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Immunology

Immunologic effects of whole-body ultraviolet irradiation: Selective defect in splenic adherent cell function *in vitro*

(antigen-presenting cells/plaque-forming cells)

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ABSTRACT **Splenocytes from mice receiving whole-body UV irradiation do not make a normal primary *in vitro* plaque-forming cell (PFC) response to the soluble T-dependent antigen trinitrophenylated poly(L-glutamic acid^{60}L-alanine^{30}L-tyrosine^{10}). This impaired immune response results from a selective loss of antigen-presenting cell function in the splenic adherent cell (SAC) population of the UV-treated mice. SACs from UV-irradiated mice are unable to reconstitute a PFC response when added to normal splenocytes passed through Sephadex G-10 (which depletes adherent cells), whereas normal SACs, when added to Sephadex G-10-passed splenocytes from UV-treated mice, do restore a PFC response. The effect of *in vivo* UV irradiation on the SAC population is indistinguishable functionally from the effect of *in vitro* UV irradiation of SACs from normal mice. Possible explanations for this selective effect of external UV irradiation on SAC function are discussed.**

Exposing animals to ultraviolet (UV) light has been shown to alter many parameters of their immunologic reactivity. In contrast to normal mice, UV-irradiated mice are unable to reject syngeneic UV-induced tumors when challenged with these tumors subcutaneously or intravenously (1, 2). A depression of secondary cytotoxic responses to UV-induced syngeneic tumor cells and to alloantigens has also been demonstrated with spleen cells from UV-irradiated mice (3). Further, a UV-treated mouse does not show a normal local graft-versus-host reaction in draining lymph nodes when injected in its footpads with allogeneic lymphocytes (4). UV-irradiated guinea pig skin is unreactive to topical sensitization with dinitrochlorobenzene, whereas sensitizing unirradiated skin in the same animal can elicit delayed type hypersensitivity (DTH) (5).

More recent studies have begun to explore the cellular basis for these various UV-induced changes in immunity. The inability to sensitize topically UV-treated mice has been recently shown to correlate with an UV-induced absence of Langerhans cells, the antigen-presenting cells (APCs) in the skin (6). Greene *et al.* (7) have further demonstrated that trinitrophenyl (Tnp)-conjugated splenic adherent cells (SACs) from mice that have been UV irradiated are incapable of sensitizing efficiently for DTH reactivity and, in fact, induce a population of antigen-specific suppressor T cells. They also showed that immunization with Tnp-conjugated SACs from normal mice stimulates a DTH response in UV-irradiated mice, a response not elicitable in these animals with Tnp-conjugated SACs from UV-irradiated mice themselves. This seems to indicate that the UV exposure does not markedly affect the T cells responsible for the DTH response.

The critical importance of APCs in regulating immunologic reactivity is now well appreciated. The evidence pointing to a defect in the antigen-presenting capability of SACs from UV-irradiated mice therefore prompted us to explore this phenomenon in a more direct fashion than is possible in a DTH system. We have investigated these effects of whole-body UV irradiation by using an *in vitro* microculture system, which allows direct examination of the functional activity of the various cell types involved in a T-dependent humoral immune response. Our experiments demonstrate that UV irradiation of mice produces a selective defect in splenic adherent cell function.

MATERIALS AND METHODS

Mice. Male or female BALB/c and A/J mice were purchased from The Jackson Laboratory or Charles River Breeding Colonies (Wilmington, MA). They were maintained in our animal facilities on standard laboratory chow and chlorinated water ad lib and used at 8–20 weeks of age.

UV Source and Treatment. UV was administered with a bank of six FS-40 "Sun Lamp" fluorescent tubes (Westinghouse, Pittsburgh, PA). These tubes provide a continuous UV spectrum with a peak at 313 nm. Tube-to-target distance measured 20 cm. UV output measured by an I.L. 443 phototherapy radiometer (International Light, Newburyport, MA) was 1.2–1.4 mJ/cm^2 per sec. UV irradiation *in vivo* was accomplished by shaving the backs of mice and exposing them under the bank of tubes ½ hour daily for 6 days and then using the mice for experiments within 5 days of the last exposure. This was found to be the minimal UV exposure necessary to prevent haptenated spleen cells from such treated animals from priming for DTH responses in UV-treated mice. Cells in medium were exposed to UV irradiation *in vitro* for 30 sec in quartz cuvettes.

Antigen. The random copolymer poly(L-glutamic acid^{60}L-alanine^{30}L-tyrosine^{10}) (GAT), molecular weight 30,000–50,000, was purchased from Vega Biochemicals (Tucson, AZ). For use in microcultures, 50 mg of GAT was conjugated with 2.5 mg of 2,4,6-trinitrobenzene sulfonate (ICN) in 5 ml of 0.1 M sodium borate buffer (pH 9.0) at 37°C for 2 hr, extensively dialyzed against phosphate-buffered saline at 4°C, filtered, and frozen until use. This conjugate (Tnp-GAT) contained approximately 1 mol of Tnp per mol of GAT.

Culture Conditions. Cells were cultured as described by Hodes and Singer (8). Tris/ammonium chloride-treated responding spleen cells (5×10^5) were cultured in 200-μl volumes in flat-bottom wells of microtiter plates (Linbro) at 37°C in a 5% CO_2/humidified air atmosphere. The medium was Eagle's minimal essential medium supplemented with 2 mM L-glutamine, penicillin at 100 units/ml, streptomycin at 100 μg/ml, 1 mM sodium pyruvate, nonessential amino acids, 15 mM Hepes buffer solution, 50 μM 2-mercaptoethanol, and 10% fetal calf serum (FCS) (M.A. Bioproducts, Walkersville, MD). The

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Abbreviations: APC, antigen-presenting cell; DTH, delayed type hypersensitivity; FCS, fetal calf serum; G-10, Sephadex G-10; GAT, poly(L-glutamic acid^{60}L-alanine^{30}L-tyrosine^{10}); PFC, plaque-forming cell; SAC, splenic adherent cell; Tnp, trinitrophenyl.

Table 1. Primary IgM plaque-forming responses *in vitro* to Tnp-GAT are T-cell dependent and require SACs bearing *I-A*-controlled determinants

Treatment of responding spleen cells*†	SACs per culture, treatment	Direct PFCs per 10^7 cultured cells‡	
Normal mouse serum + complement		275 (1.27)	
Anti-Thy 1.2 + complement		22 (2.22)	
		Exp. I	Exp. II
Untreated		1629 (1.16)	—
Passed through G-10		31 (1.77)	0
	45,000	2272 (1.24)	—
	45,000, complement alone		1218 (1.03)
	45,000, anti-I-A + complement		89 (1.49)

* A/J spleen cells were used in Exp. II, BALB/c cells in the others.
† About 5×10^5 responding cells per well were cultured with Tnp-GAT at 50 μg/ml.
‡ Geometric mean (SEM) of parallel triplicate culture groups, each group a pool of two culture wells.

Tnp-GAT concentration of 50 μg/ml was found through titration studies to be the optimal antigen concentration for PFC responses and was used for this work. Six wells were plated for each culture group and duplicate wells were pooled for assay when harvested on day 4 of culture. The cells were washed once in Hanks' balanced salt solution and assayed for PFC response.

PFC Assay. The direct (IgM) anti-Tnp PFC response was assayed by a slide modification of the Jerne hemolytic plaque assay (9). Horse erythrocytes (Colorado Serum, Denver, CO) were conjugated with Tnp by the method of Rittenberg and Pratt (10). The data are given as the geometric mean (SEM) of the parallel triplicate pooled culture groups expressed as direct PFC per 10^7 cultured cells.

Antiserum Treatment. The anti-Thy 1.2 reagent (AKR anti-C3H thymus) was the generous gift of Martin Dorf of this department. Whole spleen cells (10^8) were incubated in 0.2 ml of serum, diluted 1:5 in minimal essential medium with 1% DNase, at room temperature for 30 min. Monoclonal antibody to mouse histocompatibility antigen I-A^k (clone 11-5.2) (Becton Dickinson, Mountain View, CA) was used for treating the splenic adherent cells. Cells (10^7) and 10 μg of anti-I-A^k were incubated in 0.2 ml of minimal essential medium/5% FCS on ice for 30 min. Complement treatments for both antisera were done with 0.5 ml of Low-Tox-M rabbit complement (Cedarlane Laboratories, Hornby, ON, Canada) diluted 1:5 in minimal essential medium for 45 min at 37°C.

Adherent Cell Depletion. Sephadex G-10 (G-10) passage of spleen cells was performed by a modification (11) of the method described by Ly and Mishell (12). The columns were first equilibrated with minimal essential medium/5% FCS; cells were then loaded onto the column (10^7 cells per 10 ml of beads) and incubated for 30 min at 37°C before elution with 2 column volumes of warm minimal essential medium/5% FCS. Approximately 50% of the original cells were recovered in the eluate.

Table 2. UV irradiation *in vivo* depresses splenic plaque-forming responses *in vitro*

Source of responding BALB/c spleen cells*	Direct PFCs per 10^7 cultured cells	
	Exp. I	Exp. II
Untreated mouse	401 (1.52)	538 (2.74)
UV-irradiated mouse	24 (2.35)	38 (2.03)

See legend for Table 1.
* Splenocytes from individual mice.

SACs. Glass-adherent spleen cells were prepared by a technique slightly modified from that described by Cowing *et al.* (13). Spleens were teased to a single cell suspension in minimal essential medium/5% FCS, washed, incubated for 4 min in Tris-buffered ammonium chloride (0.83%), pH 7.65, to lyse erythrocytes, and washed again. The cells were then resuspended to 10^7 per ml of minimal essential medium/10% FCS, and 5-ml samples were added to 100-mm glass petri dishes. The cells were allowed to adhere at 37°C in a 5% CO_2 atmosphere for 2 hr; nonadherent cells were gently washed off once during that incubation with a change of medium. The dishes were washed three times with warm minimal essential medium/5% FCS, then incubated at 4°C for 15 min with cold minimal essential medium/10% FCS containing lidocaine (2.34 mg/ml). The adherent cells were then recovered from the plates by using a rubber policeman, washed, and incubated overnight in minimal essential medium/20% FCS on a rotator at 37°C. The cells received 1500 rads (15 grays) of γ irradiation before addition to culture wells.

RESULTS

***In Vitro* PFC Responses to Tnp-GAT Are T Cell Dependent and Require SACs Bearing *I-A*-Controlled Determinants.** A microculture system and PFC assay were adapted from the technique of Hodes and Singer (8) for the antigen Tnp-GAT to allow functional analysis *in vitro* of the subsets of cells involved in the immune response. As shown in Table 1, the primary PFC response *in vitro* to Tnp-GAT is T cell dependent in that anti-Thy 1.2 + complement treatment reduced the response to less than 10% of that in the control treatment group. The adherent cell dependence of this system is demonstrated by the virtual loss of a PFC response after passage of the responding cell population through a G-10 column and repletion of the response by the addition of SACs to the cell population

Table 3. SACs from UV-irradiated mice do not reconstitute plaque-forming responses of normal spleen cells passed through G-10

Treatment of responding BALB/c spleen cells	BALB/c SACs per culture, treatment	Direct PFCs per 10^7 cultured cells
Untreated		1629 (1.16)
Passed through G-10		31 (1.77)
	45,000, normal	2272 (1.24)
	45,000, UV *in vivo*	325 (1.31)

See legend for Table 1.

Table 4. Normal SACs reconstitute plaque-forming responses of normal and UV-irradiated mice

Responding BALB/c spleen cells		BALB/c SACs per culture, treatment	Direct PFCs per 10^7 cultured cells
Source	Treatment		
Normal	Untreated		509 (1.41)
	Passed through G-10		35 (6.03)
		22,500, normal	1446 (1.11)
UV-irradiated	Untreated		21 (2.65)
	Passed through G-10		9 (3.54)
		22,500, normal	534 (1.51)

See legend for Table 1.

passed through G-10. This reconstituting cell population did not contribute a significant number of functional PFC precursors to the cultures because they received 1500 rads of γ irradiation before addition to the cultures. That the cell that restores the PFC responses bears *I-A*-controlled determinants is shown in Table 1. SACs treated with complement alone reconstituted the PFC response, but after treatment with anti-I-A and complement these SACs did not restore the response.

Effect of UV Irradiation *in Vivo* on PFC Responses. UV irradiation has been shown to alter the immunologic responsiveness of mice as measured by such *in vivo* parameters as tumor rejection and DTH. Establishing an *in vitro* correlate of this altered responsiveness offered a means of identifying the cell populations affected by the UV irradiation. We therefore looked at the primary PFC response to Tnp-GAT in spleen cells from mice that had received UV irradiation. As shown in Table 2, the primary PFC responses of spleen populations from mice treated with UV irradiation were dramatically lower than responses of spleen populations from normal mice.

SACs from UV-Irradiated Mice Do Not Reconstitute the PFC Response of G-10-Passed Splenocytes from Normal Mice. The evidence suggesting effects of UV irradiation on the APC in DTH by Greene *et al.* (7) prompted us to ask if an abnormality in the splenic adherent cells of mice irradiated with UV *in vivo* could be detected in the microculture system. Normal spleen cells were passed over a G-10 column and reconstituted with SACs from normal or UV-irradiated mice. As shown in Table 3, whereas normal SACs fully restored the PFC response, SACs from mice receiving UV irradiation *in vivo* did not reconstitute a significant PFC response. This inability to reconstitute the PFC response in culture points to a functional abnormality of the SACs of UV-irradiated mice.

Normal SACs Reconstitute the PFC Response in G-10-Passed Splenocytes from UV-Irradiated Mice. The functional integrity of the T and B cells in the spleens of UV-irradiated mice was next assessed. Whole spleen cell populations from normal and UV-irradiated mice were passed through G-10 columns. As shown in Table 4, SACs prepared from normal mice reconstituted the G-10-depleted PFC response in the spleen population from normal mice. Furthermore, these normal SACs restored the PFC response of G-10-passed splenocytes from animals treated *in vivo* with UV irradiation, a population of spleen cells that, prior to G-10 passage, could not produce a significant primary PFC response. Because the PFC response of spleen cells from UV-irradiated mice could be restored simply through the addition of normal SACs, the splenic T and B cells appear to be functionally unaffected by the UV irradiation.

SACs UV Irradiated *in Vitro* Similarly Do Not Reconstitute the PFC Response. Is this selective effect of *in vivo* UV irradiation on SACs functionally distinguishable from *in vitro* UV irradiation of normal SACs? As demonstrated in Table 5, the PFC response of normal spleen cells passed over a G-10 column, although repleted by normal SACs, could not be restored by SACs derived from mice that had received UV irradiation *in vivo*. The response was also not efficiently reconstituted by normal SACs that had received a 30-sec exposure *in vitro* to UV light. This length of UV exposure was found to be the minimum necessary to inactivate stimulator cells in a mixed lymphocyte reaction (unpublished observation). Spleen cells from mice treated *in vivo* with UV irradiation were also passed over a G-10 column. Whereas a PFC response of moderate magnitude was restored by normal SACs, no PFC response was produced when either SACs from mice treated with UV *in vivo*

Table 5. Normal SACs treated *in vitro* with UV irradiation and SACs from mice treated *in vivo* with UV irradiation do not reconstitute plaque-forming responses

Responding BALB/c spleen cells		BALB/c SACs per culture, treatment	Direct PFCs per 10^7 cultured cells
Source	Treatment		
Normal	Untreated		538 (2.74)
	Passed through G-10		6 (2.47)
		45,000, normal	1030 (1.38)
		45,000, UV *in vitro*	109 (1.87)
		45,000, UV *in vivo*	233 (1.37)
UV-irradiated *in vivo*	Untreated		38 (2.03)
	Passed through G-10		0
		45,000, normal	180 (1.14)
		45,000, UV *in vitro*	0
		45,000, UV *in vivo*	0

See legend for Table 1.

Table 6. SACs UV irradiated *in vitro* and SACs from mice UV irradiated *in vivo* do not inhibit PFC responses in culture

Source and treatment of responding BALB/c spleen cells	BALB/c SACs per culture			Direct PFCs per 10^7 cultured cells
	Number	Treatment	Source	
Normal, passed through G-10	90,000	Untreated	Normal	1124 (1.61)
	45,000			1030 (1.38)
	90,000	UV *in vitro*	Normal	515 (1.23)
	45,000			109 (1.56)
	90,000	Untreated	UV *in vivo*	236 (1.15)
	45,000			233 (1.37)
	45,000	Untreated	Normal	
	+45,000	UV *in vitro*	Normal	1044 (1.01)
	45,000	Untreated	Normal	
	+45,000	Untreated	UV *in vivo*	644 (1.41)

See legend for Table 1.

or SACs themselves treated with UV *in vitro* were added to the G-10-passed spleen cells from UV-irradiated mice. Thus, normal SACs UV irradiated *in vitro* and SACs from mice UV irradiated *in vivo* appear functionally indistinguishable.

SACs UV Irradiated *in Vitro* and SACs from Mice UV Irradiated *in Vivo* Do Not Inhibit PFC Responses. The absence of a PFC response in G-10-passed spleen cell populations reconstituted with SACs UV irradiated *in vitro* or SACs from mice UV irradiated *in vivo* could reflect either an absence of functional SACs or a directly inhibitory effect of these SACs in the culture. This issue was addressed in the experiment shown in Table 6. As in previously discussed experiments, normal SACs reconstituted a PFC response in a population of normal G-10-passed spleen cells, whereas normal SACs UV irradiated *in vitro* and SACs from mice UV irradiated *in vivo* did not efficiently restore the PFC response in culture. A 1:1 cell mixture of normal SACs and normal SACs irradiated with UV light *in vitro* reconstituted the PFC response of G-10-passed normal spleen cells to the same level as that seen with an equal number of normal SACs alone. Mixing normal SACs with SACs from mice UV irradiated *in vivo* likewise reconstituted a PFC response only slightly lower than the response restored by the equivalent number of normal SACs alone. These findings are consistent with an absence of functional SACs in UV-treated mice rather than an actively inhibitory or toxic effect of the SACs in culture.

DISCUSSION

The experiments described above demonstrate that splenocytes from mice receiving whole-body UV irradiation do not make a normal primary PFC response *in vitro* to the soluble T-dependent antigen Tnp-GAT. Investigation of this phenomenon revealed that this impaired immune response resulted from a selective loss of APC function in the adherent spleen cell population of the UV-treated mice. Thus, SACs from UV-irradiated mice are unable to reconstitute a PFC response when added to G-10-passed normal splenocytes, whereas normal SACs, when added to G-10-passed splenocytes from UV-treated mice, do restore a PFC response. The effect of *in vivo* UV irradiation on the SAC population is indistinguishable functionally from the effect of *in vitro* UV irradiation of SACs from normal mice.

Previous work on whole-body UV irradiation of mice has shown these animals to have a diminished capacity to reject syngeneic UV-induced tumors (1, 2), has demonstrated that their splenocytes mount a depressed secondary cytotoxic response to syngeneic UV-induced tumors and to alloantigens (3), and has revealed diminished DTH responses to haptenated syngeneic cells (7). The evidence in DTH models (7) suggests that at least the APC is defective in UV-irradiated mice. The present studies confirm these general findings of depressed immunity in UV-treated mice and extends them to a humoral system in which the altered immunologic reactivity is reflected in decreased antibody-forming cell function. It also provides direct evidence pointing to a defect in APC function in UV-irradiated mice, in fact, suggesting that only the APC component of the immune system is significantly affected.

Furthermore, preliminary work in our laboratory has demonstrated that that GAT-primed T cells are not efficiently activated for proliferative responses by GAT-pulsed SACs from UV-irradiated mice, indicating that the APC defect(s) seen in DTH and humoral responses may be common to virtually all immune responses involving adherent cell triggering of T lymphocytes.

An understanding of how whole-body UV irradiation of a mouse selectively acts on this single splenic cell population, the APC, must take into account some well-described properties of UV light in biologic systems. Due to the short wavelength of UV light and the scatter of this light on entering the epidermis, its penetration into the mouse is but a few millimeters. Thus a direct effect of externally delivered UV light on the spleen is not possible. Toews *et al.* (6) have recently demonstrated the exquisite sensitivity of Langerhans cells to UV irradiation, raising the possibility of a generalized susceptibility of specialized macrophages and macrophage precursors to damage by UV light. Given that Langerhans cells may pick up antigen in the skin and traffic into draining lymphatics (14), a plausible explanation for the effects of UV irradiation on splenic SACs may be that UV light damages in the skin a significant number of Langerhans cells, which eventually migrate to the spleen to become nonfunctional APCs.

UV light has also been shown to affect formed elements of the blood as they circulate superficially in the extensive dermal and epidermal circulation (15). An attractive explanation for the SAC abnormality we have demonstrated is that Ia-bearing monocytic precursors to splenic APCs are damaged by UV irradiation in the dermal–epidermal circulation before eventually homing to the spleen. Indirect evidence supporting this proposed mechanism has emerged from various experimental systems. Six days after the last exposure to UV irradiation, morphologically identifiable Langerhans cells are again seen

in the skin of mice, and by 15 days they are present in normal density (6). Six days of UV irradiation are necessary before haptenated SACs from these mice lose their sensitizing potential. Haptenated SACs from UV-irradiated mice regain partial sensitizing potential for DTH by the sixth day after the last exposure to UV irradiation and appear to sensitize normally by the tenth day (unpublished observation). These findings imply that APC turnover time is approximately 1 week and that APCs are derived from what are presumably circulating precursors.

A final, less likely, explanation for the abnormality demonstrated in the SACs of whole-body UV-irradiated mice is that a humor or toxin is released in the skin upon exposure to UV light and acts at a distance on a select cell population in the spleen.

While we have demonstrated in this study a functional abnormality in APCs, we have not addressed the question of precisely which of their activities has been abrogated. Adherent cells have been shown in many *in vitro* systems to have both a nonspecific culture supportive function and an important role in antigen presentation. Reducing agents, such as 2-mercaptoethanol, and macrophage or fibroblast supernatants have been shown to restore PFC responses to particulate antigens in nonadherent responder splenocyte populations (16–18), perhaps through nonspecifically enhancing lymphocyte viability and blastogenesis. However, cell mixing experiments (unpublished observations) and *in vivo* studies suggest that such general culture effects are not the basis for the APC defect. An effect on the ability of antigen-bearing adherent cells to activate T lymphocytes therefore seems likely. Many studies have demonstrated the importance of Ia-positive macrophages in triggering T-cell responses (19, 20). This triggering, which involves both antigen and Ia recognition by the T cell, may also require an activating signal from the macrophage. UV irradiation might disturb APC function either by interfering with antigen uptake or surface display in the context of Ia or by preventing elaboration of a lymphocyte-activating substance without directly preventing antigen recognition.

A final issue to be clarified is the role played by suppressor T cells in the diminished immune responses of UV-irradiated mice. Just as the inability to reject UV-induced tumors has been shown in some systems to be related to circulating UV-induced suppressor T cells (21), and UV-irradiated mice primed with haptenated SACs from UV-treated mice give rise to hapten-specific suppressor T cells that abrogate DTH reactivity (7), the lack of *in vitro* PFC responses we found may well reflect the induction during culture of T suppressor cells in addition to a simple lack of APC stimulation of helper T-cell or B-cell function. Antigen presentation *in vitro* in the absence of adherent cells has been shown to induce a population of antigen-specific T suppressor cells (11). Use of external UV irradiation to alter a splenic population of APCs may allow further study of this phenomenon, perhaps helping us arrive at a more precise definition of the events that lead to the activation of helper and suppressor T-cell populations.

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