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Suppressor lymphocytes induced by epicutaneous sensitization of UV-irradiated mice control multiple immunological pathways

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SUMMARY

The purpose of this study was to determine whether the formation of hapten-specific suppressor T lymphocytes induced by the epicutaneous sensitization of UV-irradiated mice could suppress other hapten-specific immune responses in addition to contact hypersensitivity (CHS). Suppressor cells were induced by applying trinitrochlorobenzene (TNCB) to the unexposed skin of mice irradiated several days earlier with 40 kJ/m^2 UVB (280–320 nm) radiation. Previous work demonstrated that the spleens of such animals contain Lyt-1$^+$, 2$^-$ T lymphocytes which prevent the induction of CHS to TNCB when transferred to normal mice, and inhibit proliferation of normal lymphocytes *in vitro* to TNP-modified syngeneic cells. These studies show that addition of T lymphocytes from UV-irradiated, TNCB-sensitized mice to cultures of normal lymphocytes and TNP-modified syngeneic cells inhibited the generation of TNP-specific cytotoxic T lymphocytes (CTL). The inhibition was dose-dependent and occurred only when the suppressor cells were present during the first 24 hr of culture. The suppressor cells had no effect on the activity of preformed CTL. In addition, injection of the suppressor lymphocytes into mice at the time of i.v. injection of TNP-modified sheep red blood cells (TNP-SRBC) reduced the number of direct plaque-forming cells against TNP, but had no effect on the production of antibody against SRBC. Cells that inhibited anti-TNP antibody formation were Thy-1$^+$, Lyt-1$^+$, 2$^-$. These results indicate that hapten-specific suppressor cells from UV-irradiated mice prevent the activation of several different hapten-specific immunological pathways.

INTRODUCTION

In addition to its carcinogenic activity, ultraviolet (UV) irradiation of mice contributes to the growth of skin cancers by altering the immune system (Kripke, 1981). Long before primary skin cancers can be detected in UV-irradiated mice, the animals develop suppressor T lymphocytes which prevent the immunological rejection of UV radiation-induced skin cancers (Kripke & Fisher, 1976; Spellman & Daynes, 1977). Attempts to determine the relationship between exposure to UV radiation and altered immune responsiveness revealed that the epicutaneous application of contact sensitizing haptens to the unexposed skin of irradiated mice also induced antigen-specific suppressor T lymphocytes (Noonan, DeFabo & Kripke, 1981).

Abbreviations: CHS, contact hypersensitivity; CTL, cytotoxic T lymphocytes; E:T ratio, effector:target cell ratio; HRBC, horse red blood cells; NR, non-UV-irradiated control; NSC, normal spleen cells; PFC, plaque-forming cells; S:E ratio, suppressor:effector cell ratio; SRBC, sheep red blood cells; TNBS, 2,4,6-trinitrobenzene sulphonic acid; TNCB, 2,4,6-trinitrochlorobenzene; TNP-NSC, trinitrophenyl-modified normal spleen cells; UV Ts, ultraviolet radiation-induced suppressor T lymphocytes.

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This systemic effect of UV radiation is highly selective, however, because many other immune responses are unaffected. For example, allograft rejection (Kripke *et al.*, 1977) and the production of antibodies to sheep erythrocytes (Spellman, Woodward & Daynes, 1977) are unimpaired following exposure of mice to UV radiation.

The induction of hapten-specific suppressor T lymphocytes in UV-irradiated mice not only provides a system with which to study the activation of the suppressor pathway by UV irradiation, but it is also a novel model for investigating mechanisms of immune regulation. Recent studies indicate that, in addition to suppressing the induction of contact hypersensitivity (CHS) *in vitro*, the hapten-specific, UV-induced suppressor T lymphocytes (UV Ts) prevent the proliferation of normal lymphocytes *in vitro* in response to hapten-conjugated syngeneic cells (Ullrich, 1985). This finding suggested that the UV Ts exert their suppressive effect by blocking the proliferation of hapten-specific precursors. However, it is not clear whether only cells involved in CHS are affected, or whether other hapten-specific responses are inhibited as well. In order to answer this question, we tested the ability of the UV Ts to inhibit the generation of hapten-specific cytotoxic T lymphocytes (CTL) *in vitro* and the induction of anti-hapten antibodies following i.v. injection of TNP-coupled sheep red blood cells (TNP-SRBC). Since both

responses were suppressed, we also investigated whether i.v. immunization with TNP-SRBC would activate TNP-specific suppressor cells in UV-irradiated animals.

MATERIALS AND METHODS

Animals

Specific pathogen-free female C3H/HeNCr(MTV$^-$) mice were supplied by the National Cancer Institute, Frederick Cancer Research Facility Animal Production Area, Frederick, MD. The animals were between 10 and 12 weeks old at the beginning of each experiment.

Generation of suppressor cells

These methods have been published in detail elsewhere (Ullrich, 1985). Briefly, the animals were irradiated with 30–40 kJ/m^2 UVB (280–320 nm) during a single 3 hr exposure. Five days later, they were sensitized with antigen by the epicutaneous application of hapten (100 μl of a 3% solution of TNCB) to unirradiated skin. Six days later, their ears were measured with a micrometer (Swiss Precision Instruments, Los Angeles, CA) and each ear was painted with 10 μl of a 1% solution of TNCB. Twenty-four hours later, the ears were remeasured, and the specific ear swelling was determined. Control mice (NR) were sensitized and challenged as described above, but were not exposed to UV. If the CHS response was suppressed in the UV-irradiated mice, their spleens were removed, single cell suspensions were prepared, and the T lymphocytes were partially purified by passage over nylon-wool columns (Julius, Simpson & Herzenberg, 1973). Spleen cells from NR mice were treated similarly and used as a source of control cells in the subsequent assays.

Generation of cytotoxic T lymphocytes to hapten-modified self

A modification of the procedure of Shearer (1974) was used. Stimulator cells were prepared by conjugating normal spleen cells (NSC) with 20 mM trinitrobenzene sulphonic acid (TNBS). The cells were washed three times and cultured with an equal number (5×10^6) of normal spleen cells in RPMI-1640 medium supplemented with 10% fetal calf serum, 5×10^5 M 2-mercaptoethanol, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 10% sodium pyruvate, 10 mM Hepes buffer, 1 × vitamins and non-essential amino acids (Gibco, Grand Island, NY). After a 5-day culture period, the cells were harvested, and cytotoxicity was measured using a 4-hr ^{51}Cr-release assay. A TNP-modified, syngeneic, methylcholanthrene-induced fibrosarcoma, MCA-113, was used as the target cell. Various numbers of effector cells were cultured with 1×10^4 TNP-MCA-113 cells. The percent cytotoxicity displayed by each group was calculated as follows:

$$\% \text{ cytotoxicity} = \left(\frac{\text{experiment release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}}\right) \times 100.$$

Spontaneous release was measured after incubating the target cells without effectors. Total release was measured in wells to which a 1% solution of Triton X-100 had been added.

Depletion of lymphocyte subpopulations

Monoclonal antibodies specific for the murine thymus-derived antigen Thy-1.2 (Becton Dickinson Monoclonal Center, Mountain View, CA), and lymphocyte differentiation antigens Lyt-1.1 and Lyt-2.1 (New England Nuclear, Boston, MA), were used in the phenotyping studies. Cell suspensions containing 1×10^7 cells/ml were treated with a 1:300 dilution of anti-Thy-1.2 or a 1:10,000 dilution of anti-Lyt-1.1 or Lyt-2.1 for 30 min at 4°. The cells were washed three times and then treated with a 1:8 dilution of rabbit complement (Pel-Freeze, Rodgers, AK) at 37°. After a 1-hr incubation, the cells were washed three times in Hanks' balanced salt solution.

Determination of antibody production

The slide modification (Mishell & Dutton, 1967) of the Jerne & Nordin (1963) plaque assay was used to determine the number of plaque-forming cells (PFC) in the spleens of immunized mice. The mice were injected i.v. with 0·2 ml of a 1% solution (v/v) of TNP-conjugated sheep red blood cells (TNP-SRBC) (Rittenberg & Pratt, 1969). Five days later, the spleens of the mice were removed and the numbers of direct plaque-forming cells generated against SRBC, horse red blood cells (HRBC) and TNP-HRBC were determined.

Statistical analysis

The two-tailed Student's t-test was used to assess the significance of differences in CTL activity and numbers of PFC. All CTL data were converted into a normal distribution by an arcsine transformation before analysis by Student's t-test.

RESULTS

Suppression of the CTL response by UV Ts

The initial experiments were designed to determine the ability of UV Ts to suppress the generation of TNP-specific CTL *in vitro*. These CTL were specific for TNP since they lysed TNP-modified target cells but not unmodified ones (Fig. 1). Varying numbers of nylon wool-purified UV Ts were added to cultures containing NSC and hapten-modified stimulator cells. When UV Ts were added to the primary CTL generation cultures at the time of culture initiation, the TNP-specific CTL response was depressed (Fig. 1). The amount of suppression was directly related to the number of UV Ts added (Fig. 2). At the numbers of UV Ts employed, which yielded suppressor to effector (S:E) ratios of 1:1, 2:1 and 4:1, the observed suppression was 12%, 58% and 99% of the control response, respectively. In order to rule out the possibility that a crowding effect by the UV Ts was responsible for suppression of the CTL response, the same number of filler cells from non-irradiated, TNCB-sensitized mice (NR) was added to other culture wells. No significant diminution of the CTL response was noted at any of the S:E ratios employed. Even at the highest S:E ratio (4:1), the addition of NR lymphocytes had no effect upon the generation of a primary CTL response ($P > 0{\cdot}5$ at each S:E ratio).

We have also added mitomycin c-treated normal spleen cells or T cells from unsensitized animals to control for the effect of crowding. In neither case was the CTL response suppressed (data not shown). However, because it has been demonstrated that a supraoptimal dose of hapten can induce suppressor cells (Sy, Miller & Claman, 1977), we have chosen to use lymphocytes from non-irradiated, but hapten-sensitized, animals to ensure that the suppression observed is not due to the inadvertent

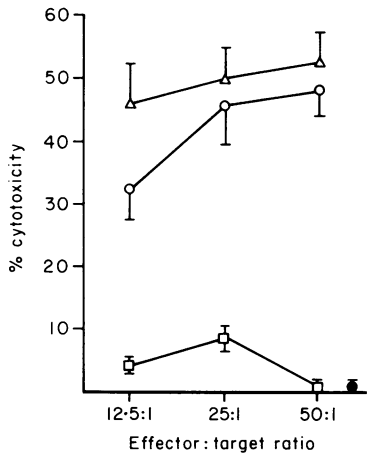

Figure 1. Suppression of CTL generation by UV Ts. Suppressor cells (UV Ts) from UV-irradiated, TNCB-sensitized mice were added to the primary CTL culture at the time of initiation. Effector cells were tested 5 days later for cytotoxicity against TNP-modified MCA-113 at effector: target cell ratios of 12·5:1, 25:1, and 50:1 in a ^{51}Cr-release assay. The number of UV Ts cells added was four times the number of responder cells present in the culture. (●) CTL response against unmodified target cells, which was identical at all of the E:T ratios; (○——○) TNP-specific CTL response generated in the absence of UV Ts cells; (□——□) CTL response generated in the presence of UV Ts; (△——△) CTL response generated in the presence of filler cells from non-irradiated (NR), TNCB-sensitized mice. These data represent the mean values ±SEM from five separate experiments.

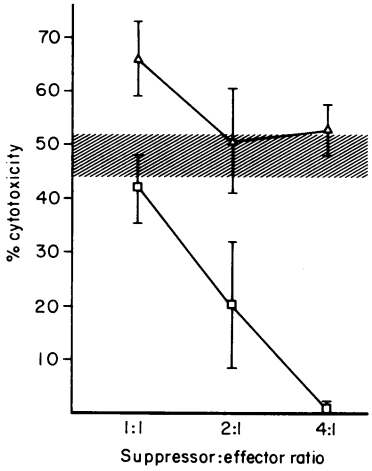

Figure 2. Dose–response of UV Ts-mediated suppression. The UV Ts cells were added to the CTL cultures in varying numbers to give suppressor:effector cell (S:E) ratios of 1:1, 2:1, and 4:1. The effectors generated were then tested in a ^{51}Cr-release assay against TNP-conjugated MCA-113 cells at a constant effector:target cell (E:T) ratio of 50:1. The shaded region represents the anti-TNP CTL response (±SEM) generated in the absence of added cells; (□——□) CTL response generated in the presence of UV Ts; (△——△) CTL response generated in the presence of filler cells from non-irradiated (NR), TNCB-sensitized mice. These data represent the mean values ± SEM from three separate experiments.

introduction of excess antigen along with sensitized lymphocytes.

Next we examined the specificity of the UV Ts. A CTL response was generated against TNP-modified or DNP-modified spleen cells *in vitro*. Suppressor cells were induced by painting mice with either TNCB or dinitrofluorobenzene (DNFB; 50 μl 0·5%) 5 days after UV irradiation. The data in Table 1 demonstrate the hapten-specificity of the UV-induced suppressor cells. The addition of cells from mice irradiated with UV and then sensitized with TNCB had no effect on the generation of CTL against DNP, but did suppress the anti-TNP response. On the other hand, cells from UV-irradiated, DNFB-sensitized mice only suppressed the anti-DNP response, indicating the hapten-specificity of these cells.

Kinetics of suppression

The addition of UV Ts into the CTL cultures at the time of their initiation suppressed the TNP-specific CTL response. In order to determine whether the UV Ts interfered with an early step in the generation of CTL, such as activation of the CTL precursors with antigen, or a later step, such as differentiation of the CTL, the UV Ts were added at various time-points after initiation of the CTL cultures. The results from this experiment are presented in Fig. 3. Suppression of the response occurred only when the UV Ts were added during the first 24 hr of culture. Maximal suppression was noted when the UV Ts were added at the beginning of the culture, and suppression gradually decreased as the time-interval between initiation of the culture and the addition of UV Ts increased. These data indicate that the UV Ts suppress an early event in the generation of CTL. In addition, we measured the ability of the UV Ts to suppress the killing of TNP-modified target cells by preformed effector cells. The UV Ts had no effect upon the CTL response when added to the 4-hr ^{51}Cr-release assay (data not shown), reinforcing the fact that the UV Ts must be added at the start of the CTL generation culture to have an effect.

Effect of UV Ts on the primary antibody response to TNP

In order to determine whether the anti-TNP antibody response was also affected by UV Ts, these cells were injected i.v. into syngeneic recipients. At the same time, the mice were immunized by the i.v. injection of TNP-SRBC. The ability of these mice to generate a primary anti-hapten antibody response was measured using a direct Jerne plaque assay. The data presented in Table 2 show that the injection of 1×10^8 UV Ts resulted in a 60% suppression of the anti-TNP PFC response (Group 6) compared to control animals that received 1×10^8 NR cells (Group 3). The data also illustrate that the UV Ts are specific for the TNP hapten. Although the anti-TNP response was significantly suppressed ($P < 0{\cdot}005$) in mice injected with UV Ts, no suppression of the anti-SRBC response was observed in these animals (Groups 1 and 4, $P > 0{\cdot}5$). Cells from UV-irradiated, TNCB-sensitized mice are capable, therefore, of suppressing the generation of both anti-TNP CTL and anti-TNP antibodies.

Cell surface phenotype of the UV Ts

In order to identify the cell involved in suppression of the anti-TNP PFC response, various subpopulations of spleen cells were

Table 1. Specificity of UV-induced suppressor cells

Culture conditions*	Specific lysis of TNP-modified target cells (%)†	Culture conditions*	Specific lysis of DNP-modified target cells (%)†
NSC+TNP-NSC	61±4	NSC+TNP-NSC	4±2
NSC+DNP-NSC	6±4	NSC+DNP-NSC	26±4
NSC+TNP-NSC+NR-TNCB	71±5	NSC+DNP-NSC+NR-TNCB	24±9
NSC+TNP-NSC+NR-DNFB	76±3	NSC+DNP-NSC+NR-DNFB	25±4
NSC+TNP-NSC+UV-TNCB	11±6 ($P<0.001$)‡	NSC+DNP-NSC+UV-TNCB	24±3
NSC+TNP-NSC+UV-DNFB	70±6	NSC+DNP-NSC+UV-DNFB	7±1 ($P<0.001$)‡

* 5×10^6 responder cells were cultured with an equal number of stimulator cells, modified with either TNP or DNP. Suppressor cells were generated by sensitizing UV-irradiated mice with 100 μl of 3% TNCB or 50 μl of 0·3% DNFB. Control cells were generated by sensitizing non-irradiated mice. A 2:1 suppressor to effector cell ratio was used in this experiment.

† A 50:1 effector to target ratio was used. Targets were MCA 113 cells modified with 20 mM TNBS or 20 mM DNBS.

‡ Student's *t*-test, cultures with UV Ts *vs* cultures with stimulator and responder cells.

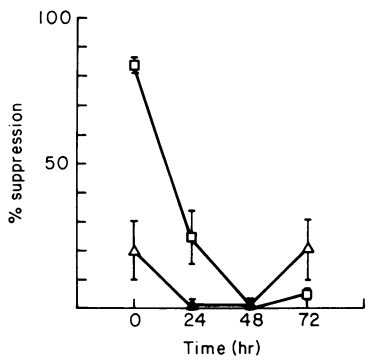

Figure 3. Kinetics of suppression. The UV Ts (□) or NR T (△) were added to cultures of NSC + TNP-NSC at the times indicated. Five days after the initiation of the cultures, the activity against TNP-modified targets was measured. The percentage suppression was calculated according to the following formula:

$$\% \text{ suppression} = 1 - \left(\frac{\% \text{ cytotoxicity generated by TNP-NSC + UV Ts}}{\% \text{ cytotoxicity generated by TNP-NSC + NSC}}\right) \times 100.$$

depleted from UV-irradiated mice by means of antibody-mediated, complement-dependent lysis. Three monoclonal antibodies were used: anti-Thy-1.2, anti-Lyt-1.1, and anti-Lyt-2.1. The spleen cells were enriched for T lymphocytes by incubation on nylon wool, and then treated with the monoclonal antibodies and complement. The treated cells, along with the immunizing antigen, were injected into normal recipients, and the anti-TNP PFC response was measured. As is seen in Fig. 4, the introduction of UV Ts caused significant suppression of the anti-TNP response compared to that of NR cells. Depletion of both Thy-1.2^+ and Lyt-1.1^+ cells from the UV Ts population abrogated the suppressive activity. Depletion of Lyt-2.1^+ cells had no effect on the suppressive activity. Since we demonstrated previously the specificity and activity of these antisera (Ullrich & Kripke, 1984; Ullrich, 1985), we conclude that a Thy-1^+, Lyt-1^+2^- cell from the UV-irradiated, TNCB-sensitized mouse transfers the suppression of anti-TNP antibody production.

Table 2. Suppression of the antibody response to TNP by UV-induced suppressor cells*

Group	Cells transferred (1×10^8/mouse)	Indicator cells	PFC/10^6 spleen cells±SEM†	$P<$‡
1	NR	SRBC	518±214	
2	NR	HRBC	41±21	
3	NR	TNP-HRBC	623±196	
4	UV	SRBC	469±156	NS§
5	UV	HRBC	21±4	
6	UV	TNP-HRBC	219±104	0·005

* Suppressor cells were generated by irradiating the mice on Day 0, followed by sensitization with 100 μl of a 3% solution (w/v) of TNCB in acetone on Day 5. Seven days later, spleen cells were obtained from UVB irradiated mice and control mice that were sensitized with hapten (NR). These cells were injected into normal mice i.v. and, at the same time, the mice were immunized by injecting 0·1 ml of a 1% solution (v/v) of TNP-SRBC. The spleens were assayed for anti-SRBC and anti-TNP PFC 5 days later.

† Mean values from five independent experiments ± the standard error of the mean.

‡ *P* values determined by two-tailed Student's *t*-test; Group 1 *vs* Group 4; Group 3 *vs* Group 6.

§ NS, not significant.

Role of epicutaneous sensitization in the induction of UV Ts

The UV Ts used in these studies were obtained following epicutaneous sensitization of UV-irradiated mice with TNCB. Previous studies (Kripke, Morison & Parrish, 1983; Ullrich, 1985) demonstrated that two signals are required to induce these suppressor cells: UV radiation and hapten sensitization. As immunization of UV-irradiated mice with a variety of other antigens does not lead to suppressor cell induction (Kripke *et al.*, 1977), it is not clear whether the hapten itself is critical for Ts induction or whether the epicutaneous route of sensitization is the important factor. In order to distinguish between these possibilities, the TNP hapten, conjugated to SRBC, was injected i.v. into UV-irradiated and normal mice. A decreased antibody

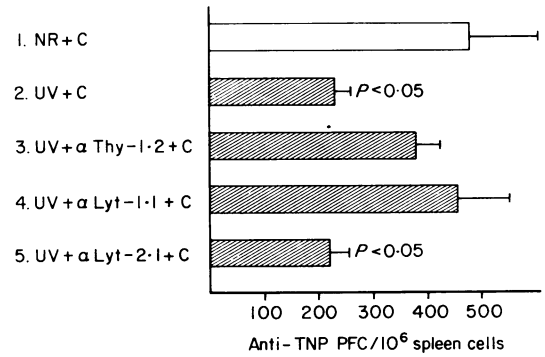

Figure 4. Phenotypic characterization of the UV Ts. Recipient mice were injected with NR cells treated with complement, UV Ts cells treated with complement alone, or UV Ts cells treated with anti-Thy-1.2, anti-Lyt-1.1, or anti-Lyt-2.1 followed by complement, along with the TNP-coupled SRBC. After 5 days, spleen cells from these mice were tested for anti-TNP PFC responses against TNP-conjugated HRBC. These data represent the mean values ± SEM from two separate experiments.

Table 3. Effect of UV-radiation on the generation of an antibody response* *in vivo*

Experiment	Indicator cells	PFC/10⁶ spleen cells ± SEM† UV	NR	P<‡
1	SRBC	429 ± 73	313 ± 35	0·05
	HRBC	33 ± 7	70 ± 19	
	TNP-HRBC	454 ± 59	320 ± 19	0·05
2	SRBC	440 ± 79	340 ± 80	0·01
	HRBC	20 ± 14	38 ± 33	
	TNP-HRBC	640 ± 100	450 ± 120	0·01

* UV = exposure to 30 kJ/m² UVB on Day 0. Four (Experiment 1) or five (Experiment 2) days later, all mice were immunized with 0·1 ml of 1% v/v TNP-SRBC. Direct plaques were measured 5 days later against the cells indicated.

† Mean values from triplicate samples ± the standard error of the mean.

‡ *P* values determined by two-tailed Student's *t*-test; UV *vs* NR.

response to TNP in the UV-treated mice would suggest that activation of Ts was a function of the hapten, rather than of the route of sensitization. No such decrease was noted, and, in fact, an enhancement of anti-TNP PFC was observed in UV-irradiated mice (Table 3). The mechanism of this slight, although statistically significant ($P < 0.05$), enhancement of the PFC response remains to be determined.

DISCUSSION

The presence of immunocompetent cells in the skin suggests that the skin is more than a passive barrier to the environment and contains within it the elements necessary to achieve protection from pathogens and perhaps neoplasia (Streilein, 1983). It is well known that UV radiation can interfere with the ability of the immunocompetent cells in the skin to respond to antigen (Bergstresser & Streilein, 1983). In addition, epicutaneous sensitization of UV-irradiated animals on unirradiated skin results in the induction of hapten-specific suppressor cells (Noonan *et al.*, 1981; Kripke *et al.*, 1983). The data presented in this paper demonstrate the wide range of activity of these cells. We found that UV Ts, which suppress CHS to TNCB *in vivo*, also suppressed the *in vitro* generation of anti-TNP CTL. The degree of suppression was directly proportional to the number of UV Ts present at the time of initiation of the culture. In addition, the transfer of the UV Ts to a normal recipient also prevented the generation of an anti-hapten antibody response *in vivo*. The phenotype of the cells that transfer suppression of both antibody production and the CHS response is Lyt-1^+2^-. Preliminary experiments also indicate that the cell that suppresses CTL activity is a Lyt-1^+2^- cell. Therefore, the activity of UV Ts is not restricted solely to the cutaneous response to antigen. Our data indicate that, once induced, UV Ts regulate several different immune responses to TNP.

This finding suggests that diverse immunological pathways may be regulated by a single type of suppressor cell, perhaps by means of its ability to inhibit the proliferation of TNP-specific lymphocyte clones. The information obtained thus far on the phenotype of the cells involved in suppression of the various effector responses is consistent with this possibility. Alternatively, epicutaneous sensitization of UV-irradiated mice may generate multiple subpopulations of TNP-specific UV Ts, each of which regulates a different anti-TNP effector response. Cloning the UV Ts should allow us to distinguish between these two possibilities. The fact that the UV Ts suppress the generation of an anti-TNP response *in vitro* should facilitate attempts to clone this cell.

It is interesting to note the similarity between the activity of the supressor cells induced by hapten sensitization of UV-irradiated animals and the suppression induced by i.v. injection of hapten-modified cells. We show that UV Ts suppress the generation of CTL and anti-hapten antibody, as well as the proliferation of T cells in response to hapten-modified self. Sunday, Benacerraf & Dorf (1981) and Sherr & Dorf (1981) demonstrated that a suppressor cell induced by the i.v. injection of hapten-modified cells also has a wide range of activity, i.e. suppression of CHS, suppression of antibody formation to hapten, and suppression of CTL. In addition, Tagert, Thomas & Asherson (1978) demonstrated that injecting TNBS i.v. also induces T cells that suppress the generation of CTL.

Suppressor T cells also can be generated by multiple applications of hapten (Thomas, Watkins & Asherson, 1979). Although there are some common features between the activity of these suppressor cells and the UV Ts, such as the suppression of anti-TNP antibody production, there are two major differences between the phenomena. Firstly, UV Ts suppressed a primary PFC response, whereas the Ts generated by multiple applications of hapten only suppressed a secondary response. Secondly, UV Ts suppressed the anti-TNP PFC response regardless of the carrier used, whereas the Ts induced by multiple applications of hapten only suppressed the response to TNP-self.

Even though antibody production against TNP is inhibited by UV Ts, i.v. immunization of UV-irradiated animals with TNP-SRBC does not result in a decreased PFC response. This indicates that the induction of UV Ts does not occur following i.v. immunization and may require sensitization via the epicutaneous route. Once induced, however, the UV Ts will then

suppress sensitization with TNP by other routes. A previous study by Jensen (1983) using the CTL system is consistent with these findings. She showed that lymphocytes taken from UV-irradiated mice could be sensitized *in vitro* to generate a primary anti-hapten CTL response. However, CTL were not generated *in vivo* after epicutaneous administration of hapten to UV-irradiated mice. Again, this suggests that once the UV Ts are induced by the appropriate mode of immunization, they can inhibit the formation of CTL. In this context, Spellman *et al.* (1984) attempted to mimic epicutaneous sensitization by applying protein antigens through scarified skin of UV-irradiated mice. They demonstrated that antibody formation in response to soluble antigen was impaired in UV-irradiated mice, compared with non-irradiated animals. Although these studies used the model of sensitization through UV-exposed skin (Towes *et al.*, 1980) as opposed to our model of sensitization through unexposed skin, they emphasize the importance of the epicutaneous route in activation of suppressive pathways in UV-irradiated animals. How UV irradiation can affect epicutaneous sensitization that occurs through unexposed skin remains an intriguing, unanswered question.

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