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LOSS OF Ia-BEARING SPLENIC ADHERENT CELLS AFTER WHOLE BODY ULTRAVIOLET IRRADIATION[1]

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Daily UV irradiation of mice results in a marked decrease in the antigen-presenting capability of SAC from these mice after 1 wk of UV exposure. To directly examine this cell population, we developed a technique for purifying SAC that involves passing mouse splenocytes through two cycles of glass adherence with an intervening incubation on rabbit anti-mouse Ig-coated dishes. This method yields an adherent cell population contaminated by less than 2% sIg-bearing and 2% Thy-1.2-bearing cells. SAC from externally UV irradiated mice prepared by this method, when pulsed with antigen, activate primed T cells to proliferate much less efficiently than SAC from normal mice. Both the proportion and absolute number of Ia-bearing cells in this purified SAC population from UV irradiated mice are considerably smaller than that seen in similarly prepared populations from normal mice. Studies looking at other characteristics of splenic macrophages, that is, polystyrene particle ingestion, antibody-coated SRBC rosetting (Fc receptor activity), esterase staining, and both light and electron microscopic morphology, showed no detectable effects of external UV irradiation. Previous adjuvant immunization was shown to override functional defects elicited by external UV irradiation. This demonstration of a UV irradiation induced selective loss of Ia bearing splenic adherent cells and the functional consequences of this loss provide further evidence for the importance of Ia-bearing accessory cells in antigen presentation of T dependent antigens, and provides insight into the origin of the immunologic defects induced by whole body UV irradiation.

The activation of primed T lymphocytes requires antigen presentation by an adherent cell population. Rosenthal and Shevach (1) first demonstrated that effective accessory cell-lymphocyte interactions occur only when these cells share genes linked to the major histocompatibility complex. This observation was extended by the demonstration that murine T lymphocytes are activated to proliferate only when antigen is presented to them on accessory cells that bear I region controlled determinants (2, 3). Others have demonstrated a similar requirement for Ia-bearing adherent cells in the triggering of T helper activity for *in vitro* plaque-forming cell (PFC) responses (4, 5) and in the elicitation of delayed-type hypersensitivity (DTH)[2] reactions *in vivo* (6).

We have recently found that the loss of immune reactivity in mice exposed to ultraviolet (UV) radiation is associated with a defect in splenic adherent cell (SAC) function. Thus, trinitrophenyl (TNP)-conjugated SAC from UV-irradiated mice are incapable of efficiently sensitizing UV-irradiated mice for DTH reactivity. However, immunization with TNP-conjugated SAC from normal mice primes UV-irradiated mice for a DTH response, indicating that the UV exposure does not markedly affect the T cells responsible for the DTH response (7). Further, SAC from UV-irradiated mice are unable to reconstitute a primary *in vitro* PFC response to a soluble T dependent antigen when added to adherent cell-depleted splenocytes (8). However, normal SAC can restore a PFC response when added to non-adherent splenocytes from UV irradiated animals, providing evidence that the T and B cells in the spleens of UV irradiated mice are functionally intact. Finally, it has been shown that the defect in this model is the antigen presenting ability of the SAC, since primed T cells are not efficiently activated to proliferate by antigen-pulsed or hapten-coupled SAC from mice exposed to UV radiation (9).

The present studies were carried out to determine the underlying cellular basis for the loss of SAC antigen-presenting function in UV-irradiated mice. These experiments demonstrate that UV irradiation of mice results in a marked loss of Ia-bearing splenic adherent cells without detectably affecting other non-Ia-bearing adherent cell (macrophage) populations. This loss of Ia bearing SAC correlates with the observed functional defect of these cells and is consistent with the established role of Ia molecules in the adherent cell dependent activation of T lymphocytes.

MATERIALS AND METHODS

Mice. Female BALB/c mice were purchased from the Charles River Breeding Colonies, Wilmington, MA. Female B10.BR mice were purchased from The Jackson Laboratory, Bar Harbor, ME. They were maintained in our animal facilities and used at 8 to 20 wk of age.

Ultraviolet (UV) light source and treatment. Ultraviolet irradiation was carried out by using a bank of 6 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 I.L. 443 Phototherapy Radiometer (International

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[2] Abbreviations used in this paper: DTH, delayed-type hypersensitivity; GAT, L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}; SAC, splenic adherent cells.

Light, Inc., Newburyport, MA) was 1.2 to 1.4 mJ/cm^2/sec. *In vivo* UV irradiation was accomplished by shaving the backs of mice and exposing them under the bank of tubes ½ hr daily for 6 days or as noted in the experimental protocol; the mice were used for experiments the day after the last exposure.

Splenic adherent cell preparation: splenic adherent cells (SAC). Adherent spleen cells were prepared as previously described (8) with the following modifications: plastic Falcon 3003 petri dishes (Becton, Dickinson Co., Oxnard, CA) were used rather than glass dishes and the adherent cells were used without overnight incubation.

Purified splenic adherent cells. A first cycle of glass adherent spleen cells was prepared exactly as previously described (8). This SAC population was then depleted of B cells by specific adherence to plastic Falcon 1005 Petri dishes coated with 1 mg/ml affinity-purified rabbit anti-mouse immunoglobulin (Ig) (35 to 50 × 10^6 cells per dish). After incubation on ice for 1 hr, the cell population that did not adhere to the Ig-coated dishes was harvested. Cells were resuspended in RPMI 1640 medium (M.A. Bioproducts, Walkersville, MD) supplemented with 10% heat-inactivated fetal calf serum (FCS) (M.A. Bioproducts), 100 units/ml penicillin, 100 μg/ml streptomycin, and 10 mM HEPES buffer solution, transferred to 100 mm glass Petri dishes at approximately 10 × 10^6 cells per dish and incubated overnight at 37°C in a 5% CO_2 atmosphere. The adherent cells were then recovered from the plates by using cold lidocaine solution and a rubber policeman. For comparative studies of SAC from normal and UV-irradiated mice, purified SAC were made starting with equal spleen cell numbers from the two groups of donors. No consistent pattern of absolute SAC yield was observed during these experiments.

Antigen pulsing of SAC. The random copolymer L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT), m.w. 30 to 50,000 (Vega Biochemicals, Tucson, AZ) was dissolved in saline containing 1% Na_2CO_3, adjusted to pH 7.0 and sterilized by filtration. SAC were pulsed with antigen by incubation for 60 min at 37°C at 10^7 cells/ml in RPMI-10% FCS containing 100 μg/ml GAT, followed by 4 washes with large volumes of cold medium.

Immunization. Animals were immunized subcutaneously in four flank sites with 100 μg of soluble GAT or in the footpads and tail with 0.2 ml of an emulsion containing 100 μg GAT in complete Freund's adjuvant with 1 mg/ml of killed *Mycobacterium tuberculosis* (H37Ra) organisms (Difco Laboratories, Detroit, MI). For priming and boosting with adjuvant alone, an equal volume of complete Freund's adjuvant containing 0.5 mg/ml *Mycobacterium tuberculosis* (CFA) (Difco Laboratories) was emulsified with saline and 0.2 ml was injected i.p.

T cell proliferation. T cell proliferation assays were carried out with nylon wool purified primed lymph node T cells exactly as previously described (9). Peripheral lymphocytes were isolated from blood by Ficoll-Hypaque gradient centrifugation and cultured with 2 to 5 μg/ml concanavalin A (Miles-Yeda, Ltd., Israel) at 2 × 10^5 cells per well in round bottomed microtiter plates (Linbro Scientific, Inc., Hamden, CN). Mitogen-stimulated cultures were pulsed with tritiated thymidine on the second day of culture.

Immunofluorescence staining. Purified SAC were prepared as described above and pelleted in 10 x 75 mm plastic tubes (Falcon 2058) at 4 × 10^5 cells/tube. Indirect immunofluorescence for I-A^k and Thy 1.2 utilized monoclonal antibodies 10.2.16 (anti-I-A^k, Salk Institute) and HO 13.4 (anti-Thy 1.2, Salk Institute) prepared from hybridoma supernatants and used at 200 μg/ml. Each cell pellet was incubated for 30 min at 4°C with 15 μl of antibody solution followed by washing and staining at 4°C with rabbit Fab'$_2$ anti-mouse Ig conjugated with fluorosceinisothiocyanate (FITC) (gift of Dr. Emil Unanue, Dept. of Pathology, Harvard Medical School). Direct immunofluorescence for Ig utilized this FITC anti-Ig alone. Cells viewed under a Leitz fluorescence microscope fitted with FITC filters were scored as positive if a continuous ring of bright membrane fluorescence was present.

Tests of macrophage function. Purified SAC were prepared as described above except that the second adherence step was performed in flat bottom tissue culture wells (Linbro Scientific, Inc.) containing a glass coverslip in the bottom of each well. Staining for nonspecific esterase was performed on the coverslip adherent cells as described by Koski *et al.* (10). Antibody-coated sheep red blood cell (SRBC) rosetting was quantitated by adding 2% SRBC (Colorado Serum Co., Denver, CO) coated with anti-SRBC 7S globulin (gift of Dr. Emil Unanue, Dept. of Pathology, Harvard Medical School) to each Linbro well; after 30 min at 37°C, coverslips were gently washed in phosphate-buffered saline (PBS) and fixed with 2% glutaraldehyde in PBS. Adherent cells with three or more SRBC attached as viewed under phase microscopy were scored as positive. Phagocytosis was evaluated by adding 10^7 polystyrene particles (1.1 μ) (The Dow Chemical Co., Indianapolis, IN) to each Linbro well during the final overnight incubation of adherent cells. After 18 hr at 37°C, coverslips were gently washed with PBS, fixed with 1% paraformaldehyde in PBS, and viewed under phase microscopy. Cells containing clusters of intracytoplasmic latex beads were scored as positive.

Internal labelling of SDS-PAGE. Purified SAC prepared as above were cultured overnight in methionine-free MEM containing 10%, dialyzed FCS and 250 μCi/ml ^{35}S methionine (New England Nuclear, Boston, MA). Adherent and nonadherent cells were separated and each was solubilized with 0.5% NP-40 in PBS containing 0.001 M PMSF. Labelled Ig was precipitated with rabbit anti-mouse Ig followed by protein A-Sepharose (Sigma Chemical Co., St. Louis, MO) and labeled I-A^k was precipitated with monoclonal antibody 10.2.16 (anti-I-A^k, Salk Institute) followed by protein A-Sepharose. Precipitates were washed extensively and dissolved in SDS sample buffer (11). SDS-PAGE and autoradiography were performed as described by Studier (12) with the use of a discontinuous pH buffer system (11).

RESULTS

Loss of splenic Ia-bearing adherent cells after UV irradiation. Based on the known importance of Ia$^+$ adherent cells in antigen presentation to T lymphocytes, we hypothesized that the defective antigen-presenting function of SAC from UV-irradiated mice might be explained by a reduced frequency of Ia$^+$ cells in the SAC population. To test this prediction directly, an adherent cell population free of contaminating Ia$^+$ B cells was prepared by modifying the technique described by Cowing *et al.* (13). The splenic cell population that adhered to glass during a 2-hr incubation was harvested and transferred to plastic Petri dishes coated with affinity-purified rabbit anti-mouse immunoglobulin. The cell population that did not adhere to these dishes was then transferred to glass Petri dishes and incubated overnight. The cells that adhered to glass after this overnight incubation were harvested. Immunofluorescent examination of macrophage populations prepared in this manner from normal mice showed only 0 to 2% staining positively for surface Ig, 1 to 2% staining positively for Thy 1.2 antigenic determinants, and 42 to 51% staining positively for Ia determinants.

To characterize this cell population further and verify minimal B cell contamination, these splenic cells were analyzed for their capacity to synthesize immunoglobulin and I-A. Both the glass adherent and nonadherent populations were incubated with ^{35}S-methionine, solubilized with NP-40, and immunoprecipitated with monoclonal Ig2a anti-I-A^k or rabbit anti-mouse Ig followed by protein A-Sepharose. SDS polyacrylamide gel electrophoresis was then performed on the precipitates. As shown in Figure 1, the nonadherent cell population actively synthesized both Ig and I-A, whereas the glass adherent population synthesized only I-A. This provided further confirmation that these glass adherent splenic cells were free of significant B cell contamination.

Finally, these adherent cells were shown to have antigen-presenting function when pulsed with soluble antigen and used to stimulate antigen-primed T cells in a proliferation assay. In fact, this purified SAC population demonstrated a greater than 10-fold enrichment for antigen-presenting capability as compared to 2-hr plastic adherent cells (data not shown).

The previously described functional defect seen in the 2-hr plastic adherent splenic cell population from UV irradiated mice was also seen with highly purified SAC. As shown in Table I, GAT primed nylon wool purified T cells are activated to proliferate much less efficiently by purified SAC from UV irradiated mice than by purified SAC from normal animals.

Such purified SAC from normal B10.BR mice and B10.BR mice exposed to UV irradiation for ½ hr daily for 6 consecutive days were then stained for surface I-A with a monoclonal anti-I-A^k sandwiched with an FITC-rabbit Fab'$_2$ anti-mouse Ig reagent. As shown in Table II, both the proportion and absolute number of I-A bearing cells in the purified SAC population from UV irradiated mice are markedly smaller than that seen in the purified SAC population of normal mice. This finding is consistent with the diminished antigen presentation capacity of SAC from UV irradiated mice.

To determine if the loss of Ia$^+$ cells in the purified SAC population of UV irradiated mice is selective, the purified SAC populations were studied for several typical macrophage characteristics. As detailed in Table III, polystyrene particle ingestion, antibody-coated SRBC rosetting (Fc receptor activity), and nonspecific esterase staining characteristics are the same for the purified SAC population of UV irradiated mice and that of normal mice. Further, scanning and transmission electron microscopic studies revealed no morphologic differences between these two cell populations. Thus, external UV irradiation of mice results within a week in a selective decrease in the number of Ia bearing SAC without detectably affecting several typical characteristics of the normal splenic macrophage population.

How does UV radiation cause a depletion of Ia bearing SAC? We then sought to determine how external UV irradiation exerts its selective systemic effect on the Ia bearing adherent cells of the mouse spleen. It has been previously demonstrated that SAC prepared from spleens of normal mice and then UV irradiated *in vitro* cannot reconstitute a primary *in vitro* PFC response to a soluble T dependent antigen when added to adherent cell depleted splenocytes (8). Because it cannot penetrate deeper than the mouse epidermis (14), a direct effect of externally delivered UV radiation on cells of the spleen is not possible. We concluded that the seemingly selective effect on a

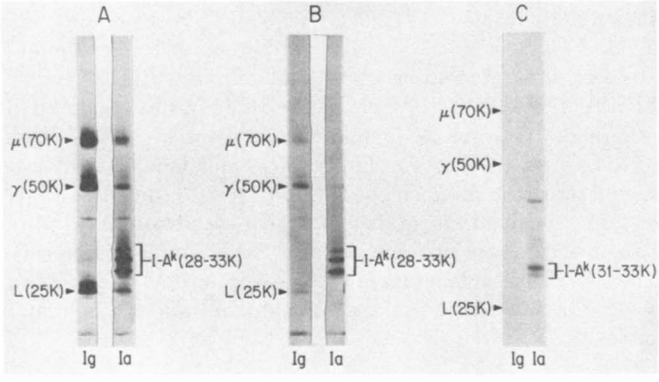

Figure 1. Sodium dodecyl sulfate polyacrylamide slab gel electrophoresis of ^{35}S methionine-labeled Ig and I-A^k molecules. After internal labeling of *A*, nonadherent cells, *B*, adherent cells (SAC), and *C*, purified SAC followed by NP-40 lysis, Ig and I-A^k molecules were precipitated as described in *Materials and Methods.* Acrylamide slab gels (5 to 15%) were run at 7.5 watts, impregnated with PPO-DMSO, and dried. Autoradiographs on Kodak X-Omat film are shown after 4 days (*A*), 10 days (*B*), and 14 days (*C*). Apparent M_R of bands indicated as determined by marker proteins.

TABLE I

Antigen-pulsed purified SAC from UV-irradiated mice do not efficiently activate primed T cells for proliferative responses

Responding Cells	Pulsed SAC	Source of SAC	
		Normal	*In vivo* UV treated
GAT/CFA primed	15×10^3 GAT pulsed	11,900 ± 2,600[a]	3,200 ± 1,800
	7.5×10^3	7,600 ± 2,900	2,100 ± 1,100

[a] Value represents ^{3}H-thymidine incorporation in wells containing pulsed SAC minus incorporation in wells containing an equal number of unpulsed SAC ± SE.

TABLE II

Surface I-A bearing cells are decreased in purified SAC populations from UV-irradiated mice

	I-A^k	
	Expt. I[a]	Expt. II[b]
	%	%
B10.BR: normal[c]	51	42
B10.BR: UV-irradiated 30 min daily for 6 days	12	9

[a] Total purified SAC recovered: normal, 3×10^6 cells (1.5×10^6 I-A^k positive); UV irradiated, 1×10^6 cells (0.12×10^6 I-A^k positive).

[b] Total purified SAC recovered: normal, 1.5×10^6 cells (0.63×10^6 I-A^k positive); UV irradiated, 3×10^6 cells (0.26×10^6 I-A^k positive).

[c] Purified SAC were prepared as described in *Materials and Methods*, stained, and examined by microscopy for immunofluorescence. Staining for I-A^k was determined by subtracting FITC-α mouse Fab^2-positive cells from anti-I-A^k + FITC-α mouse Fab^2-positive cells.

TABLE III

Purified SAC populations from UV-irradiated mice demonstrate normal polystyrene particle ingestion, antibody-coated SRBC rosettes, and esterase staining[a]

	Normal	*In Vivo* UV Treated
	%	%
Polystyrene particle (1.1 μ) ingestion	66	64
Antibody-coated SRBC rosettes	58	58
Esterase staining	67	70

[a] See *Materials and Methods* for techniques used in SAC purification and assessing macrophage function.

spleen cell population is not merely an early manifestation of a generalized bone marrow depression in UV irradiated mice on the basis of normal peripheral white blood cell numbers, normal differential white blood cell counts, and normal morphology of bone marrow aspirates in UV irradiated animals (data not shown).

The possibility that UV radiation might damage the circulating precursors to the splenic Ia bearing adherent cells while in the dermal-epidermal circulation was addressed in two experimental systems. Con A-stimulated proliferation of lymphocytes, a response known to be dependent on Ia^+ adherent cells (15), was not depressed when mice were exposed to UV radiation for 1 hr before collection of the peripheral blood cells used as the responding population, even when the addition of Con A to the cells was delayed until 24 hr after initiating the cultures to permit delayed loss of antigen presenting cell function (data not shown). We were also able to stimulate the proliferation of primed T cells with antigen pulsed peripheral lymphoid cells from mice primed and boosted with CFA. But no reproducible depression in the antigen-presenting capability of these peripheral lymphoid cells was observed if the mice were exposed to UV radiation before bleeding (data not shown).

Although we were unable to demonstrate an immediate effect of external UV irradiation on circulating antigen presenting cell function, we cannot conclusively rule it out as the mechanism by which UV irradiation induces a loss of Ia bearing SAC. To gain further information on this question, we chose to study the time course of the loss of SAC antigen-presenting function after external UV irradiation of mice. BALB/c mice were exposed for ½ hr daily to UV irradiation for 2, 4, 6, or 8 days, all groups receiving their final exposure on the day before SAC preparation. SAC from each group were prepared by a one-step adherence to plastic dishes, pulsed with GAT, and used at 6×10^4 SAC/well to stimulate proliferative responses of nylon wool purified GAT-primed T cells. As shown in Figure 2, a dramatic decrease in SAC antigen-presenting capacity occurs after the mice have been exposed to UV radiation daily for 1 wk.

In a further attempt to clarify the mechanism by which UV irradiation of mice affects Ia bearing SAC, we asked if these effects of UV irradiation are absolute, or whether they could be overcome by agents that stimulate reticuloendothelial function.

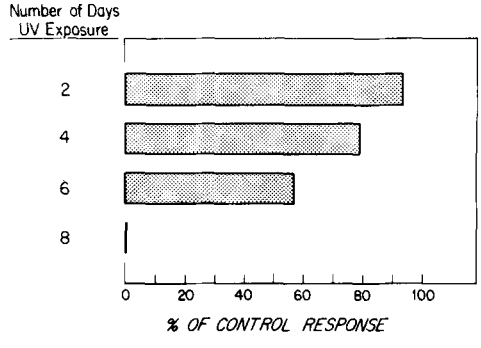

Figure 2. Antigen-pulsed splenic adherent cells from mice externally UV irradiated for 8 days do not activate primed T cells for proliferative responses. SAC were prepared by a single plastic adherence from mice exposed to external UV irradiation for 2, 4, 6, or 8 days. They were then pulsed with GAT and used at 6×10^4 SAC/well to stimulate a proliferative response in a population of nylon wool-purified GAT primed T cells. The differences between the proliferative response obtained by using antigen-pulsed and nonpulsed SAC (Δcpm) were determined for each group. The data are expressed as % control response where the control response is Δcpm with the use of SAC from mice that received no UV irradiation.

As shown in Table IV, the GAT specific proliferative response of T cells from UV irradiated mice primed with soluble GAT is half that seen in normal mice similarly primed. Priming and boosting mice i.p. with CFA before UV irradiation overrides this functional defect.

DISCUSSION

We have demonstrated that daily UV irradiation of mice results in a marked decrease in the antigen presenting capability of SAC from these mice after 1 wk of UV exposure. To directly examine this cell population, we developed a technique for purifying SAC that involves passing mouse splenocytes through two cycles of glass adherence with an intervening incubation on rabbit anti-mouse Ig coated dishes. This method yields an adherent cell population contaminated by less than 2% sIg bearing and 2% Thy 1.2 bearing cells. SAC from externally UV irradiated mice prepared by this method, when pulsed with antigen, activate primed T cells to proliferate much less efficiently than SAC from normal mice. Both the proportion and absolute number of Ia bearing cells in this purified SAC population from UV irradiated mice are considerably smaller than that seen in similarly prepared populations from normal mice. Studies looking at other characteristics of splenic macrophages, that is, polystyrene particle ingestion, antibody-coated SRBC rosetting (Fc receptor activity), esterase staining, and both light and electron microscopic morphology, showed no detectable effects of external UV irradiation.

We cannot absolutely rule out the possibility that UV irradiation of mice simply causes a loss of the normal adherence properties of a subpopulation of splenic accessory cells. This could result in an apparent loss of Ia bearing accessory cells, since our purification technique is based on adherence properties of these cells. However, because no differences were found between splenic adherent cell populations of UV irradiated and normal mice in studying general macrophage characteristics, a UV radiation-induced loss of adherence would have to be selective for the Ia^+ accessory cell subpopulation, an unlikely possibility. Furthermore, since a functional loss of SAC has been demonstrated in spleen populations not subjected to separation after external UV irradiation (8), this loss of adherence would have to be associated with loss of the antigen-presenting ability of the Ia bearing accessory cells.

Previous studies of Ia antigens on SACs have been done using A.TH anti-A.TL antibodies. Use of a monoclonal anti-I-A reagent in the present studies could conceivably result in our underestimating the true number of Ia bearing cells in the SAC population in that monoclonal antibodies detect only a single antigenic determinant. Our finding of 42 to 51% Ia bearing cells in a normal purified SAC population, however, agrees closely

TABLE IV

Adjuvant preimmunization overrides depression of priming for T cell proliferation in UV-irradiated mice[a]

Preimmunization of Responders	Antigen Added	Responding Cell Donor	
		Normal	*In vivo* UV irradiated
None	100 μg/ml GAT	20,000 ± 1,900	10,200 ± 1,000
CFA alone[b]	100 μg/ml GAT	18,500 ± 2,900	16,300 ± 1,300

[a] The data are expressed as the differences between the ^{3}H-thymidine incorporation in wells containing antigen and those without antigen ± SE.

[b] Mice primed i.p. with 0.2 ml TNP-KLH/CFA 4 wk before GAT priming and boosted with CFA alone (1:1 suspension with saline) 1 wk before GAT priming.

with studies done by using an A.TH anti-A.TL reagent (13). Further, the Ia antigen recognized by this monoclonal antibody appears to be the determinant on the adherent cell critical for presentation of GAT, the antigen used in functional experiments in the present studies, in that this antibody fully blocks the proliferation of primed T cells from H-2^k mice to GAT (unpublished observation).

The loss of functional splenic antigen presenting cells and drop in number of Ia bearing SAC in mice after 1 wk of daily exposure to UV radiation may provide insight into the kinetics of SAC turnover. These data are consistent with the possibility that the Ia bearing SAC is a short-lived cell population that is replaced within at least 7 days by stem cell precursors. UV irradiation may interfere with the normal replacement of Ia^+ adherent cells in the spleen by these precursors. This possibility is supported by several other studies. Longo and Schwartz (16) demonstrated the total loss of antigen presenting ability of mouse spleen or peritoneal washout cells 4 days after 900 to 950 R total body x-irradiation; restoration of that function in splenic populations was accomplished with bone marrow reconstitution of these animals. Beller, Scher, and Unanue (personal communication) have provided further evidence supporting this hypothesis. They have shown that although 70 to 80% of *L. monocytogenes*-induced peritoneal exudate macrophages in mice bear surface Ia determinants for a 2 to 3 wk period under normal conditions, only about 10% of these cells express Ia determinants by 4 days after sublethal total body x-irradiation. Bone marrow reconstitution of these mice restores the high proportion of Ia^+ macrophages to this exudate population. Steinman *et al.* (17) has also described a distinct Ia bearing splenic adherent cell, the dendritic cell, that turns over at least every 8 to 11 days, four times faster than the general splenic macrophage pool.

We have as yet been unable to explain the mechanism by which UV irradiation of mice affects their Ia bearing SAC. Our finding that previous adjuvant priming can override functional defects elicited by external UV irradiation may shed some light on this issue. The adjuvant may call forth an increased number of splenic Ia^+ adherent cell precursors from the bone marrow or activate resident adherent cells to maintain Ia expression. This concept is not without precedent given the finding that adjuvants have been shown to increase the representation of Ia bearing adherent cells in mouse peritoneal exudates (K. Behbehani and E. Unanue, personal communication). Studies are now underway to explore these possibilities.

The depletion of Ia bearing antigen presenting cells from spleens in UV irradiated mice may help in clarifying the events leading to T cell mediated suppression. UV irradiated mice primed with haptenated SAC from UV-treated mice give rise to hapten specific suppressor T cells that abrogate DTH reactivity (7). Further, antigen presentation *in vitro* in the absence of adherent cells has been shown to induce a population of antigen specific T suppressor cells (18). We are currently pursuing studies to define further the nature of the cellular interactions that initiate T cell suppressor pathways using the UV irradiated mouse as a model system.

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