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Suppression of lymphoproliferation by hapten-specific suppressor T lymphocytes from mice exposed to ultraviolet radiation

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Summary. Application of a contact-sensitizing agent to the skin of mice previously exposed to UV radiation at a different site results in the induction of hapten-specific suppressor T lymphocytes. When splenic lymphocytes from such mice were cultured with normal lymphocytes and hapten-conjugated splenic adherent cells, the primary proliferative response was suppressed. The cell responsible for the suppression *in vitro* was a T lymphocyte, and two signals were required for its induction, ultraviolet radiation and hapten sensitization. The T cell suppressing lymphoproliferation was specific for the hapten applied after UV radiation. The UV-induced T suppressor cell inhibited only primary lymphoproliferation; the response of lymphocytes from immunized mice was unaffected. The activity of the UV-induced suppressor cell was not affected by mitomycin C treatment. Thus, suppression of the primary proliferative response of lymphocytes to hapten-modified syngeneic cells *in vitro* correlates with *in vivo* suppression of contact hypersensitivity by these UV-induced suppressor cells. This suggests that the suppressor cells act by preventing the proliferation of hapten-specific responder clones. Use of this *in vitro* assay system should facilitate investigation of the characteristics of these cells and the mechanism by which these regulatory T lymphocytes inhibit contact sensitization.

Abbreviations: CHS, contact hypersensitivity; CML, cell-mediated lympholysis; DNFB, dinitrofluorobenzene; DTH, delayed hypersensitivity; EHAA, Eagle's Hanks' amino acid medium; MLR, mixed lymphocyte reaction; NR, non-irradiated controls; NSC, normal spleen cell; PUVA, 8-methoxypsoralen and UVA; TNCB, trinitrochlorobenzene; TNP-SAC, trinitrophenyl-conjugated splenic adherent cells; Tr, responder T lymphocytes; UV, ultraviolet; UVA, ultraviolet radiation above 320 nm; UVB, ultraviolet radiation between 280 and 320 nm.

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INTRODUCTION

Thymus-derived lymphocytes play a predominant role in the regulation of immune functions. In addition to T cells functioning as helper and effector cells for various immune funcions, certain subclasses of T cells act to suppress immune responses. Since the initial report of Gershon & Kondo (1970) of suppression of antibody synthesis by T cells, others have demonstrated the role of these cells in regulating such cellular immune responses as contact hypersensitivity (CHS) (Zembala & Asherson, 1974), mixed lymphocyte reaction (MLR) (Rich & Rich, 1974), cell-mediated lysis (CML) (Wagner *et al.*, 1976) and delayed type hypersensitivity (DTH) (Liew & Russel, 1980). The mechanism by which these regulatory cells exert their effect is complex and not completely understood. Evidence for immunoregulatory circuits involving various subsets of T cells and factors (Waltenbauch *et al.*, 1977), negative feedback (Eardly *et al.*, 1978) and

regulation by an idiotype anti-idiotype network (Jerne, 1974) have all been proposed to explain how T cells control immune responsiveness.

Immunoregulatory T lymphocytes have also been implicated in the suppression of certain immune responses by UV radiation. Among the many immunological alterations induced by UV radiation (Kripke, 1981) in mice is the systemic suppression of CHS (Jessup *et al.*, 1978) which is mediated by antigen-specific suppressor T lymphocytes (Noonan, DeFabo & Kripke, 1981a). Since UV radiation is a ubiquitous environmental factor, it is important to understand how it induces immunosuppression and how these antigen-specific T lymphocytes modulate immune responses at a cellular level. Until now, however, a major drawback to studying the characteristics and the mode of action of these suppressor T lymphocytes has been the lack of an *in vitro* assay. Previously, the activity of the UV-induced T suppressor cell was demonstrated through the use of passive transfer experiments (Kripke, Morison & Parrish, 1983) in which normal mice were first injected with the UV-induced suppressor T cells and then sensitized with a contact allergen such as trinitrochlorobenzene (TNCB). Suppression was detected by a reduction in the response of these mice to a contact allergen 6 days later.

The purpose of this study was to determine if an *in vitro* assay could be devised which would permit detection and characterization of these suppressor cells. Since the response suppressed *in vivo* was a primary response to a hapten applied epicutaneously, it seemed reasonable that a primary lymphoproliferative response to hapten-modified self, such as the one described by Shearer *et al.* (1975), might prove to be a suitable *in vitro* assay with which to test the activity of UV-induced suppressor T lymphocytes. The data presented in this paper demonstrate that UV-induced suppressor T lymphocytes do reduce a primary antigen-induced lymphoproliferation, and that this assay appears to be an accurate *in vitro* correlate for the suppression of CHS *in vivo*.

MATERIALS AND METHODS

Mice

Specific pathogen-free female C3H/HeNCr(MTV$^-$) mice were supplied by the NCI-Frederick Cancer Research Facility's Animal Production Area. The mice were 10 weeks old at the start of each experiment.

Measure of lymphoproliferation in vitro

An adaptation of the method of Shearer *et al.* (1975) was used. Spleen cells from normal mice were separated into non-adherent and adherent populations by a 1-hr incubation at 37° on large plastic petri-dishes. The non-adherent population was collected, and responder T lymphocytes (Tr) were purified by nylon wool filtration (Julius, Simpson & Herzenberg, 1973). The adherent population was removed from the plastic dish by a 15-min incubation with 12 mM lidocaine (Astra Pharmaceutical Products, Worcester, MA) and further fractionated by the use of 50% discontinuous Percoll (Sigma Chemical Co., St. Louis, MO) gradients (Glimicher *et al.*, 1981). The cells at the interface of the gradient were removed and washed, and 10^7 cells/ml were mixed with an equal volume of 20 mM trinitrobenzene sulphonic acid (TNBS), pH 7·0, for 30 min at 37°. The cells were washed and treated with 50 μg/ml mitomycin *c* per 10^7 cells. After another 30-min incubation at 37°, the cells were washed three times and resuspended in Eagle's Hanks' amino acid medium (EHAA), supplemented with 5×10^{-5} M 2-mercaptoethanol and 2·5% human AB serum. Generally, 4×10^5 trinitrophenyl-conjugated splenic adherent cells (TNP-SAC) were cultured with 4×10^5 T lymphocytes in complete EHAA for 5 days in round-bottomed, 96-well microtitre dishes. During the last 6 hr of the culture period, 1 μCi of tritiated thymidine (New England Nuclear, Boston, MA) was added to each well. The samples were harvested in an automated cell harvester, and the incorporated radioactivity was measured in an LS-350 liquid scintillation counter (Beckman Instruments, Fullerton, CA).

In some experiments, the response of immune T lymphocytes to TNP-SAC was measured. The T cells were obtained from the draining lymph nodes of mice that had been painted 4 days previously on the shaved abdomen with 100 μl of a 3% solution of TNCB in acetone. The lymph nodes were removed, single-cell suspensions were prepared, and the cells were fractionated on nylon wool columns (Julius *et al.*, 1973). The cells were resuspended in EHAA and cultured in round-bottomed microtitre dishes at a concentration of 4×10^5 cells/well.

Induction of suppressor cells by UV radiation

Two methods were used to induce suppressor cells—UVB radiation and PUVA. In the first, the dorsal fur was shaved using electrical hair clippers. The mice were then exposed to 3 hr of UVB radiation. The source of the UVB (280–320 nm) radiation was a bank

of six FS-40 sunlamps (Westinghouse, Bloomfield, NJ). The incident dose received by the mice was approximately 5 J/m^2/sec. In the second, mice were first injected intraperitoneally with 0·4 mg of the chemical photosensitizer 8-methoxypsoralen (Hoffman La Roche, Nutley, NJ) dissolved in a 2% gelatin solution. One hour later, these mice, whose dorsal fur was shaved, were exposed for 2 hr to long-wave UVA (above 320 nm, filtered through Mylar to remove wavelengths less than 315 nm). The incident dose of UVA received by each mouse was approximately 4–5 J/m^2/sec. Previous studies have demonstrated that PUVA treatment suppresses CHS and induces antigen-specific suppressor T lymphocytes (Kripke *et al.*, 1983). During the UV treatment, the animals' ears were shielded by electrical tape.

Five days after the UV treatment, the abdominal hair was removed with electrical hair clippers and the animals were sensitized with antigen by painting 100 μl of a 3% solution of TNCB in acetone on the abdominal skin. Six days after sensitization, the animals' ears were measured, and the mice were then challenged by the application of 5 μl of a 1% solution of TNCB in acetone on each surface of both ears. The next day, the ears were remeasured and the amount of swelling in response to the antigenic challenge was determined.

In certain experiments, the UV-treated mice were sensitized with 100 μl of a 3% solution of oxazolone (4-ethoxymethylene-2-phenyloxalo-5-one, Sigma Chemical Co.) in 95% ethanol applied epicutaneously. Six days later, these mice were challenged by the application of 5 μl of a 3% solution of oxazolone in olive oil on each surface of both ears. With both antigens, the change in ear swelling was determined by subtracting the ear swelling obtained in control mice which were not sensitized prior to challenge, from that obtained from animals which were. The spleens of the sensitized mice were removed and the T cells were isolated through the use of nylon wool columns (Julius *et al.*, 1973). Alternatively, the suppressor T cells were isolated by removing B cells and adherent cells through the use of an anti-immunoglobulin (Ig)-coated petri-dish (Mage, McHugh & Rothstein, 1977).

Passive transfer of the suppression induced by ultraviolet radiation

Either whole spleen cell populations, or nylon wool-purified T lymphocytes from mice that were treated with UV radiation and sensitized with antigen, were injected intravenously (i.v.) into normal recipients. Within 3 hr after 1×10^8 cells were injected i.v., the recipient animals were sensitized with antigen by being painted with 100 μl of a 3% TNCB in acetone solution on the abdominal skin. Six days later, the CHS response was measured by challenging the mice with TNCB in acetone on the ears, and the swelling in response to the antigen was determined one day later. The effect of the transfer of UV-induced suppressor cells upon the DTH was measured by injecting the hind footpads with 1×10^7 TNP-conjugated whole spleen cells and measuring footpad swelling 24 hr later.

Depletion of T lymphocytes from the suppressor cell population

Anti-T cell antibodies were used in a complement-dependent lysis. The first antibody was a rabbit anti-mouse brain-associated Thy 1 antiserum (Cederline Laboratories, Westbury, NY). The cell concentration was adjusted to 1×10^7/ml and incubated with a 1:40 dilution of the antiserum for 1 hr at 4°. The cells were washed and incubated with a 1:8 dilution of rabbit complement (Pel-Freeze, Rogers, AK) for 1 hr at 37°. The second reagent was a monoclonal anti Lyt 1 antibody (Becton-Dickinson, Mountain View, CA). An indirect cytotoxicity method was used. The monoclonal antibody was conjugated with the arsanilate hapten. The cell concentration was adjusted to 2×10^7 cells/0·1 ml, and a 1:100 dilution of antibody was added. The cells were washed and a 1:50 dilution of rabbit anti-arsanilate was added. After 30 min, the cells were washed and a 1:8 dilution of complement was incubated with the cells for 1 hr at 37°. The cells were washed and counted, and various members of cells were cultured with TNP-SAC and responder T lymphocytes in a 96-well microtitre dish.

RESULTS

In vivo suppression of DTH by UV-induced suppressor T cells

The ability of spleen cells from UV-treated and hapten sensitized mice to suppress CHS is well documented (Noonan *et al.*, 1981b; Kripke *et al.*, 1983). However, it was not clear whether the UV-induced suppressor cells were also capable of suppressing a DTH response. The data from an experiment to test the capability of these cells to suppress DTH are found in Table 1. In this experiment, the recipient mice were injected with suppressor cells and sensitized with TNCB as usual. Six days later, they were challenged in

Table 1. Passive transfer of suppression by lymphocytes from ultraviolet-treated mice

DTH*			CHS†		
Treatment of donors	Δfootpad swelling of recipients‡	% suppression§	Treatment of donors	Δear swelling of recipients‡	% suppression
TNCB	44·3±7·7¶	—	OX	14·0±1·0	-
UVB+TNCB	28·8±5·9	35 ($P<0·01$)**	UVB+OX	5·5±0·8	61 ($P<0·01$)
PUVA+TNCB	27·4±5·8	39 ($P<0·01$)	PUVA+OX	6·4±1·5	43 ($P<0·01$)

* Sensitizing dose, 100 μl of a 3% solution of TNCB in acetone; challenge, 1×10^7TNP-NSC in 50 μl, injected in hind footpad.

† Sensitizing dose, 100 μl of a 3% solution of oxazolone in olive oil; challenge, 10 μl/ear of 3% solution of oxazalone in olive oil.

‡ Response of sensitized animals minus response of unsensitized animals; footpad swelling in unsensitized mice = 10; ear swelling in unsensitized mice = 3 (5 mice per group).

§ Percentage suppression $= -\dfrac{\text{(response of recipients that received cells from UV-treated mice)}}{\text{(response of recipients that received cells from control mice)}}\times 100.$

¶ Units, cm $\times 10^{-3}$.

** P values determined by Student's t-test.

the hind footpads with 1×10^7 TNP-conjugated normal spleen cells (TNP-NSC). Twenty-four h later, the change in footpad swelling was determined by subtracting the background response exhibited by unsensitized animals from the response found in mice that were sensitized with TNCB. These data demonstrate that, in addition to suppressing CHS, the transfer of spleen cells from UVB- or PUVA-treated animals also suppressed DTH in the recipient mice.

In vitro suppression of lymphoproliferation by UV-induced suppressor T lymphocytes

An experiment was designed to examine the ability of UV-induced suppressor cells to regulate a primary

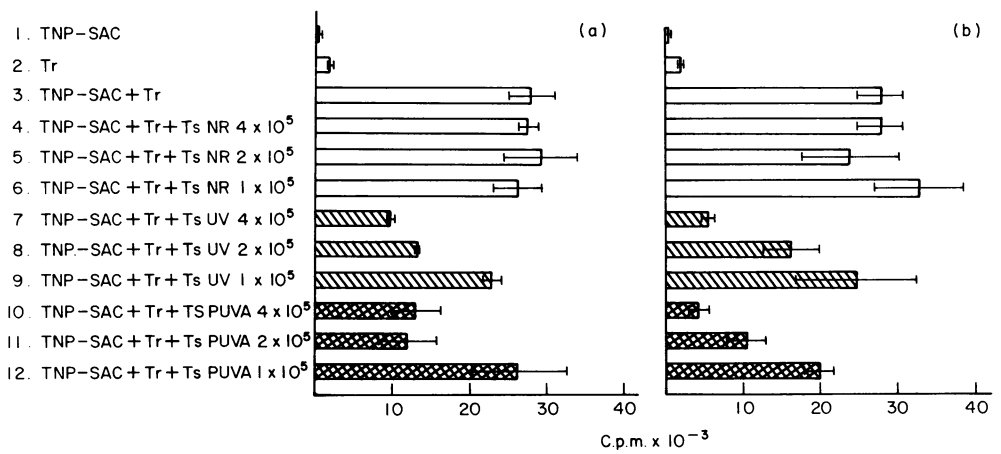

Figure 1. Suppression of primary lymphoproliferation by UV-induced suppressor cells. 4×10^5, 2×10^5 or 1×10^5 cells from NR-, UVB- or PUVA-treated mice were added to wells that contained 4×10^5 TNP-SAC and 4×10^5 nylon wool-purified spleen cells (Tr). In Fig. 1b, the NR (□), UVB (■) and PUVA (⊠) cells were treated with 50 μl mitomycin C prior to culture. The values shown are means from three experiments ± standard error of the mean. In both Figs 1a and 1b, the addition of 4×10^5 and 2×10^5 UVB- and PUVA-treated cells resulted in a significant immunosuppression ($P<0·01$, Student's t-test).

lymphoproliferation *in vitro*. Splenic adherent cells were conjugated with TNP and cocultured in 96-well dishes with nylon wool-purified T lymphocytes from unsensitized donors. To this culture system, various numbers of UV-induced suppressor cells were added. The results from this experiment are shown in Fig. 1a. The data demonstrate that T cells from either UVB- or PUVA-treated mice were capable of suppressing the lymphoproliferative response. The addition of 4×10^5 nylon wool-purified cells from UVB-treated mice resulted in a 65% suppression of the proliferation of 4×10^5 lymphocytes in response to TNP-SAC. When fewer suppressor cells were added, the level of suppression decreased (2×10^5 UV-treated cells added, Group 8, 53% suppression; 1×10^5 UV-treated cells added, Group 9, 19% suppression). The possibility that the suppression resulted from crowding was ruled out by the controls, in which T cells from animals that were not exposed to UV-radiation were added to the wells. As can be seen from Groups 4–6, the addition of NR T cells did not depress the response. Also, the viability of the cultures containing UVB-treated cells was similar to cultures containing NR cells (83% *vs* 86%). A similar suppression of primary lymphoproliferation was observed when cells from PUVA-treated mice were added to the wells. In this case, the addition of 4×10^5 wool-purified cells from PUVA-treated mice caused a 54% suppression of the proliferative response.

The data in Fig. 1b suggest that proliferation of the suppressor cells in culture was not required for the immunosuppression to occur. In this experiment, all three populations of cells, NR, UVB, and PUVA, were treated with mitomycin C prior to addition to the culture dishes. The data demonstrate that these cells were still capable of a dose-dependent suppression of primary lymphoproliferation. Thus it is apparent that treatment of donor mice with UVB or PUVA plus antigen induced a cell that is capable of suppressing a primary response to TNP-SAC *in vitro*.

The finding that lymphocytes from UV-treated animals were capable of suppressing the proliferative response (Fig. 1) suggests that this assay may serve as an *in vitro* correlate of suppression of CHS by UV-induced suppressor cells *in vivo*. However, it first was necessary to characterize further the *in vitro* suppression to ensure that the suppression of lymphoproliferation was mediated by the same UV-induced

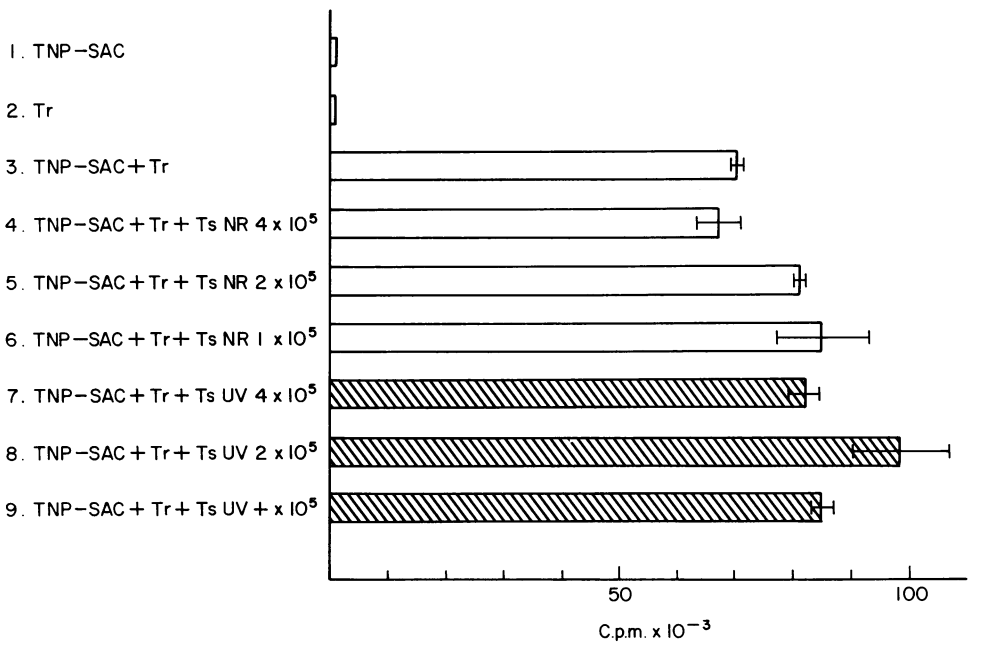

Figure 2. Failure of the cells from UV-treated mice to suppress the proliferation of primed T cells to TNP-SAC. 4×10^5 nylon wool-purified cells (Tr) from mice sensitized with TNCB were cultured with 4×10^5 TNP-SAC. Various numbers of NR (□) and UVB (■) cells were added. The data are expressed as the mean value from triplicate cultures ± one standard deviation.

cells that suppress CHS *in vivo*. Therefore, experiments were designed to determine whether the cells that suppress *in vitro* lymphoproliferation have characteristics similar to the UV-induced suppressor cells.

Failure of UV-induced cells to suppress a secondary immune response

The first characteristic examined was the ability of suppressor T cells from UVB-treated mice to suppress the secondary response. Previous *in vivo* studies established that the UV-induced suppressor T cells do not inhibit the elicitation of CHS in presensitized animals (Kripke *et al.*, 1983). The experiment was similar to the one illustrated in Fig. 1, except that the T responder cells were from mice that had been sensitized with TNCB 4 days previously. Results from this experiment are presented in Fig. 2. The data show that the suppressor T cells from UV-treated mice, which were capable of suppressing a primary response to TNP-SAC, had no effect upon TNP-SAC-induced proliferation of primed T responder lymphocytes.

Phenotype of the cells suppressing *in vitro* lymphoproliferation

Previous studies have demonstrated that the transfer of suppression of the CHS response was mediated by T lymphocytes (Noonan *et al.*, 1981a). This is consistent

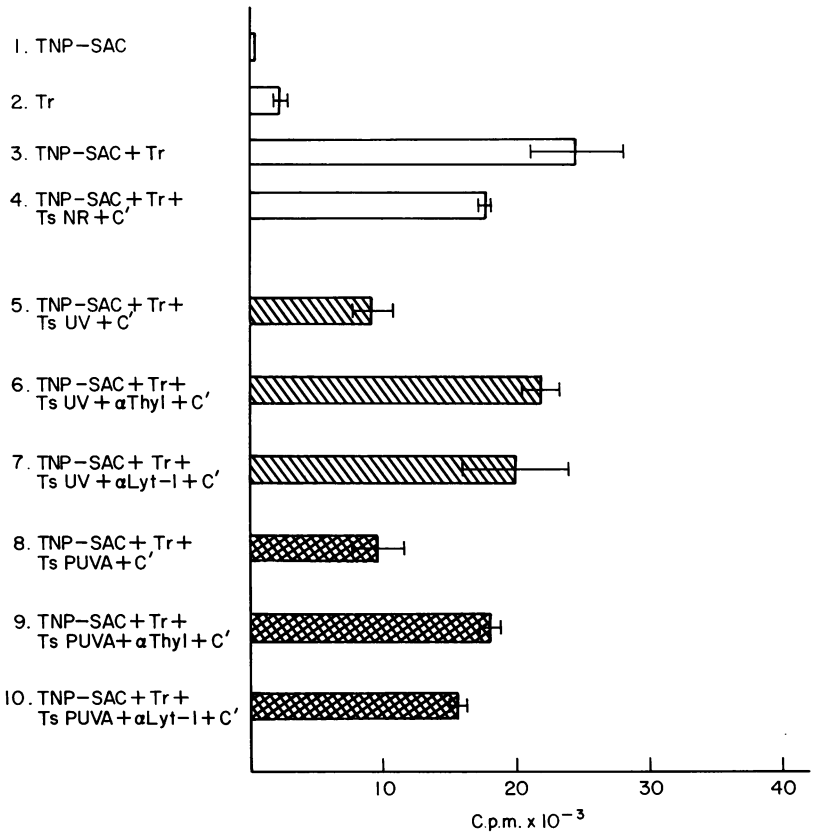

Figure 3. T lymphocytes are responsible for the immunosuppression mediated by cells from UV-treated mice. 4×10^5 TNP-SAC were cultured with 4×10^5 nylon wool-purified splenic lymphocytes (Tr). Suppressive lymphocytes from UVB- (■) and PUVA-treated (⊠) animals were treated with complement alone, anti-theta antisera plus complement, or a monoclonal anti-Lyt 1 plus anti-arsanilate and complement (see Materials and Methods) prior to coculture with Tr and TNP-SAC in a 96-well microtitre dish. The data are expressed as the mean value from triplicate cultures ± one standard error. The addition of complement-treated cells from UVB- and PUVA-treated mice resulted in a significant immunosuppression ($P < 0{\cdot}01$, Student's *t*-test). The addition of T-cell depleted UVB- and PUVA-treated cells did not result in a significant immunosuppression ($P > 0{\cdot}01$, Student's *t*-test).

Table 2. Antigen specificity of UV-induced T suppressor lymphocytes

TNP-SAC	Tr	Suppressor cells*	C.p.m. ± SD†	% suppression
+	−	−	441 ± 127	−
−	+	−	1866 ± 12	−
+	+	−	14,673 ± 3046	0
+	+	NR TNCB	26,867 ± 2373	0 (NS)‡
+	+	UV TNCB	9342 ± 719	36 ($P < 0.05$)
+	+	PUVA TNCB	5523 ± 2080	63 ($P < 0.01$)
+	+	UV OX	20,884 ± 2948	0 (NS)
+	+	PUVA OX	15,413 ± 4051	0 (NS)

* Animals received UV, PUVA or no radiation on day 0. Five days later, they were sensitized with either TNCB or oxazolone (OX). The spleens were removed 7 days later, and the T cells were purified by panning with anti-Ig-coated dishes.

† Means from triplicate cultures ± the standard deviation of the mean.

‡ P values determined by Student's t-test. NS = not significantly different from wells that contained TNP-SAC + Tr.

with the fact that the cells which suppress the primary lymphoproliferative response to TNP-SAC could be isolated by two methods that enrich for T lymphocytes: nylon wool purification (Julius *et al.*, 1973) or panning with anti-Ig-coated plates (Mage, McHugh & Rothstein, 1977). This suggests that the suppressor cells were probably T cells. In order to prove this point, we depleted the nylon wool-purified cell of T cells by using anti-T cell reagents in a complement-dependent lysis. Two reagents were used, a rabbit anti-mouse Thy 1.2 antiserum, and a monoclonal anti-Lyt 1 antibody. The data from an experiment in which the suppressor cells were depleted with these reagents are found in Fig. 3. In this experiment, the control cells and cells from UV- and PUVA-treated mice were all treated with complement alone. From Groups 5 and 8, it is evident that this treatment had no effect on the ability of 4×10^5 cells from UV- and PUVA-treated mice to suppress the response (60% and 61% suppression, respectively). Treatment of the NR cells with complement slightly diminished the response (Group 4). Treatment of the UVB cells with either the

Table 3. Two signals are required to induce the T suppressor lymphocyte

TNP-SAC	Tr	Source of suppressor cells*	C.p.m. ± SD†	% suppression
+	−	−	122 ± 95	−
−	+	−	3419 ± 634	−
+	+	−	24,420 ± 2990	0
+	+	NR + TNCB	23,067 ± 3094	6 (NS)‡
+	+	UV + TNCB	10,677 ± 1470	56 ($P < 0.01$)
+	+	PUVA + TNCB	9502 ± 2006	61 ($P < 0.01$)
+	+	UV	24,803 ± 51	0 (NS)
+	+	PUVA	37,919 ± 6060	0 (NS)

* Animals were irradiated on day 0. Five days after irradiation, they were shaved and sensitized with TNCB, or shaved only. After 7 days, suppressor cells were isolated from the spleens by nylon wool purification.

† Mean values from triplicate cultures ± the standard deviation of the mean.

‡ P values determined by Student's t-test. NS = not significantly different from wells that contained TNP-SAC + Tr.

anti-Thy 1 or the anti-Lyt 1 antisera resulted in a loss of suppression. Also, treatment of the PUVA-treated cells with anti-T-cell reagents reduced the suppression, and the response was similar to that observed when the complement-treated NR cells were added. Therefore, the data demonstrate that a T lymphocyte is required for the suppression of the proliferative response to TNP-SAC.

Antigen specificity of the suppressor T lymphocyte

The T suppressor cell involved in the UV-induced suppression of CHS is specific for the antigen that was used to sensitize the mice after UV treatment (Kripke *et al.*, 1983). An experiment was performed to see if the cell that suppresses the primary lymphoproliferation to TNP-SAC is also specific for the antigen used to sensitize the UV-treated mice. One half of the UVB- and PUVA-treated mice were sensitized with TNCB as usual, and the remaining mice were sensitized with 100 μl of a 3% solution of oxazolone. The suppressor cells were purified on anti-Ig-coated dishes (Mage *et al.*, 1977) and added to wells containing 4×10^5 TNP-SAC and 4×10^5 Tr cells. From the data in Table 2, it is evident that, to turn off the response, the suppressor cells must have been directed against the same antigenic determinants as the stimulator cells. When the suppressor cells were from UVB- or PUVA-treated animals which had been sensitized with TNCB, suppression of the response resulted. However, if the cells were from mice sensitized with oxazolone, there was no suppression. It was possible that the lack of suppression by the cells isolated from UV-treated oxazalone sensitized mice was a result of the inability to generate suppressor cells in these mice. However, if these cells were transferred to normal recipient mice which were subsequently sensitized with oxazalone, the CHS response was suppressed (Table 1). These results indicate that the reason why suppression of primary proliferation to TNP-SAC by cells from UV-treated mice sensitized with oxazolone was absent was not due to the failure to generate suppressor cells, but suggest that a requirement for antigenic specificity between stimulator and responder cells is required *in vitro*, as is the case with the *in vivo* suppression of CHS by cells from UV-treated mice (Kripke *et al.*, 1983).

Data from an experiment demonstrating that two stimuli are required to induce the suppressor cell are shown in Table 3. In this experiment, mice were first exposed to UVB or PUVA, and then sensitized with TNCB, or exposed to UVB or PUVA without sensitization with antigen. The T cells (4×10^5) from these mice were isolated and added to wells containing TNP-SAC (4×10^5) and Tr (4×10^5). The data illustrate that if the animals received both stimuli (UVB/PUVA, plus antigen), suppressor cells were induced (Groups 5 and 6). However, if the second stimulus (antigen) was not applied after the irradiation, suppressor cells were not induced in these animals (Groups 7 and 8). Therefore, it is apparent that, to induce suppressor cells which function *in vitro*, two signals are required, and the T cells induced are specific for the antigen used to sensitize the animal. This is analogous to the requirements for induction of the cells that block CHS *in vivo*.

DISCUSSION

The suppression of CHS in UV-treated mice is a prime example of the immunomodulatory effects of UV radiation. Previous studies have found that the cell responsible for the transfer of UV-induced suppression to normal recipients was an antigen-specific T lymphocyte (Noonan *et al.*, 1981a; Kripke *et al.*, 1983). This T cell suppressed the induction of the CHS response, but had no effect upon the effector cells. The data presented here show that the UV-induced suppressor of lymphoproliferation has similar characteristics. It suppresses a primary immune response (Fig. 1), but has no effect upon a secondary response (Fig. 2). It is antigen specific (Table 2), it may be isolated through the use of nylon wool columns or panning with anti-immunoglobulin-coated dishes (Table 2) and the results suggest the involvement of a T lymphocyte. This was confirmed by the loss of suppression when the cells from the UV-treated mice were treated with anti-Thy 1 antiserum and complement. In addition, the use of a monoclonal anti-Lyt 1 antibody, which has been reported to bind to the majority of peripheral T cells (Ledbetter *et al.*, 1980), also caused a loss of suppression. In addition, the requirement for two signals (Table 3) to induce the suppressor cell is also in agreement with earlier findings on the characterization of the *in vivo* UV-induced suppressor of CHS (Kripke *et al.*, 1983). The conclusion drawn from these experiments is that the suppression of lymphoproliferation by UV-induced antigen-specific T lymphocytes is an adequate reflection of the suppression of CHS *in vivo*, and may actually reflect the mode of action of these regulatory T lymphocytes.

Jensen (1983) has found that the transfer of suppressor cells from UV-irradiated and DNFB-sensitized

mice into normal recipients suppressed the development of a cytotoxic response in the recipient animals against hapten-modified spleen cells. She concluded that the UV-induced suppressor cells blocked the priming of cytotoxic cells in the recipient mice. These data agree with those presented in Figs 1 and 2, demonstrating that the UV-induced suppressor T lymphocytes inhibited a primary, but not a secondary, response.

There are certain similarities between the characteristics of the UV-induced suppressor cells and those generated by i.v. injection of DNBS (Moorhead, 1976). Both sets of suppressor cells block the induction of CHS and both affect lymphoproliferation. Also, it has been found that the use of supraoptimal doses of DNFB induces a T suppressor cell which inhibits the afferent limb of CHS (Sy, Miller & Claman, 1977). In addition, Thomas, Watkins & Asherson (1979) have demonstrated that multiple injections of picryl sulphonic acid induced a T lymphocyte which suppressed the afferent limb of CHS, interfered with blast transformation, and was antigen specific: characteristics all similar to those of the suppressor cell induced by UV. The significance of the similarities between these various suppressor cells induced by different routes of immunization and those induced by UV is not clear. Others have suggested that the defect in antigen presentation found in UV-irradiated animals (Green *et al.*, 1979; Noonan *et al.*, 1981b; Jensen, 1983) results in improper recognition of antigen and the generation of specific unresponsiveness in a manner similar to the induction of tolerance by i.v. injection of DNBS (Streilein, 1983). Further investigation into the characteristics and mechanism of action of UV-induced T suppressor cells should not only provide insight into how this environmental factor causes a selective systemic suppression of the immune response, but it should also contribute to a better understanding of immunoregulation in general.

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