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Circulating suppressor factors in mice subjected to ultraviolet irradiation and contact sensitization

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SUMMARY

BALB/c mice subjected to a single dose of UV-B radiation showed suppressed contact hypersensitivity to trinitrochlorobenzene. The *in vitro* antigen reactivity of peritoneal cells from these mice was investigated using the leucocyte-adherence inhibition assay. These cells showed a high degree of reactivity with specific antigen. However, this reactivity, but not the reactivity of cells from non-irradiated sensitized mice, could be suppressed by serum from UV-treated sensitized mice. The suppressive effect of this serum could also be demonstrated on other syngeneic systems with unrelated antigens and was partially effective with allogeneic cells, indicating a lack of antigen specificity and genetic restriction. Suppressive properties were also found in serum taken from mice 3–5 days (but not at other times) after irradiation without subsequent sensitization.

INTRODUCTION

Ultraviolet (UV) radiation, especially at wavelengths 280–320 nm (UV-B), is well known to have widespread systemic effects on the immune system (Kripke, 1984). An experimental model extensively studied by investigators in this area is the suppression of contact hypersensitivity (CHS) in mice exposed to a relatively high dose of UV-B from commercial sunlamps (Noonan, De Fabo & Kripke, 1981a).

In order to explain how superficial irradiation causes generalized suppression of cell-mediated immunity (CMI), many attempts have been made to identify the cellular and molecular sites of action. Experiments conducted *in vivo* have determined that both splenic adherent cells (macrophages) and epidermal Langerhans cells from irradiated mice are defective in antigen-presenting function (Greene *et al.*, 1979; Stingl *et al.*, 1981; Noonan *et al.*, 1981b). This defect may result from the formation in the irradiated skin of a soluble photoproduct, possibly urocanic acid, which circulates to and has effect on splenic cells (De Fabo & Noonan, 1983). Alternatively, cellular damage or the formation of a photoproduct in the skin after UV exposure may serve as a stimulus for macrophages to leave the spleen and accumulate at the site of damage, thereby being unavailable to carry out antigen-presenting duties elsewhere (Kripke, Morison & Parrish, 1983). Suppressor T (Ts) cells have also been implicated, since transfer of these cells from irradiated mice to normal mice prevents the induction of CHS (Noonan *et al.*, 1981a). It is inferred that the defect in antigen-presenting cells (APC) precedes and causes the increase in Ts cells.

In vitro experiments have employed both cells from UV-treated animals and cells irradiated after removal from their natural site (Letvin *et al.*, 1980a,b; Stingl *et al.*, 1981; Sauder *et al.*, 1981). The results generally confirm that a defect in antigen presentation leads to suppressed immunoreactivity.

Although Ts cells produce well-characterized soluble suppressor factors (SF) *in vitro*, and the systemic distribution of a suppressive molecule from irradiated skin has been postulated (De Fabo & Noonan, 1983), circulating immunosuppressive factors have received little attention. In this paper, we show that irradiation and contact sensitization of mice leads to the production of a circulating serum factor that suppresses an *in vitro* reaction of CMI.

Abbreviations: APC, antigen-presenting cell/s; CHS, contact hypersensitivity; CMI, cell-mediated immunity; LAI, leucocyte-adherence inhibition; Ox, oxazolone; PSA, picryl sulphonic acid; SF, suppressor factor/s; TNCB, trinitrochlorobenzene; TNP-BSA, trinitrophenylated bovine serum albumin; Ts, suppressor T.

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MATERIALS AND METHODS

Mice

Mature male and female BALB/c and CBA mice were obtained from the Central Animal Breeding House, University of Queensland.

UV irradiation

The dorsal fur of the mice was removed with electric clippers and, when ear swellings were to be measured, the ears were covered with black insulating tape. The mice were placed into partitioned cages with wire-mesh lids to prevent shielding by cage-mates and by food and water containers. The cages were

placed 22 cm below a bank of 10 Westinghouse FS20 sunlamps for 45 min. Approximately 87% of the radiation emitted by these lamps is in the wavelength range of 255–360 nm. The dose of UV administered (7·8 kJ/m^2) was found to produce significant immunosuppression of CHS while causing minimal skin damage. Mice were irradiated 3 days prior to sensitization (Noonan *et al.*, 1981a).

Contact hypersensitivity

CHS was induced by the method of Asherson, Zembala & Barnes (1971). The abdominal fur was shaved with electric clippers and the skin was painted with 50 μl of a 5% solution of trinitrochlorobenzene (TNCB) (picryl chloride, BDH Chemicals, Poole, Dorset, U.K.) or 50 μl of a 3% solution of oxazolone (Ox) (Aldrich Chemicals, Milwaukee, WI) in acetone. Mice were made tolerant to TNCB by a single intraperitoneal injection of a neutralized solution containing 2·5 mg of picryl sulphonic acid (PSA) (Sigma Chemical Co., St Louis, MO) followed 7 days later by skin painting with TNCB (Asherson *et al.*, 1971). The mice were tested for CHS by the application of a challenge dose of 5 μl of 1% TNCB or Ox in acetone to both surfaces of one ear. The other ear was painted with acetone as a control. Ear thickness was measured with a thickness gauge (Model 7309, Mitutoyo, Tokyo, Japan) immediately before and 24 hr after application of the challenge dose. Groups of five mice were used throughout these experiments.

LAI technique

The direct leucocyte-adherence inhibition (LAI) assay (Halliday, Maluish & Miller, 1974; Noonan & Halliday, 1978; Halliday & Noonan, 1978) involves the determination of glass adherence of sensitized peritoneal cells (PC) and the inhibition of this adherence by a relevant antigen; serum SF are detected by their ability to prevent LAI when added to a reactive mixture.

For the detection of LAI, the mixtures contained sensitized PC (10^6 cells in 0·1 ml of medium), 0·05 ml of relevant antigen at optimum concentration (or control with medium only) and 0·05 ml of normal mouse serum. For the detection of SF in the test sera, these were included in mixtures in place of normal serum. The mixtures were preincubated at 37° for 30 min and then introduced into haemocytometer chambers (four chambers for each different mixture). After a further incubation at 37° for 60 min, the total cells in each of 20 squares (a pattern of five in each chamber) were counted for each mixture. The non-adherent cells were washed away and the remaining cells were recounted in the same squares. All mixtures were coded before counting. The percentage leucocyte adherence was calculated for each mixture (18–20 values/mixture), and the mean percentage adherence and standard error (SE) determined. A reduction in adherence, consequent upon the addition of antigen to PC in a mixture with normal serum, was interpreted as LAI. If substitution of a test serum restored the adherence, this was interpreted as suppression of LAI. The statistical significance of these differences was determined by Student's *t*-test. Percentage suppression by test serum was calculated by the following formula (Halliday & Noonan, 1978):

$$\% \text{ suppression} = 100 \times \frac{\text{mean \% adherence with antigen and test serum} - \text{mean \% adherence with antigen and normal serum}}{\text{mean \% adherence with no antigen and normal serum} - \text{mean \% adherence with antigen and normal serum}}$$

Antigen preparation

Trinitrophenylated bovine serum albumin (TNP-BSA) was made by mixing 500 mg of BSA (Commonwealth Serum Laboratories, Melbourne, Australia) with 0·15 M sodium carbonate containing 100 mg of PSA at 4° overnight. The resulting solution was dialysed against saline, again at 4° (Rittenberg & Amkraut, 1966). Oxazolonated BSA (Ox-BSA) was prepared by reacting 100 mg of BSA in 10 ml of 1 M sodium bicarbonate with 12 mg of Ox in 2 ml ethanol for 1 hr at room temperature. Any undissolved material was filtered out and the remaining solution was dialysed as for TNP-BSA. Crude tumour extracts were prepared by homogenization of tumour tissue in phosphate-buffered saline followed by a series of high-speed centrifugations (Halliday *et al.*, 1974). Antigens were stored at −50°.

Serum

Mice were bled from the tail. The pooled blood was allowed to clot at 37° for 30 min, left at room temperature for a further 30 min, and centrifuged at 3000 r.p.m. for 5 min. Sera were stored frozen at −20° for short periods of time or at −50° for longer periods. Sera from irradiated and sensitized mice were collected 5 days after sensitization (8 days after irradiation). Sera from irradiated but unsensitized mice were collected at various times after irradiation as indicated in the 'Results' section.

Table 1. Depression of contact hypersensitivity to TNCB in UV-irradiated BALB/c mice

			Mean ear swelling ± SE (cm ^{3})	
UV exposure	Sensitization	Challenge	Experiment 1	Experiment 2
—	—	TNCB	3·3 ± 0·8	3·4 ± 0·7
—	TNCB	TNCB	28·4 ± 0·6	31·3 ± 0·8
45 min	—	TNCB	5·6 ± 0·6	7·8 ± 0·7
45 min	TNCB	TNCB	11·8 ± 0·7	15·4 ± 1·4

Table 2. Effect of UV irradiation on reactivity of BALB/c mice to trinitrophenylated BSA in the LAI assay

Treatment of PC donors	Antigen in LA	Serum	% adherence (mean ± SE)	LAI
—	—	NMS*	64·6 ± 4·6	
—	TNP-BSA	NMS	66·9 ± 5·4†	
TNCB	—	NMS	75·3 ± 5·4	
TNCB	TNP-BSA	NMS	51·8 ± 5·2‡	23·7
UV/TNCB	—	NMS	72·6 ± 4·8	
UV/TNCB	TNP-BSA	NMS	51·9 ± 5·6‡	20·7

* NMS, normal mouse serum.

† Not significantly different from adherence of same PC without antigen ($P > 0·05$).

‡ Significantly different from adherence of same PC without antigen ($P < 0·05$).

RESULTS

In order to establish and confirm the efficiency of the procedures of contact sensitization, elicitation of CHS reactions and suppression of these reactions by UV irradiation under our experimental conditions, numerous experiments were performed with BALB/c mice and TNCB. The results of two typical experiments are shown in Table 1; the ear swelling produced by TNCB in sensitized mice was significantly suppressed when UV treatment preceded sensitization.

In an attempt to demonstrate immunosuppression by an *in vitro* technique, PC from mice exhibiting both positive and suppressed ear-swelling reactions were tested in the LAI assay. There was no apparent difference between the two PC populations, both having hightly significant reactivity with antigen (Table 2). Normal mouse serum was a component of the cell mixture tested for LAI reactivity.

When serum from UV-suppressed mice was added to LAI-reactive PC from TNCB-sensitized mice in place of normal serum, no suppression of LAI was observed (Table 3). When the same serum was added to reactive PC from irradiated and TNCB-sensitized mice, the reaction was significantly suppressed, as is also shown in Table 3. In the same table, results are given for serum from TNCB-sensitized mice (no suppression) and serum from 'tolerant' mice (suppression). On several occasions, sera with these suppressive properties have been found not to have effects on leucocyte adherence in the absence of antigen (data not shown).

Table 3. Suppressive effect of serum from irradiated and sensitized BALB/c mice on LAI reactivity

Treatment PC donors	Antigen in LAI	Treatment of serum donors	% adherence (mean ± SE)
TNCB	—	—	76·2 ± 4·1
TNCB	TNP-BSA	—	42·2 ± 6·2*
TNCB	TNP-BSA	UV-TNCB	52·2 ± 4·2*
UV/TNCB	—	—	72·6 ± 4·8
UV/TNCB	TNP-BSA	—	51·9 ± 5·6*
UV/TNCB	TNP-BSA	UV/TNCB	68·5 ± 3·9†
UV/TNCB	TNP-BSA	TNCB	51·2 ± 5·2*
UV/TNCB	TNP-BSA	PSA/TNCB‡	72·5 ± 3·2†

* Significantly different from adherence without antigen ($P < 0·05$).

† Not significantly different from adherence without antigen ($P > 0·05$).

‡ Serum from tolerant mice (showing reduced ear swelling reaction after pretreatment with PSA).

Since circulating suppressor factors in other situations have been found to be antigen (hapten)-specific and subject to MHC-restriction in their *in vitro* effects (Noonan & Halliday, 1980; Koppi, Halliday & McKenzie, 1981), we tested serum from UV-suppressed mice for these properties. In Table 4, serum from mice treated with UV and TNCB is shown to be highly suppressive with TNP-reactive PC and also with PC sensitized to and reacting with unrelated antigens. Both PC donors and serum donors were syngeneic BALB/c mice. When PC and sera were obtained from allogeneic mice (CBA and BALB/c respectively, as in Table 5), suppression was still observed although at a lower level than previously.

The above evidence for lack of specificity of the SF suggested experiments in which serum donors were UV-irradiated only without contact sensitization. Table 6 shows that serum from such mice, obtained 3 and 5 days after irradiation, was highly suppressive with syngeneic PC. Suppressive activity had not appeared at Day 1 and was no longer found after 7 days.

DISCUSSION

The search for circulating suppressor factors in mice immunosuppressed by UV irradiation was prompted by our previous experience with such factors in tumour-bearing mice (Halliday *et al.*, 1974; Koppi *et al.*, 1981) and in animals with antigen-induced tolerance (Halliday & Walters, 1974; Halliday & Noonan, 1978). A persistent observation in these experimental systems was the presence of antigen-reactive lymphocytes as detected *in vitro*, accompanied by SF in serum which could block the lymphocyte reactivity. With tolerance in CHS to

Table 4. Lack of antigen-specificity of UV-induced suppressor factor from BALB/c mice

Treatment of PC donors	Antigen in LAI	Treatment of serum donors	% adherence (mean ± SE)	Suppression (%)
UV/TNCB	—	—	74·5 ± 3·3†	
UV/TNCB	TNP-BSA	—	53·2 ± 5·9*	
UV/TNCB	TNP-BSA	UV/TNCB	70·7 ± 5·5†	82·2
Bladder carcinoma	—	—	84·9 ± 3·2	
Bladder carcinoma	Carcinoma ext.	—	66·4 ± 2·8*	
Bladder carcinoma	Carcinoma ext.	UV/TNCB	84·6 ± 3·1†	98·4
UV/Ox	—	—	72·3 ± 2·4	
UV/Ox	Ox-BSA	—	55·3 ± 3·1*	
UV/Ox	Ox-BSA	UV/TNCB	72·1 ± 5·2†	98·8

* Significantly different from adherence without antigen ($P < 0·05$).

† Not significantly different from adherence without antigen ($P > 0·05$).

Table 5. Lack of genetic restriction of UV-induced suppressor factor from BALB/c mice

Treatment of PC donors (CBA)	Antigen in LAI	Treatment of serum donors (BALB/c)	% adherence (mean ± SE)	Suppression (%)
TNCB	—	—	91·7 ± 2·9	
TNCB	TNP-BSA	—	69·2 ± 3·2*	
TNCB	TNP-BSA	UV/TNCB	79·2 ± 4·3	44·4
Fibrosarcoma	—	—	87·4 ± 5·2	
Fibrosarcoma	Fibrosarcoma ext.	—	67·6 ± 4·8*	
Fibrosarcoma	Fibrosarcoma ext.	UV/TNCB	78·6 ± 4·9	55·5

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

Table 6. Time-course of appearance of suppressor factor in serum of BALB/c mice subjected to UV radiation only

Treatment of PC donors	Antigen in LAI	Treatment of serum donors	% adherence (mean ± SE)
UV/TNCB	—	—	71·4 ± 4·2
UV/TNCB	TNP-BSA	—	49·2 ± 2·9*
UV/TNCB	TNP-BSA	UV only Day 1‡	48·0 ± 4·1*
UV/TNCB	TNP-BSA	UV only Day 3	71·0 ± 3·2†
UV/TNCB	TNP-BSA	UV only Day 5	66·0 ± 5·4†
UV/TNCB	TNP-BSA	UV only Day 7	50·0 ± 4·5*
UV/TNCB	TNP-BSA	UV only Day 9	51·3 ± 4·5*

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

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

‡ Days after UV irradiation.

TNCB, these SF were also shown to be functional *in vivo* in suppressing skin reactions in suitably prepared animals (Halliday & Noonan, 1978).

Our detection of apparently competent PC in irradiated mice with suppressed CHS was not unanticipated, being consistent with the above observations. The effects of UV on CMI thus appeared not to reside in the cells involved in LAI assays. However, these assays are dependent on T lymphocytes plus APC in the form of adherent macrophages or monocytes, at least where soluble tumour-related antigens are concerned (Koppi & Halliday, 1982; Thomson & Halliday, 1985). The results, therefore, seemed to be incompatible with previous findings by others (see 'Introduction') that APC were defective after UV treatment. It is possible that the LAI assay as conducted here will function adequately with low levels of APC. This may have been the basis of early claims (Koppi, Maluish & Halliday, 1979) for APC-independence of LAI, whereas later work using more efficient depletion techniques clearly showed the need for these cells (Koppi & Halliday, 1982). Recent experiments in our laboratory, using hapten-coupled spleen cells as APC in LAI assays, suggest that a cellular defect can indeed be detected after irradiation; similar cells were used by Noonan *et al.* (1981b) in their *in vivo* experiments.

The observation that the reactivity of PC from irradiated TNCB-sensitized mice could be suppressed by serum from UV-suppressed mice, whereas PC from mice sensitized without prior irradiation could not, is also consistent with previous results. Reactive PC from mice tolerized to TNCB could be suppressed in the LAI reaction by serum from similarly tolerant mice, whereas PC from TNCB-sensitized mice could not (Halliday & Noonan, 1978).

In contrast to serum SF found in either tolerant or tumour-bearing mice, the UV-related factors appear to lack antigen specificity and MHC-restriction. Partial suppression was observed repeatedly when serum from UV-treated allogeneic mice was used (Table 5), suggesting that there may be a restricted component in a mixture of SF.

Irradiated mice exhibit their deficiency in CHS only if they are exposed to the sensitizing antigen 3–5 days after irradiation (Noonan *et al.*, 1981b). It is intriguing that only during this period do these mice have circulating SF (Table 6). Without subsequent skin-painting with TNCB, the suppressive activity of serum declines. The application of TNCB at 3 days either prolongs the production of a non-specific SF or initiates the production of a second, antigen-dependent (but not antigen-specific) SF. The temporal relationship between circulating SF and *in vivo* unresponsiveness, at critical times when CHS is being induced (3 days after UV) or elicited (8 days after UV), suggests a real function in addition to that detected *in vitro*.

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