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Suppression of contact hypersensitivity by short-term ultraviolet irradiation: I. Immunosuppression by serum from irradiated mice

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SUMMARY

Serum from UV-irradiated mice was shown to be immunosuppressive *in vitro* and *in vivo*. It suppressed leucocyte adherence inhibition reactions of cells from sensitized syngeneic and allogeneic mice, and suppressed the development of contact hypersensitivity after passive transfer to mice. Supernatants of cultures of spleen cells from irradiated mice were also suppressive. The suppressive factors in sera and culture supernatants were non-dialysable. The suppressive effect of UV irradiation was abrogated by cyclophosphamide, but this restored reactivity was still inhibited by serum from irradiated donors; this suggests that the serum factor requires T suppressor cells for its production but not for its action. The level of interleukin 1 (IL-1) was not raised in serum from UV-irradiated mice; thus the serum factor appears not to be IL-1.

Keywords ultraviolet radiation immunosuppression suppressor factors

INTRODUCTION

The suppression of contact hypersensitivity (CHS) by ultraviolet (UV) radiation has proved to be a convenient model for the study of systemic UV-induced immunosuppression. Although much research has been conducted in this area of photoimmunology, the exact mechanism of immunosuppression is still unclear.

Several workers have noted the existence of antigen-specific T suppressor (T_s) cells following exposure of animals to UV and antigen (Greene *et al.*, 1979; Noonan, De Fabo & Kripke, 1981a), but how a superficial process such as skin irradiation can result in the systemic induction of T_s cells has been a matter of speculation. One theory proposes that UV radiation is taken up in the skin, producing a soluble mediator which then travels to its site of action in lymphoid tissues; here T_s cells are induced by a mechanism which may involve defective antigen-presenting cells (Noonan, De Fabo & Kripke, 1981b; De Fabo & Noonan, 1983; Kripke & Morison, 1985). The T_s cells presumably act by elaborating suppressor factors (SF).

Gahring *et al.* (1986) postulated that increased concentrations of interleukin 1 (IL-1) after UV irradiation may result in the desensitization of IL-1-sensitive cells, thus inducing immunosuppression. A serum SF was reported to appear very soon after irradiation (2 to 6 h); it suppressed CHS and delayed-type hypersensitivity (DTH) reactions, and induced splenic suppressor cells when injected intravenously (Swartz, 1984, 1986).

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We have described the presence of a factor in mouse serum collected 3 to 5 days after UV irradiation that is suppressive in the leucocyte adherence inhibition (LAI) assay (Harriott-Smith & Halliday, 1986). This study further examines the serum factor and investigates its relationship to previously reported SF.

MATERIALS AND METHODS

Mice

Mature female BALB/c and CBA mice were obtained from the Central Animal Breeding House, University of Queensland. Unless stated otherwise, experiments were conducted with BALB/c mice.

UV irradiation

The procedure for UV-irradiation of the mice has been described previously (Harriott-Smith & Halliday, 1986). Briefly, the dorsal fur of the mice was clipped and the ears covered with electrical tape to prevent non-specific inflammation. The clipped mice were placed in partitioned cages below FS20 sunlamps and received a single dose of UV radiation of approximately 7·8 kJ/m^2. Mice were contact-sensitized 3 days after irradiation.

Contact sensitization

Groups of five mice were contact-sensitized by painting the clipped abdomen with 50 μl of a 5% solution of trinitrochlorobenzene (TNCB) (picryl chloride, BDH Chemicals, Poole, Dorset, England) or a 3% solution of oxazolone (Ox) (Aldrich Chemicals, Milwaukee, WI, USA) in acetone (Noonan &

Halliday, 1978). Reactivity was determined 5 days after sensitization by measurement of ear thickness immediately before and 24 h after application of a challenge dose of 5 μl of a 1% solution of TNCB or Ox in acetone to each side of the ear. Ear thickness was measured with a dial micrometer (Mitutoyo Pocket Gauge, Mitutoyo Manufacturing Co., Tokyo, Japan). The mean ear swelling for each group of mice was calculated by subtracting ear thickness before challenge from ear thickness 24 h after challenge. The statistical significance of the difference between the mean ear swellings of control and test mice was calculated by Student's *t*-test.

Antigen preparation

Triphenylated bovine serum albumin (TNP-BSA) was made by mixing 500 mg of BSA (Commonwealth Serum Laboratories, Melbourne, Vic., Australia) with 10 ml of 0·15 M sodium carbonate containing 100 mg of picryl sulphonic acid (Sigma Chemical Co., St Louis, MO, USA) for 1 h at room temperature. The resulting solution was dialysed against several changes of saline at 4°C for 24 h.

Leucocyte adherence inhibition (LAI)

The direct LAI assay has already been described (Halliday, Maluish & Miller, 1974; Noonan & Halliday, 1978; Harriott-Smith & Halliday, 1986). The technique involves determining the adherence to glass of sensitized peritoneal cells (PC) and the inhibition of this adherence by a relevant antigen; SF are detected by their ability to prevent LAI when added to a reactive mixture.

For the detection of LAI, the mixtures contained PC (10^6 cells in 0·1 ml of medium) from irradiated and sensitized (UV/TNCB) mice, 0·05 ml of relevant antigen (TNP-BSA) at optimal concentration (or control with medium only) and 0·05 ml of normal mouse serum (NMS). For the detection of SF in test sera, these were included in place of the NMS. For detection of SF in spleen cell culture supernatants, PC were suspended in 0·1 ml of supernatant instead of medium, and NMS was again included in the mixtures so that serum concentration was always constant. Mixtures were incubated and adherence assayed as previously described (Harriott-Smith & Halliday, 1986). A reduction in adherence, consequent upon the addition of antigen to PC in a mixture with NMS, was interpreted as LAI. If the addition of a test serum or supernatant restored the adherence, this was interpreted as suppression of LAI. The statistical significance of these differences was determined by Student's *t*-test.

Serum

Mice were bled from the tail. Pooled sera were stored at -50°C. For passive transfer experiments, undiluted serum (250 μl) was injected into the tail vein of each recipient immediately before sensitization.

Cyclophosphamide

An aqueous solution of cyclophosphamide (Cy) (Endoxan-Asta, Bristol Laboratories, Crows Nest, NSW, Australia) was injected intraperitoneally at a dose of 300 mg/kg body weight, 3 days before irradiation.

Spleen cell culture supernatants

Spleens were removed aseptically from donor mice, forced through a stainless steel 40-gauge mesh and suspended in Minimal Essential Medium (MEM) (Eagle's, modified) (Flow Laboratories, Irvine, Scotland) containing 5% fetal calf serum, 1×10^{-5} M 2-mercaptoethanol and antibiotics. After removal of erythrocytes by treatment with sterile ammonium chloride (0·147 M), leucocytes were washed and finally resuspended to a concentration of 1×10^7 cells/ml in the above cell culture medium. Two ml of this suspension were incubated in 50 ml plastic tissue culture flasks (Nunc, Roskilde, Denmark) for 48 h at 37°C in a 5% CO_2 atmosphere. At the end of the incubation, the cultures were transferred into centrifuge tubes and centrifuged at 500 ***g*** for 5 min to pellet the cells. Supernatants were decanted into sterile containers and, when not tested immediately, were stored at -50°C.

Fractionation of serum or culture supernatant.

For testing in LAI assays, whole serum or SC culture supernatant (from control or irradiated mice) was concentrated in Minicon B-15 concentrators (Amicon, Fawkner, Vic., Australia) and the resulting dialysed material (solutes with mol. wt >15000) was restored to the original volume. For testing in thymocyte proliferation assays, whole serum (from control or irradiated mice) was separated into two mol. wt fractions ($<10,000$ and 10,000–30,000) using Centricon-30 and Centricon-10 micro-concentration units (Amicon).

Thymocyte proliferation assay for IL-1

Thymuses from CBA mice were removed aseptically and were forced through a stainless steel mesh to produce a cell suspension. Erythrocytes were removed by treatment with 0·147 M ammonium chloride. Cells were washed three times with MEM (as above), and adjusted to a concentration of $1{\cdot}5 \times 10^7$ cells/ml in the above medium containing 25 μg/ml of phytohaemagglutinin (PHA-P, Commonwealth Serum Laboratories). Controls contained no PHA. Whole sera or serum fractions to be assayed were filter-sterilized and 100 μl volumes added to triplicate wells of a 96-well plate (Linbro flat-bottomed tissue culture plates, Flow Laboratories, McLean, VI, USA). Doubling dilutions of the whole sera in medium were made down the plate, before 100 μl of the thymocytes were added; serum fractions were used undiluted. Doubling dilutions of recombinant IL-1 (Immunex Corp., Seattle, WA, USA) were used as standards. Plates were incubated for 72 h at 37°C in 5% CO_2; 1 μCi per well of tritiated thymidine ([methyl-^{3}H]-thymidine, Amersham, Sydney, NSW, Australia) was added for the final 6 h of the incubation. When the incubation was complete, the cells were harvested from the plates onto glass fibre filters using a Titertek Cell Harvester 550 (Flow Laboratories, Irvine, Scotland). Filter discs were mixed with 3 ml of scintillation fluid (PCS II, Amersham) in disposable scintillation vials. Samples were counted on a 1219 RackBeta Spectral Liquid Scintillation Counter (LKB Wallac, Copenhagen, Denmark). Results are reported in the form of a stimulation index (observed DPM/DPM of thymocytes cultured with PHA alone).

RESULTS

We first determined whether serum from irradiated mice, shown previously to be suppressive in syngeneic LAI reactions *in vitro*,

Table 1. Effect of serum from irradiated mice on LAI reactivity of syngeneic and allogeneic PC

Strain of PC donors	Antigen (TNP-BSA)	Strain of serum donors	Treatment of serum donors	% Adherence (mean ± s.e.)
BALB/c	−	BALB/c	—	65·4 ± 3·9
BALB/c	+	BALB/c	—	40·5 ± 4·0*
BALB/c	−	BALB/c	UV	66·0 ± 4·2
BALB/c	+	BALB/c	UV	61·3 ± 4·5†
BALB/c	−	CBA	—	74·9 ± 3·4
BALB/c	+	CBA	—	57·8 ± 4·8*
BALB/c	−	CBA	UV	72·5 ± 3·1
BALB/c	+	CBA	UV	71·1 ± 4·5†

Mice to be used as PC donors were treated with UV and 3 days later with a sensitizing dose of TNCB (UV/TNCB). Five days later, their PC were incubated in mixtures with and without antigen, and with serum from normal or irradiated syngeneic or allogenic mice. Adherence of PC was determined as described (Harriott-Smith & Halliday, 1986).

* Significantly different ($P<0.05$) from adherence without antigen (PC reactive).

† Not significantly different ($P>0.05$) from adherence without antigen (PC suppressed).

Table 2. Effect of SC culture supernatants from irradiated mice on LAI reactivity of sensitized (UV/TNCB) PC

Treatment of PC donors	Antigen (TNP-BSA)	Treatment of SC donors	% Adherence (mean ± s.e.)
UV/TNCB	−	−	67·7 ± 5·5
UV/TNCB	+	−	30·5 ± 3·9*
UV/TNCB	+	UV	67·4 ± 4·7†

* Significantly different ($P<0.05$) from adherence without antigen (PC reactive).

† Not significantly different ($P>0.05$) from adherence without antigen (PC suppressed).

suppressed allogeneic LAI assays. Serum from irradiated CBA mice was tested for its ability to suppress the LAI reactivity of irradiated and sensitized BALB/c mice and *vice versa.* As can be seen in Table 1, there is no apparent genetic restriction in the activity of the serum.

Previously defined SF have been found in the supernatants of SC cultures (Koppi & Halliday, 1983), a property which can be very useful in the production of large quantities of SF as well as in determining the cellular origin of the SF. We examined supernatants of cultured SCs for *in vitro* suppressive activity (suppression of LAI). As shown in Table 2, culture supernatants of SC from irradiated mice were highly suppressive in LAI, compared to supernatants of normal SC.

Serum and culture supernatant were further subjected to dialysis (15,000 mol. wt cut-off) and again tested for suppression of LAI reactivity (Table 3). The SF in both cases appeared to be non-dialysable.

Table 3. Effect of dialysis on suppressive activity of serum and SC culture supernatant in LAI with sensitized (UV/TNCB) PC

Antigen (TNP-BSA)	Serum or culture sup.	Dialysed or non-dialysed	% Adherence (mean ± s.e.)
−	Normal serum	Non-dialysed	74·1 ± 3·2
+	Normal serum	Non-dialysed	45·0 ± 4·4*
+	Normal serum	Dialysed	38·3 ± 3·7*
+	UV serum	Non-dialysed	71·6 ± 4·1†
+	UV serum	Dialysed	64·7 ± 5·2†
−	Normal sup.	Non-dialysed	67·7 ± 4·5
+	Normal sup.	Non-dialysed	30·5 ± 3·9*
+	Normal sup.	Dialysed	41·8 ± 3·1*
+	UV sup.	Non-dialysed	67·4 ± 4·7†
+	UV sup.	Dialysed	61·9 ± 4·3†

Sera and SC culture supernatants from normal and UV irradiated mice were tested before dialysis (non-dialysed) and after dialysis (dialysed), in mixtures with sensitized PC and antigen.

* Significantly different ($P<0.05$) from adherence without antigen (PC reactive).

† Not significantly different ($P>0.05$) from adherence without antigen (PC suppressed).

Table 4. Effect of passive transfer of serum from irradiated mice on CHS response to TNCB in syngeneic and allogeneic mice

Strain of serum donors	Strain of recipients	Ear swelling (μm) (mean ± s.e.)	% suppression by serum
−	BALB/c	233 ± 17	
BALB/c	BALB/c	144 ± 13*	38·2
CBA	BALB/c	163 ± 13*	30·0
−	CBA	223 ± 14	
CBA	CBA	166 ± 15*	25·0
BALB/c	CBA	137 ± 15*	38·6

Donor mice were UV-irradiated, and bled for serum 3 days later. Recipient mice were injected i.v. with serum, immediately sensitized with TNCB, and challenged on the ears 5 days later.

* Significantly different from control ear swelling ($P<0.01$).

Having established the existence of a non-restricted, immunosuppressive factor(s) active *in vitro,* we attempted to detect an *in vivo* function. To this end, CBA and BALB/c mice were used as donors and recipients of serum, which was injected immediately before sensitization with TNCB. The development of CHS was assayed by ear-swelling tests whose results are shown in Table 4. The ability of serum, collected 3 days after UV irradiation of donor mice, to suppress CHS in both syngeneic and allogeneic recipients, is clearly demonstrated.

To investigate further the mechanism of CHS suppression by serum, recipient mice were pre-treated with Cy. Table 5 shows the results of this procedure with two different contact sensitizers. In each case, the suppressive effect of UV irradiation (without serum transfer) was abrogated by Cy, but the CHS of Cy-treated and UV-irradiated mice was still susceptible to suppression by serum from irradiated donors.

Table 5. Effect of Cy, UV, and serum transfer on CHS reactions to TNCB and Ox in BALB/c mice

Cy pre-treatment (day −3)	UV (day 0)	Serum transfer (day 3)	Sensitization and challenge (days 3 and 8)	Ear swelling (μm) (mean ± s.e.) (day 9)	% suppression of CHS
−	−	−	TNCB	365 ± 8	
−	+	−	TNCB	153 ± 9*	58·1
+	+	−	TNCB	355 ± 13	2·7
+	+	+	TNCB	210 ± 10*	42·5
−	−	−	Ox	383 ± 5	
−	+	−	Ox	280 ± 7*	26·9
+	+	−	Ox	400 ± 7	−4·4
+	+	+	Ox	310 ± 3*	19·1

Serum was collected from donor mice 3 days after UV irradiation.
* Significantly different from control ear swelling ($P < 0{\cdot}001$).

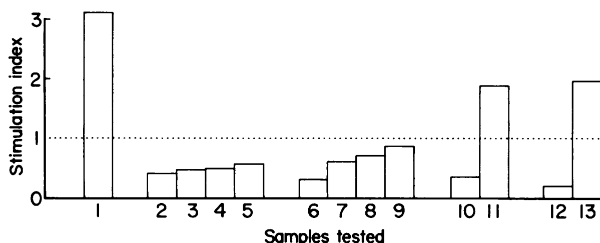

Fig. 1. Thymocyte proliferation assay for IL-1 in sera and separated fractions. Samples tested are numbered as follows: 1, recombinant IL-1 (25 units/ml); 2–5, NMS (diluted 1/2–1/16); 6–9, serum from mice UV irradiated 3 days previously (diluted 1/2–1/16); 10 and 11, separated fractions of NMS (mol. wt < 10,000 and 10,000–30,000, respectively); 12 and 13, similar fractions of UV serum.

The possibility that serum from irradiated mice contained IL-1, which could have an indirectly immunosuppressive effect, was examined by the thymocyte proliferation assay. Recombinant IL-1 induced readily detectable dose-dependent proliferation in thymocyte cultures containing PHA (data not shown). However, mouse serum obtained 3 days after UV treatment had no such effect; it consistently produced a low level of proliferation as did normal mouse serum, at levels below those of cultures without serum (Fig. 1). As mouse serum is known to contain substances which are suppressive in proliferation assays (Nelson & Shneider, 1974), sera from normal and irradiated mice were fractionated into two mol. wt ranges; low (< 10,000) and medium (10,000–30,000). With a mol. wt of approximately 15,000, any IL-1 present in the sera should be contained in the 10,000–30,000 fraction. The low mol. wt fractions from irradiated and normal mice were both highly suppressive in the thymocyte proliferation assay (Fig. 1). Although the 10,000–30,000 mol. wt fraction of the serum from irradiated mice gave a positive (> 1) stimulation index (2·04 × background proliferation), this was not significantly different from the analogous normal serum fraction, which gave a stimulation index of 1·92.

DISCUSSION

The immunosuppressive activity of serum from UV-irradiated mice was described previously in relation to suppression of LAI reactions *in vitro*. Suppression was observed with serum obtained 3 to 5 days after irradiation, but not at earlier or later times (Harriott-Smith & Halliday, 1986). We have now shown that similar serum suppresses allogeneic LAI reactions as well as the induction *in vivo* of CHS to both TNCB and Ox. Serum from irradiated BALB/c mice suppressed LAI and CHS in CBA mice, and *vice versa*, indicating that the factors had no restriction imposed by any of the genetic differences between these inbred strains (H-2, Igh, etc.). Whether the effects on LAI and CHS are due to the same or different factors is not known and may only be determined when the responsible substances are further characterized. Swartz (1984, 1986) reported suppression of CHS and DTH reactions by serum obtained 2 to 6 h after irradiation. However, differences in time of appearance of the factor and the quantity of serum used (a minimum of 400 μl of serum was required to transfer suppression) would seem to distinguish Swartz's factor from the SF reported here.

Hapten-specific T_s cells are known to develop in the spleens of irradiated mice when both UV radiation and contact-sensitizing haptens are administered (Greene *et al.*, 1979; Noonan, De Fabo & Kripke, 1981b). Similarly, mice tolerized to TNCB by an intraperitoneal injection of picryl sulphonic acid before epicutaneous sensitization produce splenic T_s cells (Zembala & Asherson, 1973; 1976). Tolerized mice have been shown to produce an antigen-specific, genetically-restricted serum factor that is both suppressive in LAI reactions and is capable of passively transferring suppression to susceptible mice (Noonan & Halliday, 1978; 1980; Halliday & Noonan, 1978). This factor falls within the well-characterised series of antigen-specific T_s factors described by several groups of workers (Dorf & Benacerraf, 1984; Asherson, Colizzi & Zembala, 1986). The serum factor induced by UV alone is distinct from the antigen-specific factors, and its molecular relationship to the latter has yet to be determined.

Among the physiologically active molecules identified in the blood of UV-treated animals are IL-1 and epidermal cell-derived thymocyte activating factor (ETAF) (Gahring *et al.*, 1984). ETAF is produced by epidermal keratinocytes and is functionally indistinguishable from IL-1. An increase in the concentration of circulating IL-1/ETAF following UV irradiation (peaking at 3 days after UV) is believed to result in an acquired reduction in the ability of IL-1-sensitive cells to be

stimulated by IL-1 and, consequently, in immunosuppression (Gahring *et al.*, 1986). We have been unable to detect significant amounts of IL-1 in serum containing suppressive activity, obtained 3 days after irradiation, or in fractions of that serum. It is possible that the dose of UV radiation we used (7·8 kJ/m^2, compared with 30 kJ/m^2 used by Gahring *et al.*), although immunosuppressive, was insufficiently inflammatory to induce IL-1 production. However, when we increased the dose to 31·2 kJ/m^2, IL-1 production was not increased (unpublished findings). Narrow band irradiation studies have enabled the wavelengths responsible for the suppression of CHS to be separated from those causing inflammation and gross skin damage (Noonan, De Fabo & Kripke, 1981a); thus although the effects of inflammation may contribute to UV-induced suppression of CHS, it appears that they are not entirely responsible.

Preliminary evidence for the production of the UV-induced serum factor by T_s cells has been obtained by the use of Cy. This substance, when administered in appropriate dosage schedules, has been shown to eliminate T_s cells preferentially (Polak & Turk, 1974; Mitsuoka, Mitsuo & Morikawa, 1976). Such a mechanism is consistent with the observation (Table 5) that Cy pre-treatment prevented UV from suppressing CHS induction. Nevertheless, Cy pre-treated and UV-irradiated mice were still susceptible to suppression of CHS induction by passive transfer of UV serum. This demonstrates that the serum factor, although probably requiring T_s cells for its production, no longer requires them for its suppressive action.

Additional evidence for the site of origin of the UV-induced factor was obtained from SC cultures. Supernatants from cultures of SC from irradiated mice (but not from normal mice) caused suppression of LAI reactivity, again implicating splenic suppressor cells in the production of the circulating serum factor. Both the serum factor and the supernatant factor were found to be non-dialysable (mol. wt > 15,000). Further work is required to establish the identity or non-identity of the two factors.

In the following paper, a photoproduct (*cis*-urocanic acid) produced by skin irradiation is shown to have immunosuppressive effects similar to those described here for UV irradiation itself.

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