the autosomal dominant inheritance of a particular type of pigmented lesion. These studies raise the possibility that melanomas arise from susceptible target cells that are not evenly distributed over the body. Thus the absence of melanomas on the most protected parts of the body could result from the absence of target cells in these areas, rather than from the lack of exposure to UV light (Clark WH Jr: Unpublished data). Further, the distribution of melanomas on sunlight-exposed areas of the body might be due to the fact that these are the areas in which the precursor melanocytic lesions are located. Thus the distribution of melanomas would be related to the location of the precursor melanocytic lesion and only secondarily to sunlight exposure, by virtue of its influence on the distribution of the precursor melanocytic lesions.

An important contribution toward the unraveling of this confusing picture was made by Clark and his associates (18) in their classification of melanoma into three distinct diseases, based on histologic appearance and biologic behavior. In addition to the practical clinical value of this classification for patient management, it provides the basis for a conceptual advance regarding the etiology of melanoma. If melanoma represents a collection of cancers with varied biologic and histologic features, then it is not unreasonable to suppose that the different categories might be associated with different inductive events. In their study of melanoma of the lentigo maligna type, Clark and Miilm (19) have provided evidence to support the hypothesis that different types of melanoma may have different origins. This type of melanoma, which constitutes about 10% of cutaneous melanomas in humans, occurs almost exclusively in heavily sun-exposed areas of the body (head, neck, and back of the hands). It occurs at a median age of 70 years in people who exhibit other signs of sunlight-induced skin damage and it follows a relatively slow, benign course. Although the rarity of this neoplasm makes statistical evaluation difficult, there seems little doubt that this type of melanoma is directly related to chronic exposure to sunlight (19). Thus the division of melanoma into subgroups has made it possible to associate at least one type of melanoma with an etiologic agent. Unfortunately, the picture is not so clear with nodular and superficial spreading melanomas, which are the more frequent and more serious types.

ANIMAL MODELS

One approach to answering the question of how UV radiation is involved in the induction of melanoma is through the use of animal models. Several models have been developed; however, in only one case has the induction of melanoma been associated with UV radiation. Two systems have been used to study the genetic regulation of melanomas. One is the platyfish system, in which a tumor-producing gene and corresponding regulatory genes have been identified (20). The other is the Sinclair miniswine system, in which the development of melanomas is also genetically determined (21). In neither of these cases is the development of melanoma associated with UV light. Melanomas have been induced in three different rodent systems. Epstein et al. (22) reported the induction of a very low incidence of melanomas in noninbred hairless mice treated with a single dose of DMBA, followed by chronic exposures to UV light. A high incidence of malignant melanomas was induced by Vesselinovitch et al. (23) in noninbred Syrian white hamsters by neonatal administration of urethan. Finally, Clark et al. (24) recently described the induction and development of melanomas in guinea pigs following chronic topical application of DMBA; thus they confirmed the earlier reports of Berenblum (25) and Edgcomb and Mitchelich (26). Among all of these systems, UV light was involved in melanoma induction only in the single experiment by Epstein et al. (22). In that study, the melanomas arose following UV irradiation of benign pigmented nevi, which had been induced by the application of DMBA. Unfortunately, because these tumors were produced in noninbred hairless mice, they could not be transplanted and thereby preserved for further study.

INDUCTION OF A TRANSPLANTABLE MURINE MELANOMA

Recently, work from this laboratory has suggested some new alternatives for how UV radiation might participate in the development of malignant melanoma. First, UV light might initiate neoplastic trans-
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forms that require promotion by a chemical agent for the full expression of malignant disease. This type of "cocarcinogenesis," in which two agents are required to produce cancer, could account for the epidemiologic findings of the obscure involvement of UV light in the induction of melanoma (13). A small number of high-intensity exposures to UV light could produce an initial neoplastic transformation that required chemical promotion for melanoma development. Thus the current increase in melanoma need not be due to increased UV radiation but could be due to changes in exposure to environmental chemicals. This concept is based on our recent discovery of a malignant melanoma on a C3H/HeN(MTV) mouse that had been treated with a short course of intense exposures to UV light, followed by chronic skin painting with croton oil.

This animal (#1735) was 1 of 40 that had been used to investigate certain aspects of two-stage carcinogenesis in which UV light was used as an initiator and croton oil as a promoter. The mice were given 10 (5 times/wk for 2 wk) 1-hour exposures to a bank of 6 Westinghouse FS40 sunlamps, as previously described (27). Each treatment consisted of a total UV dose of $1 \times 10^{6}$ J/m$^2$ over the wavelength range of 280–340 nm. Beginning 2 weeks after the last UV treatment, 0.025 ml of 2.5% croton oil (Sigma Chemical Co., St. Louis, Mo.) in electronic grade acetone (Fisher Scientific Co., Fair Lawn, N.J.) was delivered with an Eppendorf pipette to the scapular region of the mice twice per week. In the 92d week of this experiment, a raised, black lesion appeared in the scapular area on 1 of the animals. By this time, most of the mice in the study had died, either from other types of tumors or from natural causes.

Because of the extreme rarity of murine melanomas and because of the importance of developing additional models to investigate the role of UV light in melanoma induction, we have made a special effort to determine whether or not this tumor is, in fact, a malignant melanoma. Histologic sections of the primary tumor were not definitive (fig. 1); although melanin-containing cells were abundant in the tumor, nonpigmented areas with the appearance of a fibrosarcoma were also prominent. Bilateral metastases of pigmented cells were found at necropsy in the superficial draining lymph nodes (fig. 2), although no such cells were found in the lungs, liver, or spleen of the primary host upon light microscopic examination. Because the tumor arose in an inbred strain, we could see whether the melanin-containing component of the tumor persisted upon serial transplantation. The primary tumor was removed from its host, minced, and transplanted sc into syngeneic immunosuppressed and normal C3H/HeN(MTV) recipients. Figure 3 shows the histologic appearance of the tumor in its first transplant generation; pigment-containing cells are still prominent in the tumor. Electron microscopic evaluation of this tumor showed that the dark cells contained membrane-bound granules with the appearance of typical melanosomes (fig. 5). Tests of the tumor tissue in the dopa (3,4-dihydroxyphenylalanine) reaction also indicated that the pigment was actually melanin. Further evidence for the diagnosis of this tumor as a malignant melanoma is given by figure 4, which shows a lung metastasis in the third transplant generation in an immunosuppressed syngeneic mouse given an sc tumor transplant.

In spite of the tumorigenic and metastatic nature of the melanotic lesion, its heterogeneous appearance persisted; in subsequent passages, the tumors became predominantly nonpigmented. In an attempt to determine whether this was a mixed tumor in which the melanotic and amelanotic cells were stable independent tumors, we established cells from the first transplant generation in tissue culture. Figure 6 shows such a culture a few days after plating. After a cell line was established, single cells were isolated and propagated by a double cloning method (28). Thus far, three of these clones have been injected iv into syngeneic mice, and all clones produced mixed pulmonary tumor colonies of the type shown in figures 7 and 8. Although this development does not rule out the possibility that this tumor was of truly mixed origin, it shows that a tumor of mixed appearance could have arisen from a single progenitor cell. In either instance, there is little doubt that the tumor is, at least in part, a malignant melanoma. For the following reasons, this tumor was most likely induced by either one or both of the treatments given to the original host: 1) Although only a few spontaneous murine melanomas have been identified, no cases of spontaneous melanoma have been reported to occur in the C3H strain (29). 2) The tumor arose in an area of hyperpigmentation at the site of the croton oil application. One of these hyperpigmented areas was removed at week 109 from another of the 40 animals in the original experiment. A histologic section of this skin is shown in figure 9. Thus the rarity of spontaneous melanomas and the occurrence of this melanoma in the treated area make it unlikely that this was a naturally occurring lesion. However, determining what role UV light played in the induction of this tumor, if any, will require a large, carefully controlled study of all the variables in the treatment protocol.

ACCELERATED GROWTH OF TRANSPLANTED MELANOMAS IN UV-IRRADIATED HOSTS

A second possible role for UV radiation in the development of melanoma is one in which UV light affects tumor growth but not tumor induction. A series of studies performed over the past several years has demonstrated that UV radiation of mice induces a systemic alteration that interferes with tumor rejection (30). This systemic alteration is immunologic and can be adoptively transferred with lymphoid cells (31, 32). As a result of this immunologic alteration, UV-irradiated mice cannot resist transplants of syngeneic UV light-induced skin tumors that would be immunologically rejected by unirradiated mice. Recently, we tested the specificity of this immunologic effect and
found that only the growth of UV light-induced skin tumors is accelerated in UV-irradiated mice, with one striking exception: A spontaneous melanoma of C57BL/6 mice (B16 melanoma) grew more rapidly and with a higher frequency in syngeneic mice that had been treated for 2-4 months with UV radiation, relative to the growth in unirradiated mice [(33); Kriple ML, Lill PH: Unpublished data]. At present, we do not know whether the accelerated growth of the melanoma is immunologically mediated or due to other alterations in UV-irradiated mice. Nonetheless, this finding suggests that UV radiation might enhance the subsequent growth of a melanoma that arose from other, unrelated causes.

DISCUSSION

In considering the ways in which UV light could contribute to melanoma incidence, we can identify, on the basis of experimental or clinical observations, at least four possibilities. First, UV radiation could induce melanoma directly. Support for this possibility comes from the association of human melanoma of the lentigo maligna type with chronic, long-term exposure to UV light (19). The occurrence of melanoma in xeroderma pigmentosum patients also may be an example of this pathway. The inability of cells from these patients to repair DNA that is damaged by UV light suggests that these UV-induced lesions could be responsible for melanoma induction. Unfortunately, no animal models for this type of melanoma exist; no melanomas have been induced in animals with chronic UV irradiation, nor do animals have a syndrome that resembles xeroderma pigmentosum.

Second, UV radiation could act as an initiator of a malignant transformation that requires additional promotion for expression of the disease. No direct evidence has been found to support this possibility. However, the hypothesis is attractive because we know that UV light can damage DNA directly and can serve as an initiator for the development of other types of skin tumors (34, 35). The C3H murine melanoma that we described here may be an example of this mechanism. However, additional studies will have to be undertaken to determine whether UV light actually serves as an initiator in this system.

Third, UV light could function as a promoter for cells that were initiated by other means. In this instance, UV light would affect the initiated cells directly, perhaps by its ability to cause proliferation of melanocytes (36). The murine melanomas induced by Epstein et al. (22) with DMBA and UV light probably fall into this category.

The fourth possibility is illustrated by our experiments with the B16 melanoma (33), in which UV light has an indirect effect on melanoma growth. In this instance, UV radiation would not affect the potential tumor cells directly but would produce a systemic alteration that was conducive to tumor growth. This systemic alteration could be immunologic, like the one we have described for nonmelanoma skin tumors in mice (31); alternatively, it could be a biochemical alteration in which skin photoproducts provide a nutritive advantage for proliferating tumor cells.

In addition to these mechanisms, some human melanomas possibly occur without any participation of UV radiation. Most experimental melanomas fall into this category. Some are genetically regulated (those in the platyfish and miniswine systems), some are chemically induced (those in the guinea pig and hamster), and some have occurred spontaneously (transplantable murine melanomas). Whether or not UV radiation can influence the growth or development of melanoma in these systems has not been investigated, and this might prove to be a profitable direction for future studies.

In any event, it is not unreasonable to suppose that at least some human melanomas might also arise for reasons that do not include UV light. Supporting this possibility is the report by Bahn et al. (37) on the occurrence of melanoma in workers exposed to a polychlorinated biphenyl compound.

Thus several examples of different pathways that could lead to the common end point of melanoma exist. A demonstration that a melanoma arises via a particular pathway does not exclude the possibility that other melanomas arise by alternative pathways. For example, we know that murine fibrosarcomas can be induced by UV light, ionizing radiation, chemical carcinogens, and oncogenic viruses or can arise spontaneously late in life. Thus a search for a single etiologic agent for murine fibrosarcomas would be futile. By analogy, human melanomas might have in common only the cell affected in the neoplastic process and need not share an etiology in every case. Additional division of human melanomas into subgroups based on biologic behavior, histologic features, and antigenic properties may permit the assignment of etiologic agents to particular categories of melanoma.

In summary, results from human epidemiologic studies and lower animal experiments suggest that malignant melanoma could arise by several different mechanisms. At present little solid information regarding the possible ways in which UV light could participate in this form of carcinogenesis is available. However, several clues from animal studies regarding a possible role for UV light have been found. One could pursue these clues by designing animal experiments 1) to answer specific questions about the influence of UV light in the induction of melanomas by other agents; 2) to investigate the effects of UV radiation on the growth of established melanomas; and 3) to test critically the systems suggesting that UV light can initiate or promote melanoma induction. In addition, further analyses of the characteristics of both the tumor and of the host may provide new subdivisions of the disease whose etiology can be identified.

REFERENCES

FIGURE 1.—Primary tumor (#1735) from C3H/HeN(MTV') mouse. H & E. X 150

FIGURE 2.—Lymph node of primary host. Note heavily pigmented cells. H & E. X 150

FIGURE 3.—First transplant generation of melanotic tumor. Note persistence of pigmented cells in the tumor that is growing subcutaneously in an immunosuppressed syngeneic recipient. H & E. X 150

FIGURE 4.—Pigmented tumor cells in the lung of recipient bearing third transplant generation tumor. One tumor fragment had been implanted sc in this animal 8 wk earlier. H & E. X 150
FIGURE 5.—Electron micrograph of typical pigmented melanoma cell from first transplant generation of #1735 in immunosuppressed syngeneic mice. × 4,000
FIGURE 6.—First transplant generation tumor cells in primary culture. Note heterogeneity with regard to melanin granules. Unstained culture. X 400

FIGURE 7.—Lung from C3H/HeN(MTV-) mouse inoculated iv with 1X10⁶ cloned #1735 tumor cells. Note heterogeneity of tumor colonies in lungs. H & E. X 60

FIGURE 8.—Higher magnification of tumor colonies in lung of mouse inoculated with clone of #1735 cells. H & E. X 150

FIGURE 9.—Skin from mouse treated with UV light and croton oil. Note unusual aggregations of dermal melanocytes. H & E. X 150