Suppressor T Lymphocytes Control the Development of Primary Skin Cancers in Ultraviolet-Irradiated Mice

Abstract. *Exposure of mice to ultraviolet radiation results in the development of suppressor T lymphocytes in lymphoid organs, followed by the appearance of primary skin cancers. The presence or absence of these suppressor lymphocytes determines whether or not primary cancers will develop in the ultraviolet-irradiated skin. This demonstrates the importance of immunological regulatory pathways in carcinogenesis and provides an example of immunological surveillance.*

Although suppressor T lymphocytes can regulate the immune response to transplanted tumors (*1*), their involvement in carcinogenesis has not been established. We assessed the role of these immunological regulatory cells in the development of primary skin cancers induced by ultraviolet (UV) radiation. Previous work demonstrated that mice exposed repeatedly to UV radiation develop suppressor T lymphocytes in their lymphoid organs. These lymphocytes appear before primary skin cancers are apparent and are detected by their ability to prevent the immunological rejection of transplants of highly antigenic, syngeneic UV-induced tumors (*2*). We found that the development of primary cancers in UV-irradiated skin was dramatically enhanced by the presence in the host of suppressor T lymphocytes induced by UV radiation. Furthermore, when the lymphocytes were present in the host at the beginning of a course of UV irradiation, the latent period of tumor development decreased significantly.

We used two approaches to assess the role of suppressor T lymphocytes in UV carcinogenesis. In the first, C3H/HeN(MTV$^-$) mice were given lethal doses of x-radiation, and their lymphoid organs were repopulated with spleen and lymph node cells from syngeneic normal mice or mice that had been irradiated with sunlamps (FS40, Westinghouse) for 1 hour three times per week for 12 weeks. Lymphoid cells from such UV-irradiated mice contain suppressor T lymphocytes (*2*). Four weeks later, the recipients were grafted with 2.5 by 4.0 cm pieces of dorsal skin from syngeneic mice that had been exposed to UV irradiation for 16 weeks; the grafts were monitored weekly for tumor development (*3*). In this way it was possible to separate the carcinogenic effects of UV radiation on skin from its systemic immunological effects. The diagnosis of all tumors was confirmed by microscopic examination of tissue sections. Most of the tumors were classified as fibrosarcomas, and only a few were squamous carcinomas or a mixture of these two types; the proportions of the tumor types were the same in the various treatment groups. The development of primary skin cancers induced by UV radiation in the grafted skin is illustrated in Fig. 1, in which the results of two independent experiments are combined. Few tumors developed in the UV-irradiated skin grafted to the mice that received only normal lymphoid cells. In contrast, many more tumors developed in the skin grafted to mice that had received lymphoid cells from UV-irradiated donors or a mixture of lymphoid cells from UV-irradiated donors and normal mice. Thus, even though the skin grafts had received equivalent carcinogenic exposures to UV radiation before grafting,

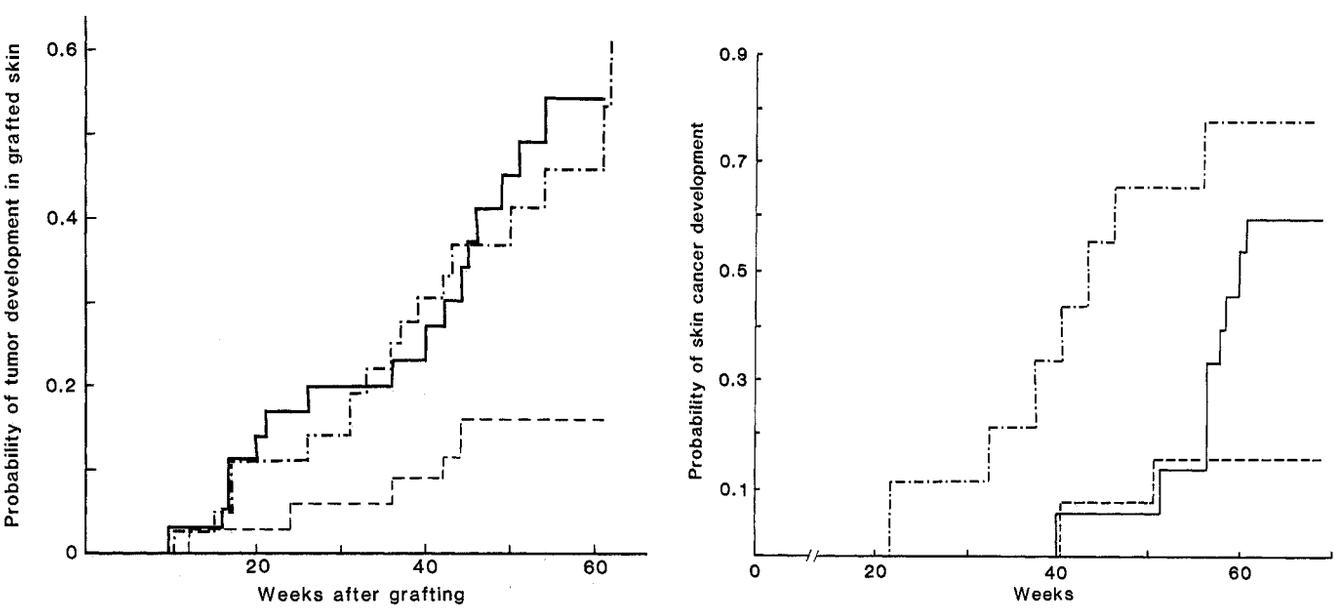

Fig. 1 (left). Development of primary tumors in UV-irradiated dorsal skin grafted to syngeneic mice containing normal lymphoid cells (- - - -), lymphoid cells with suppressor T lymphocytes from UV-irradiated mice (—·—·), or a mixture of the two populations (———). Skin graft donors had been exposed to UV radiation from FS40 sunlamps for 1 hour, three times per week for 16 weeks before grafts were removed. Syngeneic C3H/HeN(MTV$^-$) recipients had been given lethal doses of x-radiation (800 R) to destroy their lymphoid tissue and then repopulated with 5×10^7 lymphoid cells from normal or UV-irradiated donors, or both, 2 weeks before receiving grafts. The probability associated with these results under the assumption of no difference in the rate of tumor development between the group given normal lymphoid cells ($N = 38$) and those given lymphoid cells from UV-irradiated donors ($N = 36$), or from the mixed population ($N = 39$), is 0.013 and 0.010, respectively, as determined by the Kruskal-Wallis test (*9*). Fig. 2 (right). Development of primary skin cancers in UV-irradiated mice injected with normal T lymphocytes (- - - -), injected with T lymphocytes from UV-irradiated mice (—·—·), or not injected (———). At weeks 0, 3, 6, and 9, the first two groups received a single intravenous injection of 5×10^7 lymphoid cells composed of 80 to 95 percent T lymphocytes. The mice in all groups had their backs shaved once per week and were exposed to FS40 sunlamps for 1 hour, three times per week for 12 weeks. The probability associated with these results under the assumption of no difference in the rate of tumor development between the group given T lymphocytes from UV-irradiated mice ($N = 9$) and those given normal lymphocytes ($N = 12$), or no lymphocytes ($N = 15$), is 0.003 and 0.01, respectively, as determined by the Kruskal-Wallis test (*9*).

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the subsequent appearance of most primary tumors in the grafts depended on the presence of lymphoid cells from UV-irradiated mice in the recipient animals.

In the second approach, C3H/HeN(MTV$^-$) mice were given intravenous injections of 5×10^7 T lymphocytes that had been partially purified by separation on nylon wool columns (*4*). We had shown earlier that this procedure enriches the proportion of T lymphocytes in the population to about 85 percent and enhances the suppressive activity of the preparation (*3*). The T lymphocytes were obtained from normal or UV-irradiated C3H/HeN(MTV$^-$) mice and were injected at weeks 0, 3, 6, and 9. From weeks 0 to 12, these animals and mice that had not received injections were exposed to UV radiation for 60 minutes three times per week. The rate of primary skin tumor development in these three groups is illustrated in Fig. 2. Mice that received T lymphocytes from UV-irradiated donors developed more tumors than uninjected mice or mice given normal T lymphocytes; all tumors produced in this experiment were fibrosarcomas. Furthermore, skin cancers began to appear at around week 20 in the mice injected with the cell population containing suppressor T lymphocytes induced by UV radiation, but only after week 40 in the other two treatment groups. This result implies that nascent transformed cells are eliminated or held in abeyance by immunological means during the long latent period of UV carcinogenesis and that these transformed cells can develop into visible neoplasms only after the accumulation of an effective number of suppressor T lymphocytes.

Thomas and Burnet (*5*) proposed that the immune system may function as a defense mechanism against newly arising transformed cells. Support for this theory, which is called the theory of immunological surveillance, has been provided by studies of tumor systems involving oncogenic viruses (*6*), but its applicability to nonviral carcinogenesis remains controversial. Our demonstration that the latent period for photocarcinogenesis can be reduced by adding to the hosts T lymphocytes that specifically suppress the immune response against tumors induced by UV radiation suggests that immunological surveillance occurs during ultraviolet carcinogenesis and is partly responsible for the long latent period that accompanies the induction of these skin cancers. Immunological surveillance is important in this particular tumor system because these skin cancers are extremely antigenic (*7*) and thus are capable of being recognized and eliminated by the immune system. The fact that the skin is a particularly reactive tissue with special immunological capabilities (*8*) may contribute to the successful functioning of immunological surveillance in skin carcinogenesis.

We conclude that the suppressor T lymphocytes induced in mice by UV radiation not only inhibit the rejection of tumor transplants (*2*), but also play a decisive role in carcinogenesis. The demonstration that suppressor lymphocytes can affect the development of primary tumors in situ illustrates the importance of immunological regulatory mechanisms in the control of cancer growth in the primary host.

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Biplexiform Cells: Ganglion Cells of the Primate Retina That Contact Photoreceptors

Abstract. *Golgi-impregnated biplexiform cells of the macaque retina are neurons with cell bodies in the ganglion cell layer, an axon in the nerve fiber layer, and dendrites in the inner plexiform layer that are postsynaptic to amacrine cell processes and bipolar cell axon terminals. In these features they resemble conventional ganglion cells, but they also have processes that arise from the main dendritic arborization, extend to the outer plexiform layer, and are postsynaptic to rod photoreceptor terminals as central elements at the ribbon synaptic complex. However, ordinary retinal ganglion cell dendrites ramify in the inner plexiform layer and do not contact photoreceptors. Thus, biplexiform cells represent a previously undescribed class of neuron, part of whose synaptic input could bypass the commonly described interneuron circuitry of the vertebrate retina.*

The vertebrate retina is generally considered to consist of five major classes of neuron, each of which may contain many different structural and functional types (*1–3*). Light is transduced by photoreceptors to electrical signals. These signals are transmitted by bipolar cell interneurons to ganglion cells, whose axons convey visual information to the brain. Horizontal cells and amacrine cells (*4*) interact in this chain at the levels of the photoreceptor-bipolar cell synapse (outer plexiform layer, OPL) and the bipolar-ganglion cell synapse (inner plexiform layer, IPL), respectively. Ganglion cells have never been observed to contact photoreceptors directly. Here, I report a new class of retinal neuron found in Golgi preparations of the rhesus monkey retina which has the characteristic features of a ganglion cell, but which also directly contacts photoreceptors. These newly identified neurons are termed biplexiform cells since their processes arborize and are postsynaptic in both the OPL and IPL.

Retinas of rhesus monkeys (*Macaca mulatta*) were Golgi-impregnated and prepared as whole flat-mounts (*5*, *6*). Eight of 14 retinas contained Golgi-impregnated biplexiform cells; the most found in any one retina was six and the total number was 19. Biplexiform cells, as viewed in flat preparations (Fig. 1) and radial sections (Fig. 2A), have relatively small 9-μm round, smooth cell bodies which are located in the ganglion cell layer. Three or four main dendritic processes, 1.5 to 2 μm in diameter, arise from the cell body and subsequently give rise to secondary and tertiary branches forming, overall, a loose wavy, radiate dendritic pattern, about 100 μm in span, confined to the inner one-half of the IPL. Four axons of somatic origin were impregnated up to 10 μm, whereas three

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