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Latency, Histology, and Antigenicity of Tumors Induced by Ultraviolet Light in Three Inbred Mouse Strains[1]

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SUMMARY

The carcinogenic effects of chronic exposure to ultraviolet radiation were compared in three inbred mouse strains with different coat colors. Albino mice (BALB/cAnN) developed tumors earlier than agouti [C3H/HeN (mammary tumor virus negative) hereafter called C3H$^-$] or black (C57BL/6N) mice, and a large proportion of the tumors in the albino strain arose on the ears, in contrast to the other strains) in which dorsal tumors predominated. The most common histological types of tumor observed were fibrosarcomas, followed by squamous cell carcinomas. The types and frequency of types were comparable among the three strains. On the basis of a comparison of tumor growth in normal and immunosuppressed syngeneic recipients, primary tumors from the albino strain were the least antigenic as a group, whereas the C3H$^-$ tumors exhibited the greatest degree of antigenicity. In BALB/cAnN mice 33% of the ultraviolet-induced tumors tested failed to grow in normal syngeneic mice although they grew in immunosuppressed recipients. In C57BL/6N mice 54% and C3H$^-$ mice 75% of the tumors tested were not transplantable in normal syngeneic recipients and grew only in immunologically deficient hosts.

INTRODUCTION

Evidence from human epidemiological studies suggests that genetically determined factors contribute to the susceptibility of individuals to UV-induced skin cancer (19). In addition to the obvious factor of skin pigmentation, there may be other variables, such as levels of DNA repair enzymes, production of epidermal growth factors, or host immunological competence, that combine to determine individual susceptibility. In spite of the importance of identifying such variables, there is little experimental work in animal models addressed to this issue.

Previous studies have indicated that host immune defense mechanisms may play an important role in experimental UV carcinogenesis. Skin tumors induced in C3Hf mice by chronic UV irradiation were found to be highly antigenic; most tumors were immunologically rejected after transplantation to normal syngeneic recipients and grew only in immunosuppressed hosts (10). A subsequent study showed that these highly antigenic tumors were able to grow in their primary hosts because the UV irradiation produced a systemic change in the animals that interfered with tumor rejection (12). The fact that these tumors were so highly antigenic suggested that host defense mechanisms may be of critical importance in the development of these skin cancers (4). There is a good possibility that our findings in the murine system on tumor antigenicity and the systemic immunological effects of UV treatment (4) will be applicable to UV-induced skin cancers in humans. Although there are several fundamental differences between UV carcinogenesis in humans and rodents, particularly with regard to the histological types of tumors (3), the mechanism of transformation and the immunological parameters are likely to be universal. Several studies present similar reports of the association between UV radiation and the immune system in rodents and humans. Nathanson *et al.* (15) and Koranda *et al.* (9) reported that treatment of mice with immunosuppressive agents accelerated the development and increased the incidence of UV-induced skin tumors. Their experimental studies corroborate the clinical observation that renal transplant patients who receive chronic immunosuppressive therapy seem to develop a high frequency of UV-associated skin cancers (13, 14, 20). In addition, studies with guinea pigs showed that UV treatment suppressed the elicitation of a contact hypersensitivity response to 2,4-dinitrochlorobenzene at the site of irradiation (6). Similarly, the cutaneous delayed hypersensitivity reaction to streptokinase-streptodornase was suppressed in a human subject after UV irradiation (7).

This study is the 1st step in an investigation of the influence of genetic background on the susceptibility of mice to UV carcinogenesis. Because there is reason to believe that immunolgical factors can contribute to tumor induction, it was desirable to test the generality of the earlier observations in C3Hf mice (10, 12) by examining the antigenicity of tumors from other strains. In these experiments the tumor incidence, the site and rate of tumor development, and the histological types of tumors were compared in 3 inbred strains of mice with different coat colors [BALB/cAnN (here after referred to as BALB/c) albino; C3H/HeN mammary tumor virus negative (hereafter referred to as C3H$^-$) agouti; and C57BL/6N (hereafter referred to as C57BL/6) black]. In addition, the relative antigenicity of primary tumors induced in each strain was assessed by comparing tumor growth in normal and immunosuppressed syngeneic recipients.

MATERIALS AND METHODS

Mice. Specific-pathogen-free mice of the inbred strains, C3H$^-$, C57BL/6, and BALB/c, and athymic nude mice (nu/

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nu) on a random-bred Swiss background [N.NIH(S)] were supplied by the Frederick Cancer Research Center Animal Production Facility. They were given free access to chlorinated water and Purina mouse chow.

UV Irradiation. The light source was a bank of 6 Westinghouse FS40 sunlamps, which delivered an average dose rate of ~2.8 J/sq m/sec over the wavelength range of 280 to 340 nm. (This range included approximately 80% of the total energy output of the lamps.) Measurements were made with an International IL 700/760/780 light spectroradiometer system containing a PM270DCM149 detector with a spectral range of 240 to 810 nm. The mice were housed 5/cage on a shelf 20 cm below the fluorescent bulbs, and the cage order was systematically rotated prior to each treatment to compensate for the uneven lamp output along the shelf. Room lights were on an automated cycle of 12 hr of light and 12 hr of dark. The mice were shaved with Oster electric clippers with a No. 40 blade once per week. Dorsal hair was removed from the base of the tail to the nape of the neck and to the lateral midlines.

Tumor Induction. Beginning at 8 weeks of age, mice were irradiated for 1 hr 3 times per week (Monday, Wednesday, Friday). This treatment continued until an animal developed a tumor of sufficient size (approximately 10 mm in diameter) for biopsy and transplantation. All animals were inspected once a week for skin tumors, and the location and growth rate of each tumor were recorded. Tumor biopsies were fixed in Bouin's solution and stained with hematoxylin and eosin for microscopic examination.

Tumor Transplantation. Primary tumors were removed, cut into 1-cu mm fragments, and transplanted to groups of normal and immunosuppressed syngeneic mice and to nu/nu recipients. Tumor fragments were implanted s.c. in 1 flank of each recipient with a trocar. Mice were immunosuppressed by thymectomy at 4 to 5 weeks of age and whole-body X-irradiation 24 hr prior to transplantation (2 to 4 weeks after thymectomy). $C3H^-$ and BALB/c mice received 450 rads, and C57BL/6 mice recieved 500 rads from a Phillips MG 301 X-ray unit. The recipients were inspected once a week for tumor development for at least 3 months, and the tumor sizes were recorded. Progressively growing tumors are defined as tumors that increase in size until the host dies or the observation period ends.

Statistical Tests. The method of Kaplan and Meier (8) was used to describe the patterns of tumor development in the carcinogenesis study. This is a life table analysis and thus takes into account animals that die before developing a tumor. The results are expressed as the probability that an animal may have a tumor as a function of the duration of UV treatment. Differences between strains were tested with a censored rank order test according to the method of Breslow (1). Differences in tumor incidence between groups were analyzed by the χ^2 test (2), and differences in tumor latent periods were analyzed by the Mann-Whitney test (17).

RESULTS

Tumor Induction. The rate of tumor development for BALB/c, $C3H^-$, and C57BL/6 mice is shown in Chart 1. The latent period is defined here as the time between the 1st UV treatment and the appearance of a visible skin tumor. These data include only the 1st tumor that appeared on an animal, although some mice developed additional primary tumors. Thus, what is reported here is the minimal time required for animals to become tumor bearers. Because the development of subsequent tumors does not influence this determination, these tumors are not included in Chart 1. All 3 strains of mice were susceptible to carcinogenesis by UV, but tumors developed earlier in the albino strain (BALB/c) than in the other strains ($p < 0.001$ for BALB/c *versus* $C3H^-$ and BALB/c *versus* C57BL/6). This strain also had a higher proportion of ear tumors than had the other strains. Table 1 shows that 89% of the tumors that arose in BALB/c mice appeared on the ears, whereas only 42% of the tumors in C57BL/6 mice and 39% in $C3H^-$ mice originated on the ears ($p < 0.001$ for BALB/c *versus* $C3H^-$ and BALB/c *versus* C57BL/6). To see whether the shorter time to tumor appearance (latent period) in BALB/c mice could be related to more rapid tumor development on the ears than on other sites, the latent periods of ear tumors were compared to those of other tumors within each strain. No difference was detectable between the latent periods of ear *versus* dorsal-plus-other tumors in any strain. Thus, the accelerated development of tumors in BALB/c mice does not seem to be related to the fact that most of the tumors originated on the ears.

The data for the rate of tumor appearance (Chart 1) include all 1st tumors regardless of histological type. In all 3 strains there was no detectable difference between the latencies of fibrosarcomas and squamous carcinomas. For example, in the BALB/c strain, 34 of the 46 1st tumors were examined microscopically. Of these, 22 were fibrosarcomas with an average latent period of 21 (range, 17 to 28) weeks. The remaining 12 were squamous carcinomas with an average latency of 23.5 (range, 17 to 28) weeks. Because these latent periods did not differ statistically, they were combined in Chart 1.

Approximately 75% of the primary tumors were examined microscopically. In the remaining cases the animals died before biopsy material was obtained. There is no reason to

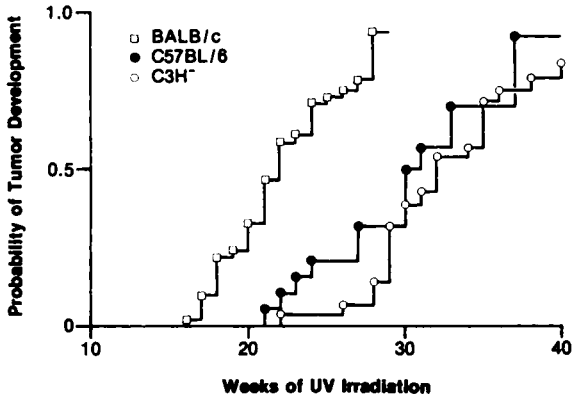

Chart 1. Rate of tumor appearance in 3 strains of mice. Animals were treated with UV irradiation for 1 hr 3 times per week, and their dorsal hair was clipped weekly. Groups consisted of 51 BALB/c females, 19 C57BL/6 males, and 28 $C3H^-$ females.

assume that this is not a random and representative sampling of tumor types. Table 1 summarizes the histological data; all tumors are included regardless of their order of appearance, because the histological type of a tumor was independent of its order of appearance. Of the tumors examined the majority were classified as fibrosarcomas. Squamous cell carcinomas were also present and, with few exceptions, arose on the ears of the mice. A few squamous cell carcinomas were classified as mixed type because they contained a prominent spindle cell component. Representative sections of each tumor type are shown in Figs. 1 to 3. There were no apparent differences in morphological types or statistically significant differences in the frequency of types among the 3 strains.

Tumor Transplantation. Primary tumors from each strain were transplanted s.c. to groups of 3 to 5 normal and immunosuppressed syngeneic recipients. Some of the BALB/c and C3H⁻ tumors were also transplanted to nu/nu mice. The tumors were classified as transplantable if they grew in at least 1 recipient. These data are summarized in Table 2. In BALB/c mice 33% of the tumors failed to grow in any normal syngeneic mice, although they grew in immunosuppresed recipients. In C57BL/6 mice 54% of the tumors failed to grow in normal mice, and in C3H⁻ mice 75% of the tumors were not transplantable in normal recipients. In all 3 strains fewer of the tumor implants grew progressively in normal mice than in mice immunosuppressed by thymectomy or in nu/nu mice. There was no detectable relationship between the site of a primary tumor and its transplantability in normal syngeneic hosts within strains. Most of the tumors transplanted were fibrosarcomas; only 2 C3H⁻ and 2 BALB/c tumors were squamous cell carcinomas. Thus, in this study it is not possible to compare the transplantation of tumors of different histological types. Such a comparison was reported in an earlier study in C3Hf mice (10).

A comparison of the latent periods of tumors that grew in normal mice with those of tumors that failed to grow is shown in Table 3. In BALB/c mice there was no correlation between latent period and transplantation of tumors in normal recipients. However, tumors from the C57BL/6 strain that failed to grow in normal mice generally had longer latent periods than those that grew in normal recipients. The transplantation of tumors from C3H⁻ mice shows no correlation with latent period in this study. However, only the earliest one-third of the tumors were tested (those arising during Weeks 22 to 30), and this may not be a representative sample of the entire population.

DISCUSSION

This study indicates that there are several differences in the responses of 3 inbred mouse strains to UV carcino-

Table 1

Site and histological type of tumors produced by chronic UV irradiation

Strain	No. of mice[a]	No. of tumor bearers	Site	No. of tumors	No. examined histologically	Squamous cell carcinoma	Fibrosarcoma	Mixed type[b]
BALB/c	51	46	Ear	58	41	10	28	3
			Back	6	6	1	5	0
			Eyelid	1	1	0	1	0
				65	48	11	34	3
C57BL/6	19	15	Ear	8	5	1	4	0
			Back	11	7	0	7	0
				19	12	1	11	0
C3H⁻	28	24	Ear	11	8	3	3	2
			Back	13	12	0	10	2
			Eye	3	2	0	2	0
			Forepaw	1	1	0	1	0
				28	23	3	16	4

[a] Number alive at the time the 1st tumor appeared.
[b] Predominantly squamous cell carcinoma with spindle cell component.

Table 2

Transplantation of primary tumors in normal and ATX[a] mice

Strain	No. of transplantable tumors/no. tested			No. of progressively growing implants/total implants		
	ATX	Normal	nu/nu	ATX	Normal	nu/nu
BALB/c	21/23 (91)[b]	16/24 (67)[c]	12/12 (100)	78/90 (87)	37/119 (31)	54/57 (95)
C57BL/6	13/13 (100)	6/13 (46)[d]		41/48 (85)	17/62 (27)	
C3H⁻	15/16 (94)	4/16 (25)[e]	12/12 (100)	54/73 (74)	15/80 (19)	34/37 (92)

[a] ATX, immunosuppressed by thymectomy.
[b] Numbers in parentheses, percentage.
[c] $p < 0.05$ *versus* ATX (χ^2 test).
[d] $p < 0.01$ *versus* ATX.
[e] $p < 0.001$ *versus* ATX; $p < 0.001$ *versus* BALB/c normal.

Table 3
Latent periods of primary tumors

In Group A are tumors that failed to grow when transplanted to normal syngeneic recipients. In Group B are tumors that grew progressively in normal syngeneic recipients.

	Group A		Group B	
	No.	Median time (wk)	No.	Median time (wk)
BALB/c	8	21.5 (17-26)[a]	16	20 (16-26)
C57BL/6	7	30 (27-37)[b]	6	24 (21-30)
C3H⁻	12	28.5 (23-30)	4	27 (22-30)

[a] Numbers in parentheses, range.
[b] $p = 0.004$ *versus* Group B C57BL/6 (Mann-Whitney test).

genesis. The black (C57BL/6) and agouti (C3H⁻) strains were quite similar in most respects, but several striking differences were observed in the albino (BALB/c) strain. The BALB/c mice developed tumors much earlier than did the other strains and showed a marked predilection for the development of ear tumors. These differences could very well be due to the quantity and distribution of pigment in skin and hair. Although there is little pigment in mouse skin, it is abundant in hair and hair follicles. Even with weekly shaving of the hair, there may be enough stubble remaining on the black and agouti mice to filter out a portion of the incident radiation. However, within a strain, hair and pigment distribution do not seem to be the critical factors in the susceptibility of different sites to tumor induction. There is little hair or pigment in the ears of C57BL/6 mice, yet the latency of tumors arising on the ears did not differ from the latency of back tumors.

The histological types and proportions of each type of tumor produced were quite comparable among the 3 strains. Thus, the predominance of fibrosarcomas characteristic of UV carcinogenesis in haired albino mice (3, 5, 18) was also observed here in the nonalbino strains. Whether these fibrosarcomas are truly of dermal, rather than epidermal, origin was not addressed here and probably cannot be determined on the basis of light microscopic evaluation.

A single study by Rusch and Baumann (16) in 1939 also compared tumor development in 3 mouse strains. These authors found that 2 strains of albino mice (strains A and C) developed more tumors much more rapidly than C57 mice in response to UV radiation. However, these mice were not shaved prior to irradiation; therefore, only ear tumors and a few eye and tail tumors were produced. The authors also noted that 4 strain A tumors transplanted to groups of 5 strain A recipients produced a transplantable tumor in only 1 of the 20 mice. Although bacterial contamination is suggested as the cause of the low frequency of transplantation, it is extremely likely that this was due to immunological rejection of the tumors because of their high antigenicity (10).

In this study the failure of some tumors to grow in normal mice cannot be explained on the basis of a low growth potential or bacterial contamination because these tumors grew readily in immunosuppressed mice. This indicates that these tumors were immunologically rejected in the normal recipients and are thus highly antigenic. The term, "antigenic," is used here in preference to "immunogenic," because the latter term implies that one has tested the ability of the tumors to immunize a host to make an accelerated secondary response. Because a secondary challenge was not performed in these experiments, the term antigenicity seems more appropriate. An earlier study (10) showed that the regression of tumors in normal mice was indeed accompanied by the development of specific memory response and that these tumors are thus immunogenic as well, at least in C3H mice. However, because this was not tested with the C57BL and BALB/c strains, the more conservative term, antigenicity, is used.

Because fewer of the tumors were rejected in the BALB/c strain, these tumors are less antigenic as a group than are the C3H⁻ tumors. The tumors from C57BL/6 mice seem to fall between those from the BALB/c and C3H⁻ strains, although the proportion of tumors that were rejected by normal mice did not differ statistically from that of either BALB/c or C3H⁻ tumors.

In comparing the antigenicity of tumors induced in different strains of mice, it must be emphasized that antigenicity is a relative, rather than an absolute, measurement. It depends on both the chemical nature and structure of the antigen and the quality of the immune response directed against it. Thus, we cannot strictly compare the absolute antigenicity of BALB/c tumors with that of C3H⁻ tumors unless they are tested in the same recipient (*i.e.*, in an F_1 hybrid of these strains). Nonetheless, we can conclude that, within the strain of origin, more of the C3H⁻ tumors are highly antigenic than are the BALB/c tumors.

Previous work from this laboratory showed that chronic UV irradiation produced a systemic alteration in C3H mice that resulted in their failure to reject these highly antigenic syngeneic UV-induced tumors (11, 12). Because the severity of this alteration increases with increasing UV exposure, it is tempting to attribute the difference in the antigenicity of BALB/c and C3H⁻ tumors to immunoselection. The C3H⁻ mice are irradiated for a longer period of time before tumors appear, and this could permit the development of a larger proportion of highly antigenic tumors in these mice than in the BALB/c strain. If immunoselection occurs during UV carcinogenesis, one might also predict that within a strain the most antigenic tumors would have the longest latent periods. This was the case in the C57BL/6 mice but not in the BALB/c mice. Obviously, additional testing is required to settle this issue. Nonetheless, it is clear that UV carcinogenesis in mice is influenced not only by coat color and pigment distribution but also by other factors, such as immunological ones, for which further clarification of roles is required.

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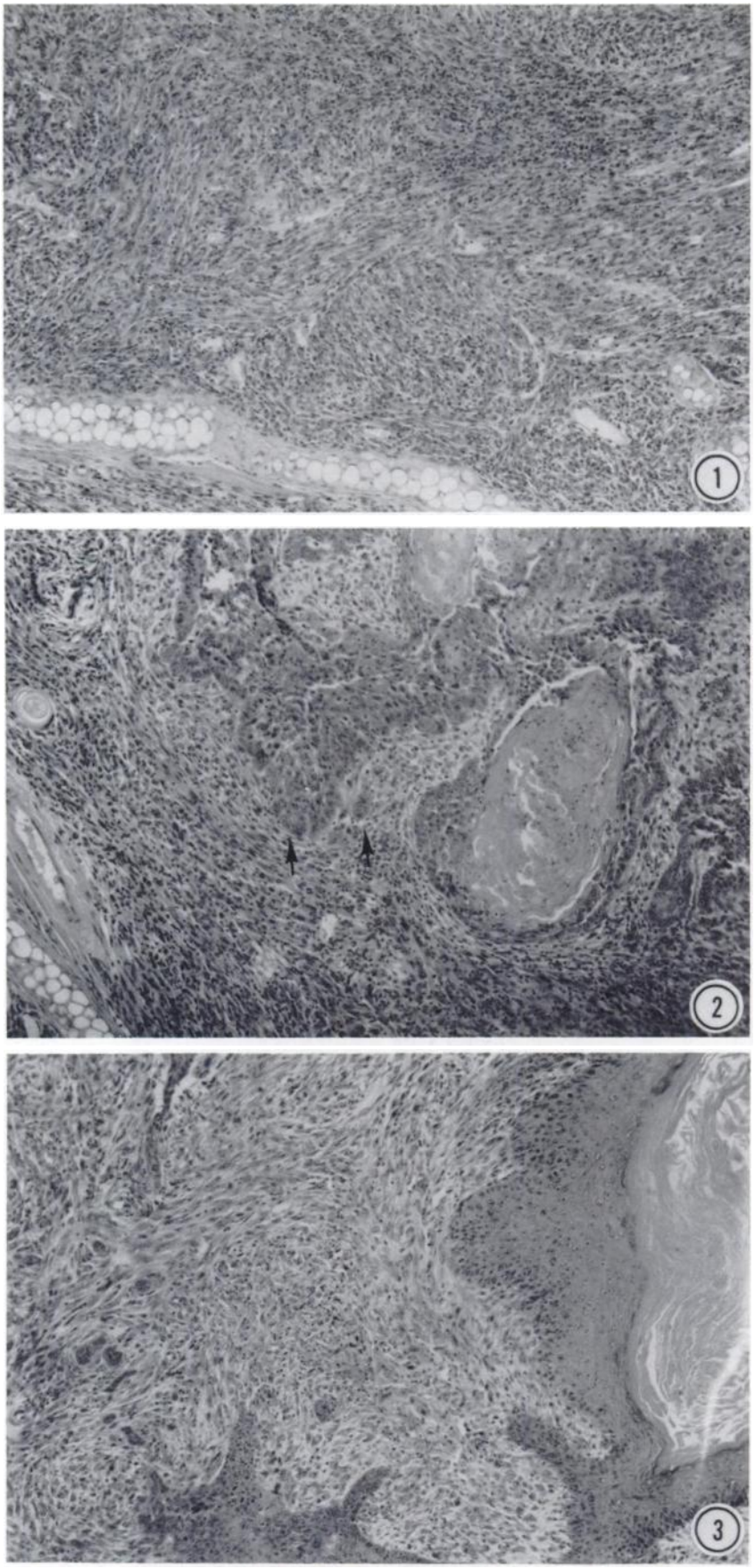

Fig. 1. UV-induced invasive fibrosarcoma on the ear of a BALB/c mouse. H & E, × 125.

Fig. 2. UV-induced squamous cell carcinoma on the ear of a BALB/c mouse. Note invasiveness of epithelial cells (*arrows*) and keratin pearl (*left edge*). H & E, × 125.

Fig. 3. UV-induced squamous cell carcinoma with prominent spindle cell component (*center*). H & E, × 125.