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Immunology

Impairment of antigen-presenting cell function by ultraviolet radiation

(suppressor cells/ultraviolet carcinogenesis)

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ABSTRACT **UV light irradiation of BALB/c mice was found to result in impairment of antigen-presenting cell function. Adherent trinitrophenyl-derivatized cells from the peritoneal exudate cell population or the spleen of UV-treated donors could not induce hapten-specific delayed hypersensitivity responses in UV-irradiated syngeneic mice, whereas adherent trinitrophenyl-derivatized cells from normal mice were able to do so. The failure to induce immunity in UV-treated mice by utilizing UV-treated adherent antigen-presenting cells was associated with the development of antigen-specific suppressor T cells. The implication of these results for UV-induced carcinogenesis is discussed.**

UV light irradiation plays an immunologic role in the induction of certain murine fibrosarcomas and squamous carcinomas (1–3) in addition to its carcinogenic action. UV-irradiated mice are unable to reject UV-induced tumors that are highly antigenic and that are rejected by normal syngeneic recipients. Recent work suggests that the lack of tumor rejection is due to the presence of suppressor T lymphocytes (T_s) in the lymphoid organs of UV-irradiated mice (4–6). The origin of T_s and their relationship to UV irradiation is incompletely understood. However, a recent suggestion that processing of antigen is deficient early in the course of UV irradiation (4) raised the possibility that T_s induction might result from a defect in the afferent limb of the immune response. To resolve this issue, we have evaluated the effects of UV irradiation on cellular immunity to hapten conjugates of syngeneic cells and have investigated the mechanism of this process.

There is substantial evidence that antigen-presenting cells (APCs) participate critically in the generation of immune responses (7). Indeed, the APC has been proposed as the site of expression of immune response (*Ir*) genes in some antigenic systems (8–10). Other studies have revealed that *H-2* genetic identity between the antigen-coupled APC and the recipient is required for maximal induction (11, 12) and transfer of T cell-dependent delayed type hypersensitivity (DTH) responses. Moreover, similar genetic identity at the *I* region of *H-2* between APC and T helper cells is required for maximal priming effects (13–15).

Previous work has shown that UV irradiation of guinea pig skin rendered it unreactive to topical immunizing doses of dinitrochlorobenzene (16). Moreover, UV irradiation of a skin test site in a human subject markedly decreased the development of the local DTH reaction (17). Furthermore, recent studies in which lymphoid cells were exposed to UV irradiation *in vitro* (18–20) demonstrated that UV treatment rendered them incapable of serving as allogeneic stimulators in a mixed leukocyte reaction despite the fact that the *H-2* encoded antigens on such cells were not discernibly affected or diminished. Still more recently, human lymphoid cells exposed to UV irradiation *in vitro* were reported not to efficiently stimulate or present hapten to antigen-reactive cells in an *in vitro* proliferative assay (21).

Therefore, the available information suggests that certain critical capacities of immunocytes to participate in cell–cell interactions are impaired as a consequence of UV irradiation. The experiments presented herein were designed to investigate the effect of UV irradiation on APC function in the cellular immune response to hapten-derivatized cells.

To examine directly the APC function in UV-irradiated mice, we used adherent-hapten-derivatized lymphoid cells of BALB/c mice to induce T-cell-dependent contact sensitivity or DTH reactions *in vivo*. Our experiments indicate that the UV irradiation *in vivo* causes a significant defect in effective antigen presentation. The APC defect not only prevents stimulation of a normal response but also favors the development of antigen-specific T_s which further dampen reactivity.

MATERIALS AND METHODS

Mice. BALB/c mice 8–10 weeks of age were obtained from Jackson Laboratory or were supplied by the Frederick Cancer Research Center Animal Production Facility (Frederick, MD).

UV Irradiation. The UV light source was a bank of six unfiltered Westinghouse FS40 lamps that delivered an average dose of 2.0 J/m^2 per sec over the wavelength range 280–340 nm, which included 80% of the total energy output of the lamps. The mice were housed, five per cage, on a shelf 20 cm below the lamps, and the cage order was systematically rotated before each treatment to compensate for the uneven lamp output along the shelf. The dorsal hair of the mice was removed with electric clippers once per week. Shaven animals received three 1-hr exposures per week for 4 consecutive weeks.

Antigens. 2,4,6-Trinitrobenzenesulfonic acid, 2,4,6-trinitrochlorobenzene (TNCB), and 1-fluoro-2,4-dinitrobenzene (DNFB) were purchased from Eastman. Spleen cells coupled with 2,4,6-trinitrobenzenesulfonic acid were prepared as described (13). Briefly, cell suspensions of spleens were made by gently grinding the organs in chilled Hanks' buffered saline with glass homogenizers. Erythrocytes were lysed by treatment with 0.83% ammonium chloride in Tris buffer (pH 7.6). The cell suspensions were adjusted to 5×10^7/ml and mixed with equal volumes of 10 mM trinitrobenzenesulfonic acid in Hanks' buffered saline adjusted finally to pH 7.4 with 1 M NaOH. Cells were incubated at room temperature for 30 min with gentle stirring. The cell suspensions were washed with Hanks' solution once, with 10 mM glycylglycine in Hanks' solution at pH 8.0, and then three times with chilled Hanks' solution to remove free trinitrobenzenesulfonic acid.

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Abbreviations: APC, antigen-presenting cell; DNFB, 1-fluoro-2,4-dinitrobenzene; DTH, delayed type hypersensitivity; TNCB, 2,4,6-trinitrochlorobenzene; T_s, suppressor T cells; PEC, peritoneal exudate cells; Tnp, trinitrophenyl.

Preparation of Purified Adherent Cells. Lidocaine hydrochloride (Xylocaine) was a gift from Astra Pharmaceutical (Worcester, MA). Eagle's minimal essential medium containing 10% fetal calf serum, 10 mM Hepes (Microbiological Associates, Bethesda, MD), and 4.2 mM sodium bicarbonate was used as a medium. Unimmunized BALB/c mice received 1.5 ml of a 10% protease peptone solution, and the resulting peritoneal exudate cells (PEC) were obtained by peritoneal lavage 3 days later (13). Alternatively, spleen cells were obtained from normal mice. PEC or spleen cells were plated on plastic petri dishes at 10^8/10 ml of medium. After incubation for 1 hr at 37°C, the nonadherent cells were removed by two washes with chilled medium. The procedure was repeated twice and the adherent cells (macrophages) were dislodged by incubation in the medium containing 12 mM lidocaine followed by four or five washes with chilled medium. Greater than 90% of the adherent cells from PEC populations displayed morphological characteristics of macrophages whereas a more variable number of splenic adherent cells had these features.

Immunization and Challenge. To induce DTH to trinitrophenyl (Tnp)-derivatized cells, mice were injected subcutaneously at two separate sites on the dorsal skin with the numbers of Tnp-conjugated cells indicated in each experiment. Contact sensitivity was obtained by the application of 7% TNCB in acetone/olive oil, 4:1 (vol/vol) to the shaved abdomens in a total volume of 100 μl. In some experiments, 0.5% DNFB was applied to the shaved abdomen on two successive days (15). For challenge, 1% TNCB or 0.2% DNFB in olive oil was applied to the left ear. The extent of the ear swelling was expressed as the difference in thickness between the challenged left and untreated right ear measured with an engineer's micrometer 24 hr after challenge. In the case of footpad challenge, 10^7 Tnp-derivatized normal BALB/c splenocytes were injected in 25 μl into the footpad (14). Foot thickness measurements were made 24 hr later and are expressed in units of 10^{-4} in. Both the ear swelling and footpad swelling reactions have been shown to be T-cell-dependent classical DTH reactivities (13, 14).

Transfer of Immune or Suppressor Cells. Five days after immunization with Tnp-cells of different types, the draining lymph nodes and spleens were removed. Single-cell suspensions were prepared as above. To transfer immunity, 5×10^7 immune lymph node cells were injected intravenously into normal age-matched syngeneic recipients. Recipients of immune lymph node cells were challenged within 1 hr of transfer and assayed for DTH 24 hr later. To detect suppressor T cells, 5×10^7 splenocytes were transferred to syngeneic recipients which were then immunized with 7% TNCB or 0.5% DNFB and challenged 5 days later.

Anti-Thy 1.2 and Complement Treatment. AKR anti-Thy 1.2 antiserum and complement treatment were performed precisely as described (13, 14).

Statistical Analysis. The statistical significance of the results obtained was calculated by using Student's *t* test as computed by the Wang programmable computer. The arithmetic mean and SEM are indicated.

RESULTS

Tnp-Derivatized Adherent Cells Induce T-Cell-Dependent Responses. We have previously shown that the subcutaneous injection of Tnp-derivatized normal spleen cells into syngeneic recipients stimulates hapten-specific DTH. Now we have evaluated the APC function of adherent Tnp-derivatized cells from the spleens or PEC of normal or age-matched UV-treated BALB/c mice. These Tnp-conjugated cells were injected into normal syngeneic recipients which were challenged 5 days later. Tnp-derivatized adherent spleen or PEC cells from normal mice induced highly significant DTH responses in syngeneic recipients (Table 1). Similarly conjugated nonadherent spleen or PEC cells from such mice, at the cell numbers used, did not sensitize. Moreover, we consistently found that adherent spleen cells from UV-treated mice (group VIII) were less efficient in inducing Tnp-specific reactivities than were cells from normal mice (group VII). Although equal numbers of Tnp-derivatized adherent UV-treated spleen cells were less efficient (50–60%), larger numbers of such cells induced responses of magnitude equivalent to those stimulated by smaller number of normal cells (data not shown).

Failure of Tnp-Derivatized UV-Treated Adherent Cells to Sensitize UV-Irradiated Recipients. To define further the effect of UV irradiation, hapten-conjugated APC obtained from either UV-treated or normal mice were used to sensitize normal or UV-treated recipient mice. DTH reactivity was assessed by injecting Tnp-derivatized splenocytes obtained from normal BALB/c mice into the footpad 5 days after immunization. As shown in Fig. 1, 10^7 Tnp-conjugated normal spleen adherent cells induced significant DTH responses in normal or UV-treated recipients. However, Tnp-derivatized APC obtained from UV-treated mice induced weaker responses in normal mice and no discernible reaction in UV-treated recipients. Whereas adherent Tnp-coupled normal splenocytes comprise APC capable of inducing sensitivity in normal or UV-irradiated recipients, hapten-derivatized cells from UV-irradiated recipients do not sensitize to the same degree.

Adoptive Transfer of Tnp-Specific DTH Reactivity from Normal or UV-Irradiated Mice. We next evaluated whether immune lymphocytes could be demonstrated in normal or UV-irradiated mice immunized with UV-treated or normal APCs. Five days after immunization with 10^7 Tnp-derivatized spleen adherent cells obtained from normal or UV-irradiated mice, the lymph nodes were removed and single-cell suspensions prepared. Immune lymphocytes (5×10^7) were transferred into normal syngeneic recipients which were challenged within 1 hr of transfer and assayed for DTH responses 24 hr later. Lymph node lymphocytes from normal or UV-treated mice immunized with APC from normal mice were capable of adoptively transferring Tnp-specific reactivity (Fig. 2). Furthermore, despite developing slightly weaker DTH responses, lymphocytes from normal mice immunized with APC

Table 1. Induction of T-cell-dependent DTH responses with Tnp-derivatized APCs

Group	Immunizing cell*	Cell number	Mean ± SEM†	*P*‡
		Exp. 1		
I	Spleen	3×10^7	48.0 ± 3.0	<0.001
II	Spleen adh	10^6	51.0 ± 1.0	<0.001
III	Spleen nonadh	10^6	21.0 ± 2.0	NS
IV	PEC adh	10^6	60.0 ± 1.8	<0.001
V	PEC nonadh	10^6	20.0 ± 2.0	NS
VI	—	—	18.0 ± 1.0	—
		Exp. 2		
VII	Spleen adh	10^7	57.0 ± 4.0	<0.001
VIII	Spleen adh UV	10^7	30.5 ± 6.0	<0.05
IX	—	—	4.0 ± 1.0	—

Challenge was application of 1% TNCB in olive oil to the ear on day 5. Measurement with an engineer's caliper was performed 24 hr later.

* Adherent (adh) or nonadherent (nonadh) spleen cells or PEC were derivatized with 10 mM trinitrobenzenesulfonic acid.

† The difference between the challenged ear and the unchallenged ear is recorded in units of 10^{-4} inch.

‡ *P* values are for the means of experimental versus control groups and are calculated by two-tailed Student *t* test. NS, not significant.

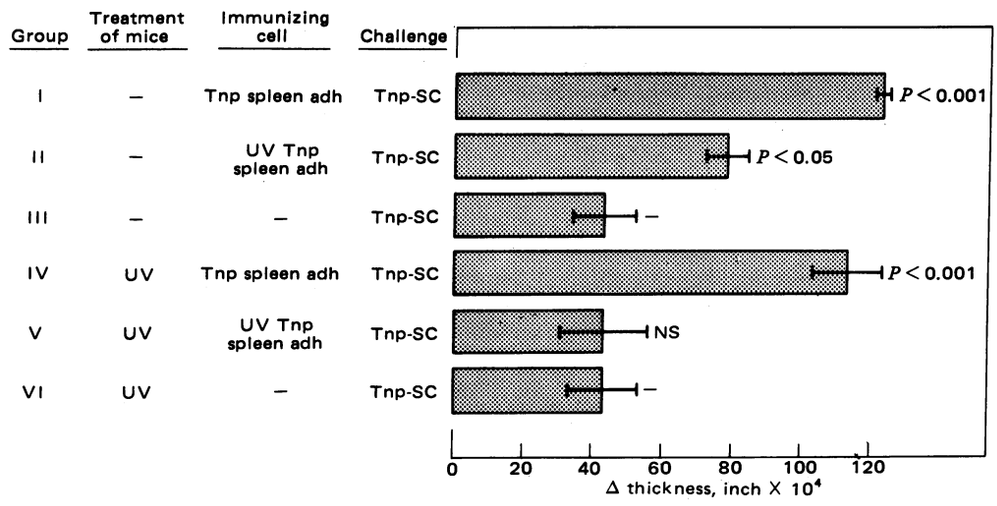

FIG. 1. Induction of DTH by adherent Tnp-derivatized cells from normal or UV-treated BALB/c mice. Adherent cells $(10)^7$ from normal or UV-irradiated mice were Tnp-derivatized and injected subcutaneously into normal or UV treated recipients. Five days later mice were challenged by injection of 10^7 normal Tnp-coupled splenocytes (Tnp-SC) into the footpad. DTH responses were assayed 24 hr later and are depicted as the difference in thickness between the challenged and unchallenged footpads. Control mice (groups III and VI) only received 10^7 Tnp-coupled splenocytes in the footpads. Groups consisted of 5–10 mice. Means ± SEM are shown.

from UV-irradiated mice were still capable of transferring discernible although somewhat decreased sensitivity to normal recipients. However, the injection of Tnp-derivatized APC from UV-treated donors into UV-treated mice did not result in the induction of transferable immunity.

UV-Irradiation-Induced Deficiency in Sensitization is Associated with Stimulation of Suppressor Cells. The failure of UV-treated mice to develop sensitivity after immunization with Tnp-coupled APC from UV-treated donors was investigated further in the following series of experiments. Normal or UV-irradiated recipients were immunized with 10^7 Tnp-derivatized adherent cells from normal or UV-treated donors. To search for the presence of suppressor cells, 5 days later the spleens of the mice were removed and 5×10^7 spleen cells were transferred to normal syngeneic recipients. These recipient mice were then sensitized by the application of 7% TNCB to the skin. Five days later the mice were challenged. Splenocytes from UV-irradiated mice immunized with APC from normal donors had no discernible suppressive effect on the generation of Tnp-specific DTH responses (Fig. 3). However, splenocytes from UV-irradiated mice immunized with Tnp-conjugated APC obtained from UV-treated donors markedly suppressed the DTH response of syngeneic recipients.

Suppression Is Antigen Specific and Is Mediated by T Cells. To identify the phenotype and specificity of the suppressor cells, splenocytes from mice immunized as described in the preceding section were treated with AKR anti-Thy 1.2 serum or normal AKR mouse serum and guinea pig complement prior to adoptive transfer. Groups of recipient mice were immunized with 0.5% DNFB in lieu of TNCB as a control for specificity. The suppressor cells induced by immunization of UV-irradiated mice with Tnp-coupled APC from UV-treated donors are T cells as shown by their sensitivity to treatment with anti-Thy 1.2 and complement (Fig. 4). Furthermore, the suppression induced is Tnp specific because reactivity to the closely related contactant DNFB was not affected by the suppressor cells transferred.

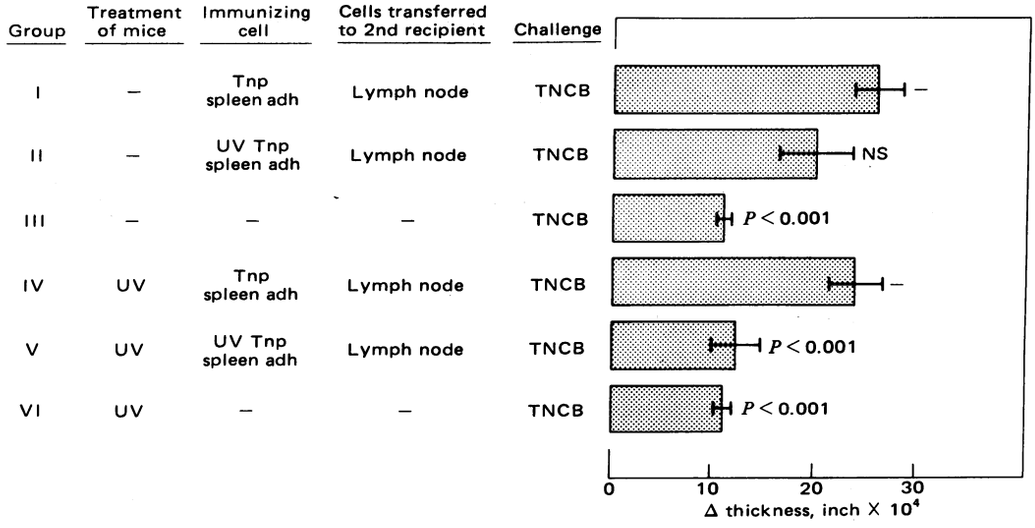

FIG. 2. Generation of transferable T-cell-dependent immunity after immunization with Tnp-derivatized adherent cells. Normal or UV-irradiated BALB/c mice were immunized with 10^7 Tnp-derivatized adherent spleen cells obtained from normal or UV-treated donors. Five days after immunization, lymph node cells were obtained and 5×10^7 lymphocytes were transferred intravenously to syngeneic recipients. Recipients were challenged, within 1 hr of transfer, by the application of 10 μl of 1% TNCB to the ear. DTH responses were evaluated 24 hr after challenge, and differences between challenged and unchallenged ear thickness are indicated. Challenge only with 1% TNCB (groups III and VI) represents negative controls. Groups consisted of five mice. Means ± SEM are shown.

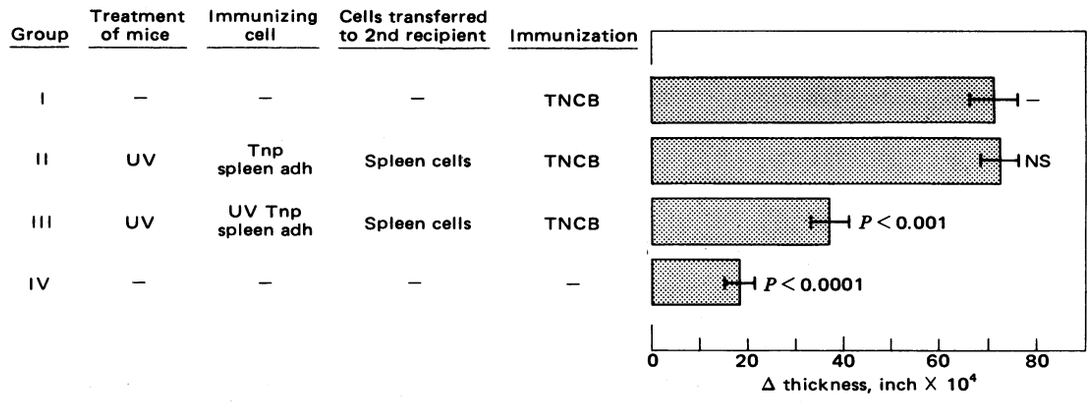

FIG. 3. Suppressor cells are generated in UV-treated recipients immunized with Tnp-derivatized adherent cells obtained from UV-irradiated donors. UV-treated BALB/c mice were immunized subcutaneously with 10^7 Tnp-derivatized adherent cells obtained from normal or UV-treated donors. Five to 7 days later, 5×10^7 splenocytes were transferred to normal syngeneic recipients. Recipients of splenocytes or normal BALB/c mice used as positive controls (group I) were immunized with 7% TNCB applied epicutaneously on the shaved abdomen. Five days later, mice were challenged on their ears with 1% TNCB; responses were measured 24 hr later. Negative controls consisted of challenge only (group IV). Means ± SEM are shown.

DISCUSSION

The results of our experiments designed to investigate the effect of UV irradiation on DTH reactivity indicate that Tnp-conjugated APC from mice irradiated with UV light lose their capacity to sensitize effectively. The UV-irradiated mice immunized with such cells develop antigen-specific suppressor T cells which further decrease hapten-specific DTH responses. Immunization with Tnp-derivatized APC from normal mice circumvents UV-induced defects in UV-treated recipients and stimulates DTH responses indistinguishable from those seen in normal mice. The APC defect in the UV-treated mice must be extensive in spite of the limited penetration of UV irradiation. This conclusion is based on the failure of Tnp-derivatized APC from UV-treated donors to sensitize such mice, whereas immunization of normal mice with such UV-treated Tnp-conjugated cells induced significant albeit decreased sensitivity. The decreased response observed in normal mice immunized with UV-treated Tnp-conjugated cells may indeed be due to the representation of antigen in normal animals by their own APC. In this regard, it should be noted that the cells from normal or UV-irradiated donors used for immunization were derivatized with equivalent amounts of hapten. If some APC in the UV-treated recipients of Tnp-conjugated cells from UV-treated mice could similarly represent antigens, we should have observed some significant level of reactivity after immunization with hapten-coupled APC from UV-treated mice. The failure to observe such a response indicates an extensive defect in antigen presentation in UV-irradiated mice.

The apparent defect in effective antigen presentation observed in the splenic adherent cells of UV-irradiated mice cannot be easily explained. The APCs in the skin, identified as Langerhans cells (22, 23), are probably the most susceptible targets of the UV irradiation and of the impairment of antigen presentation function. The apparent systemic effect of UV treatment as manifested in PEC or spleen APC may reflect the possible circulation of APC between central and peripheral areas. The existence of such a circulation of APC remains to be established experimentally. It is evident, nevertheless, that the

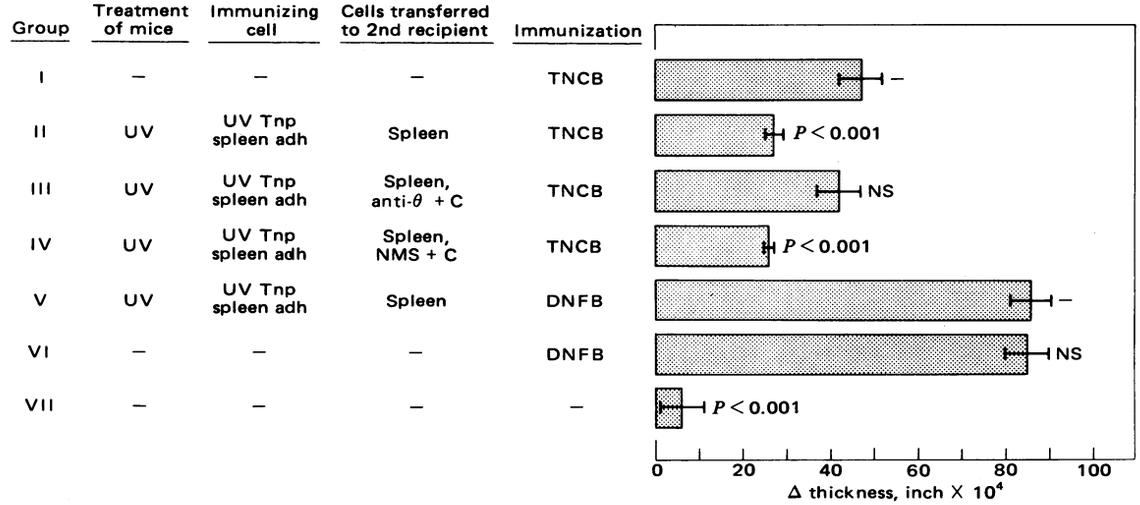

FIG. 4. Suppressor cells are T cells and are antigen specific. UV-treated mice immunized 5 days previously with 10^7 Tnp-coupled adherent cells obtained from UV-irradiated mice were donors of spleen cells; 5×10^7 spleen cells or 5×10^7 spleen cells after treatment with anti-Thy 1.2 antiserum and complement (anti-θ + C) or normal AKR serum and complement (NMS + C) were transferred intravenously to syngeneic recipients which were immunized with 7% TNCB. Another group of mice received 5×10^7 suppressor cells and were immunized with 0.5% DNFB epicutaneously. Positive control mice (groups I and VI) were immunized with the indicated antigen and received no cells. All mice were challenged with the homologous antigen 5 days later and assayed for DTH reactions 24 hr thereafter. Negative control for DNFB (group VII) received challenge only with 0.2% DNFB. Groups of five mice were used throughout. Means ± SEM are shown.

defect in antigen presentation, illustrated in these experiments, results in the activation of specific suppressor T cells which further decrease immune reactivity. This interpretation is in agreement with the numerous observations that associate the stimulation of specific suppressor T cells with inappropriate antigen presentation by APC (24, 25).

What is the target of the UV-irradiation effect on the APCs? It has been previously shown that mixed leukocyte reaction and *I* region-associated functions of lymphoid tissue cells are defective after UV irradiation of these cells *in vitro* (18–20). However, there is no evidence that the Ia molecules themselves are structurally or functionally affected by UV irradiation. The lesion may indeed result from the inability of UV-treated APCs to provide the required stimulus to the T lymphocyte with which it interacts specifically. It is further possible that this ineffective antigen presentation, occurring as a consequence of UV irradiation-induced functional changes, stimulates the generation of suppressor T cells rather than Lyt 1+ effector cells (12).

The effect of UV irradiation on APC may also explain in part the presence in UV-irradiated mice of suppressor T cells that inhibit the rejection of UV-induced tumors (2–4, 26). However, we must also account for the observation that such suppressor T cells are detected after UV treatment, previous to tumor implantations. To explain this finding, we propose that, in addition to the effect on antigen presentation documented in this study, UV irradiation may cause the generation of mutations in cells in the skin. Many new antigenic specificities would be generated as a result of such mutations; these antigens would be normally presented by functional APC. But, because antigen presentation is affected in an UV-treated animal in a manner that stimulates suppressor cells, many suppressor T cells specific for UV-induced antigens would be expected to be generated in UV-treated animals. Such antigens could also be expressed in UV-induced tumors. UV-induced tumors are indeed known to express both unique and shared determinants (26). Thus, suppressor T cells induced in UV-irradiated mice are probably antigen-reactive cells specific for determinants present on many UV-induced tumors.

It is also well established in other systems that, for effective tumor immunity, tumor antigens may be presented on *I-A*$^+$ APC (27). Therefore, it is probable that, upon tumor challenge in UV-treated recipients, UV-induced tumor antigens can only be presented in the skin on Langerhans cell-like APC (22, 23, 28) which have had their function impaired by UV irradiation. As a consequence, further stimulation of suppressor T cells, specific for unique and shared tumor antigens, would result. Such suppressor cells are of critical importance in limiting host response against these tumors, as demonstrated by recent experiments from this laboratory showing that the administration of anti-I-J antiserum, specific for suppressor T cells, can cause significant retardation in the growth of UV-induced tumors in UV-treated recipients (29).

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