Analysis of the Effect of a Sunscreen Agent on the Suppression of Natural Killer Cell Activity Induced in Human Subjects by Radiation from Solarium Lamps

Peter Hersey, F.R.A.C.P., Malcolm MacDonald, B.Sc., Christine Burns, B.Sc., Steven Schibeci, B.Sc., Helen Matthews, R.N., and Frank J. Wilkinson, B.Sc.

Oncology and Immunology Unit, Royal Newcastle Hospital, Newcastle, N.S.W., and CSIRO Division of Applied Physics (FJW), National Measurement Laboratory, Sydney, N.S.W., Australia

Previous studies in rodents have shown that ultraviolet radiation (UVR) may have direct effects on the immune system in the skin and at higher doses may induce systemic suppression of immune responses. We have previously shown that UVR from sun or solarium beds may induce systemic effects in human subjects. The purpose of the present study was to examine whether these systemic effects in human subjects could be prevented by use of commercially available sunscreen agents. Groups of 12 normal subjects were exposed to radiation from solarium lamps after application of a sunscreen agent or the base used in its preparation. Twelve half-hourly exposures induced a depression of natural killer (NK) cell activity against a melanoma and the K562 target cell which was not prevented by use of the sunscreen agent. Changes in functional activity were accompanied by a reduction in NK cell numbers assessed by Leu-11 monoclonal antibodies against the labile Fc receptor. Application of the sunscreen agent also did not protect against effects of solarium exposure on recall antigen skin tests and immunoglobulin production in vitro in pokeweed mitogen-stimulated cultures of B and T cells. These results suggest that further evaluation of the wavelength spectrum of UVR and the effectiveness of sunscreen agents in prevention of UVR-induced effects on the immune system is needed. *J Invest Dermatol 88:271–276, 1987*

Previous studies have shown that chronic exposure of mice to ultraviolet B (UVB) irradiation induced systemic changes in the immune system that resulted in an inability to reject highly antigenic transplanted UV-induced tumors [1–4] and to develop delayed type hypersensitivity (DTH) skin responses to dinitrochlorobenzene (DNCB) applied to the skin [5–7]. These changes appeared to be associated with the development of suppressor T cells [7–9]. The current hypothesis is that induction of these cells in turn is the result of UV-induced changes in antigen-presenting cells (APC) in the skin either by direct effects of UV radiation (UVR) [10–16] or due to formation of a chemical photoproduct such as *cis*-urocanic acid [17] from epithelial cells in the skin.

A number of studies suggest that similar changes may occur in human subjects. Treatment of patients with psoriasis with both oral methoxypsoralen and near-UVR was found to depress DTH responses to DNCB [18] and to reduce the number of Langerhans cells in the skin [19]. Exposure of human subjects to UVR from the sun [20] or from solarium lamps [21] was also shown to induce a number of changes in the immune system that could conceivably influence the host response against skin tumors such as melanoma.

The influence of sunscreens on these changes in mice or human subjects is not clear. Para-aminobenzoic acid (PABA)-containing sunscreens were shown to protect mice from development of UV-induced skin tumors and to protect against actinic skin damage [22]. These preparations did not, however, protect mice from the UV-induced susceptibility of mice to transplanted UV-induced tumors [23] and did not protect against UV-induced depression of DTH responses [24]. In view of the questions raised by these studies the present study sought to extend these findings by examining the effects of sunscreen agents on several of the UV-induced changes in the immune system in humans.

MATERIALS AND METHODS

Subjects Studied These were normal volunteers from the local community. Pertinent details of subjects in each group are summarized in Table I.

Manuscript received March 26, 1986; accepted for publication August 22, 1986.

This work was supported by funds provided by the New South Wales State Cancer Council and, in part, by I.C.I. Australia and the National Health and Medical Research Council.

Reprint requests to: Dr. Peter Hersey, Oncology and Immunology Unit, David Maddison Clinical Sciences Building, Royal Newcastle Hospital, Newcastle, N.S.W. 2300, Australia.

Abbreviations:
APC: antigen-presenting cells
DNCB: dinitrochlorobenzene
DTH: delayed type hypersensitivity (skin tests)
FCS: fetal calf serum
FITC: fluorescein isothiocyanate
IL-2: interleukin 2
M.Abs: monoclonal antibodies
NK: natural killer (cells)
PABA: para-aminobenzoic acid
PHA: phytohemagglutinin
PWM: pokeweed mitogen
UVR: ultraviolet radiation

0022-202X/87/$03.50

Table I. Summary of Details of Subjects Entered into the Sunscreen Study

	No. of Subjects	Age	Sex		Sun Exposure[a]		Skin Type[b]		
			M	F	Summer	Winter	II	III	IV
No UVR + screen	12	26.5 ± 6.2	5	7	129 ± 103	55.5 ± 81	3	3	6
UVR + base	11	26.8 ± 8.0	4	7	285 ± 285	156 ± 228	1	3	7
UVR + screen	11	23.7 ± 5.3	8	3	173 ± 112	66.5 ± 66	3	4	5

[a]Sum of hours exposed to sun from October to March and April to September, respectively.
[b]Skin types were classified as described by Pathak [26]: II = Very sensitive, always burns easily, tans minimally. III = Sensitive, burns moderately, tans gradually. IV = Moderately sensitive, burns minimally, tans easily.

Study Design The study was conducted in August and early September prior to onset of summer. Exposure to sun over the winter months April to August had been minimal (Table I). Volunteers were randomized to 3 groups of 12 subjects. The control group did not receive UVR exposure but they were requested to apply the sunscreen lotion each day for 12 days similar to the test subjects. The other two groups received 12 half-hour exposures on consecutive days from a Wolf "Karibik" solarium bed equipped with 6 × 2 meter-long Rellarium 100 W low-pressure mercury vapor lamps above and below the subject. One group was treated with the sunscreen agent described below and the other with the base used for preparation of the sunscreen. The spectral irradiances from these tubes is described elsewhere [25] and a representative emission spectrum together with the transmission spectrum of the sunscreen agent is shown in Fig 1. In brief, approximately 1.03% of the UVR was emitted in the wavelength band 280–315 nm (UVB); only 0.03% of this was emitted in the band 290–300 nm. The remainder was recorded as UVA with peak of emission at 350–360 nm. In terms of erythemal responses, approximately 36% of the emission was in the wavelength band 280–315 nm (UVB). The UVR output from the bed was measured at 8–10 mW/cm^2 using an International Light model IL700 UV radiometer with UVA and UVB sensors. A 30-min exposure gave 18 J/cm^2 equivalent to 185 mJ UVB and 17.82 J of UVA. This was close to 1 minimal erythemal dose (MED), assuming 20–100 J/cm^2 of UVA is needed to produce 1 MED [26] and that UVB contributed 36% of the erythemal response. (Note: most of the UVB was in the 310–315 wavelength band, which induced lower erythemal responses than lower wavelengths in the UVB spectrum.)

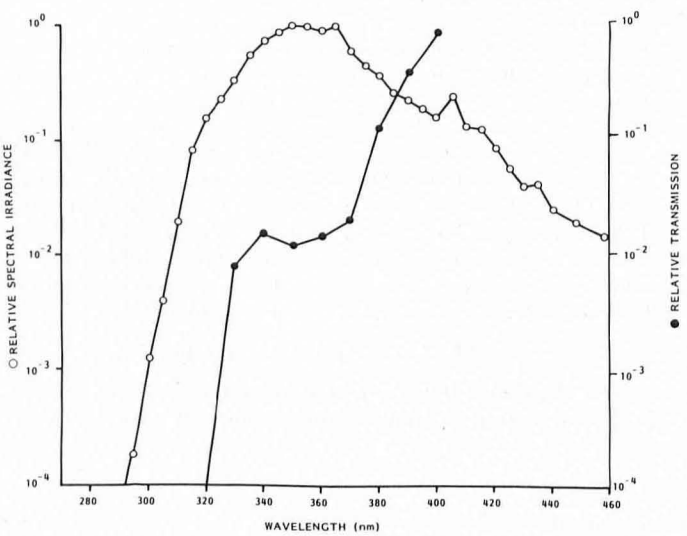

Figure 1. Representative spectral irradiance (*open circles*) from a UVA fluorescent lamp as used in these studies and the transmission spectrum (*closed circles*) through a 0.008-mm layer of the sunscreen agent used in the study.

Blood tests were conducted immediately prior to the commencement of the exposures and 1, 7, 14, and 21 days after the last exposure. Dinitrochlorobenzene was applied 1 day after the last exposure and, if no response was noticed, challenge doses were applied at 12 days. Recall antigen skin tests were applied 1 day after the last exposure.

The sunscreen used contained 8% 2-ethyl hexyl dimethyl *p*-aminobenzoate (octyl dimethyl PABA), 2% 2-hydroxy-4-methoxybenzophenone, 2% butyl methoxydibenzoyl methane in an emulsion base. The latter contained methyl and propyl paraben, cetearyl octanoate, carbomer 941, isopropyl isostearate, phenyldiethicone, stearic acid, glyceryl stearate and polyethylene glycol-100 stearate, sodium hydroxide, gluteraldehye (25%), and chemoderm 100 (fragrance). The sunscreen and base were applied in a visibly even film at 20 ml/m^2 immediately prior to each exposure. The sunscreen had a sun protection factor of 15 by the Australian standard AS 2604-1983.

Natural Killer Cell Activity The methods used to measure NK activity against the melanoma target cell MM200 and the K562 myeloid line in ^{51}Cr-release assays are described elsewhere [20,21]. Lytic units were defined as the number of effector cells required to lyse 20% of the target cells and were expressed per 10^6 of the lymphocyte populations [LU(20%/10^6)]. They were calculated by a computer program that analyzed data directly from the gamma counter using a least squares fit of log (Y/1-Y) against log of the effector cell number on the x axis (where Y = percent specific cytotoxicity) after methods described by Pross et al [27].

Estimation of Lymphocyte Subpopulations Total T cells and T-cell subsets were measured with monoclonal antibodies (M.Abs) against T3, T4, T8, and T11 antigens using the Ortho series of reagents [28] and the NK subset with Leu-11 M.Abs from Becton Dickinson [29]. The second antibody used was fluorescein isothiocyanate (FITC)-labeled sheep antimouse immunoglobulin (Silenus, code DF, Victoria). Phycoerythrin-conjugated Leu-15 and FITC-labeled OKT8 were used in an attempt to identify suppressor T-cell subsets. B cells were identified with FITC-labeled sheep antihuman Ig (Wellcome Diagnostics, code MF01, Rosebery, N.S.W.). The percentage of cells staining with the various antibodies was determined using the Spectrum III Ortho flow cytometer with a Lexal model 75 ion laser by methods described elsewhere [30,31].

Interleukin 2 (IL-2) Production and Assay Blood lymphocytes, 4 × 10^6 in 2 ml of RPMI [no fetal calf serum (FCS)], were incubated with 1% phytohemagglutinin (PHA) (Wellcome Pharmaceuticals, Code HA15) for 36 h in flat-bottomed Bijou bottles. The supernatants were collected and assayed at 4 dilutions for mitogenic activity against the NK-7, IL-2-dependent, murine cell line as described elsewhere [32]. All supernatants were assayed at once against the NK-7 cells to reduce variability. The IL-2 produced by 2 × 10^6 MLA-144 cells [33] in 1 ml of RPMI + 5% FCS was used as an independent control for day-to-day variation.

Assay of Ig Production in Pokeweed Mitogen (PWM)-Stimulated Cultures of T and B Cells The methods for these assays are as described elsewhere [20,21] except that suppressor

Table II. Effect of Solarium Exposure With or Without Sunscreen on Natural Killer Cell Activity

Group	No. of Subjects	Days after Exposure					F	*p*
		Pre	1	7	14	21		
Target cell MM200								
No UVR + screen	6	5.9 ± 0.4	5.7 ± 0.5	5.8 ± 0.5	5.8 ± 0.5	5.9 ± 0.4	0.18	NS
UVR + base	6	6.1 ± 0.3	4.9 ± 0.5	5.4 ± 0.5	5.6 ± 0.3	6.0 ± 0.3	9.33	< 0.01
UVR + screen	6	5.8 ± 0.2	4.9 ± 0.1	5.3 ± 0.3	5.5 ± 0.2	5.8 ± 0.2	19.5	< 0.01
Untreated controls	9	5.6 ± 0.5	5.5 ± 0.4	5.4 ± 0.7	5.4 ± 0.6	5.4 ± 0.5	.13	NS
Target cell K562								
No UVR + screen	6	11.7 ± 0.6	11.5 ± 0.7	11.6 ± 0.6	11.7 ± 0.7	11.7 ± 0.7	0.13	NS
UVR + base	6	12.2 ± 0.6	10.8 ± 0.8	11.2 ± 0.7	11.6 ± 0.8	12.1 ± 0.6	4.78	< 0.01
UVR + screen	6	11.9 ± 0.7	10.7 ± 0.5	11.1 ± 0.6	11.3 ± 0.6	11.7 ± 0.7	3.3	< 0.05
Untreated controls	9	11.5 ± 0.9	11.2 ± 0.6	11.0 ± 0.7	11.3 ± 0.6	11.3 ± 0.7	0.64	NS

Values indicated are means ± 1 SD of lytic units/20%/10^6 blood lymphocytes in ^{51}Cr-release cytotoxic assays. F and *p* values determined by one-way analysis of variance using the "Minitab" statistical package (Pennsylvania State University). NS = not significant.

cells were removed by panning methods as described elsewhere [34] instead of by irradiation. In brief, lymphocytes (2×10^7) were reacted with OKT8 M.Ab, washed, and then added for 1 h at 4°C to goat antimouse Ig-coated Petri dishes. Nonadherent cells were removed by gentle pipetting. The undepleted population was treated similarly except they were not reacted with OKT8 M.Ab. The T-cell numbers in the depleted population were not made up to the same numbers as in the undepleted populations but were adjusted in proportion to total yield of T8-depleted compared with undepleted T-cell populations obtained from the Petri dishes. At 7 days the culture supernatants were harvested and assayed for IgA, IgG, and IgM production by using a Hyland PDQ laser nephelometer. Suppressor T-cell activity in these cultures was estimated by comparison of Ig production in cultures where suppressor T cells had been depleted with that in cultures where suppressor activity was still present. Ratios greater than 1 indicated significant suppressor T-cell activity.

Skin Tests Responses to recall antigens were determined using the "multitest" DTH test kit produced by Institut Merieux, Lyon, France (May and Baker, Victoria, Australia). This contains 7 antigens and a control. The tests were applied to the forearm as described by the manufacturer 1 day after cessation of the solarium exposure and read 48 h later. The average diameter of induration of positive responses was measured in millimeters and individual response added to give a score [35]. Antigens in the kit were tetanus toxoid, diphtheria toxoid, *Streptococcus* antigen, tuberculin old, *Candida albicans, Trichophyton* and *Proteus* antigen.

The DNCB skin tests were conducted as described previously [20,21] by application of 1000 and 100 μg DNCB in 100 μl of acetone to the volar aspect of the forearm in a 1 cm-diameter glass ring.

Statistical Analysis Differences between the values for tests carried out at the different time periods were analyzed by one-way analysis of variance and paired *t*-tests using the Minitab statistical package from the Pennsylvania State University. Skin test results were analyzed in contingency tables.

RESULTS

Effects of Solarium Exposure on NK Activity Exposure of normal subjects to radiation from solarium lamps was associated with depression of NK activity against the melanoma (MM200) and to a lesser extent against the K562 target cells. This was most evident on days 1 and 7 after the 12 daily exposures and had returned to pretreatment levels by day 21. The results for all groups in the study are summarized in Table II. These indicate that exposure to radiation from solarium lamps was associated with depression of NK activity irrespective of whether the sunscreen agent was applied before each exposure or not.

Table III. Comparison of Suppressor Cell Activity and Total Immunoglobulin Production in Solarium-Exposed Subjects Treated With Sunscreen or Sunscreen Base (Placebo)

Group	No. of Subjects		Days after Solarium Exposure					F	*p*
			Pre	1	7	14	21		
Total Ig									
UVR + base	2		1.06 ± 0.04[a]	1.04 ± 0.01	1.24 ± 0.01	1.06 ± 0.04	1.4 ± 0.2	6.67	0.05[b]
UVR + screen	4		1.33 ± 0.3	1.25 ± 0.3	1.15 ± 0.1	1.05 ± 0.18	1.05 ± 0.15	1.08	NS
Untreated controls	9		1.22 ± 0.42	1.31 ± 0.71	1.1 ± 0.52	1.34 ± 0.26	1.34 ± 0.26	0.2	NS
IgG									
UVR + base	2	T8⁻	96.8 ± 28.5[c]	64.0 ± 29.0	69.0 ± 4.8	49.6 ± 14.3	98.8 ± 3.4	2.44	NS
			91.6 ± 23.2	61.4 ± 27.5	55.6 ± 4.5	46.4 ± 11.9	69.4 ± 7.2	1.94	NS
UVR + screen	4	T8⁻	67.5 ± 26.0	55.1 ± 20.8	35.8 ± 8.4	43.9 ± 3.8	31.3 ± 4.8	3.57	< 0.05[d]
			55.2 ± 28.5	47.7 ± 26.7	30.8 ± 4.2	43.0 ± 10.0	30.0 ± 4.1	1.43	NS
Untreated controls	9	T8⁻	32.7 ± 12.6	43.3 ± 22.6	36.0 ± 20.4	36.7 ± 24.4	43.8 ± 12.0	0.3	NS
			26.9 ± 6.4	33.1 ± 16.6	32.9 ± 22.9	27.5 ± 17.8	32.8 ± 6.0	0.5	NS

[a]Values indicated are ratios of Ig production in T8-depleted (T8⁻) cultures divided by that in undepleted cultures.
[b]Results indicate paired *t*-test on pretreatment vs 7-day values.
[c]Values indicated are total Ig (mean ± 1 SD) (μg/ml) produced in 7-day PWM-stimulated cultures. T8⁻ indicates cultures in which T8⁺ suppressor cells were depleted by panning methods.
[d]Paired *t*-test analysis of pretreatment cf day 21 values.
NS = not significant.

Skin Test Responses in Subjects After Exposure to Solarium Lamps Six of 11 subjects receiving the sunscreen base and 7 of 11 receiving the sunscreen lotion needed reapplication of DNCB to detect a response. However, 6 of 12 subjects receiving the sunscreen lotion who were not exposed to UVR also needed to be rechallenged. There were no statistical differences between the groups by contingency table analysis. These unexpected results may have been due to poor penetration of DNCB into the skin due to residual base from the sunscreen lotion in the skin.

Analysis of recall antigen skin test results revealed an apparent depression of DTH responses in the UVR-exposed groups irrespective of whether sunscreen or base was applied. The mean score ± 1 SD of 12 subjects treated with the sunscreen but not receiving UVR was 12.0 ± 8.4 whereas it was 9.0 ± 8.1 and 8.2 ± 5.3 in the groups receiving UVR + base alone or UVR + sunscreen, respectively. These differences were not significant by *t*-test of the data, although when the untreated controls were combined with a control group of 9 not receiving the lotion or exposure to the solarium, differences approached significance by *t*-test. (Mean of 21 normal controls was 12.0 ± 6.4.) The *t* value for comparisons with the group receiving UVR + sunscreen was 1.76 giving a *p* value of 0.045 by single tailed *t*-test or 0.09 by 2-tailed test.

Effects of Solarium Exposure on Suppressor Cell Activity and In Vitro Immunoglobulin Production in PWM-Stimulated Cultures of B and T Cells As shown in Table III, in subjects treated with the base only there was an increase in suppressor cell activity attributable to an increase in the ratio on day 7. Studies were, however, carried out on only 2 subjects in this group so that the significance of the results is uncertain. The most marked effects were on total immunoglobulin production. Both groups exposed to UVR showed a depression in Ig production, which had returned to pretreatment values in the groups applying the base only but remained depressed in the group receiving the sunscreen agent. Similar changes were not seen in the untreated controls. The changes in total Ig were due mainly to a change in IgG and IgM levels. IgA values showed very little variation (data not shown).

Effects on IL-2 Production Assays were carried out on all subjects in the study. There were no significant changes in IL-2 production after the solarium exposures in any of the groups.

Blood Lymphocyte Populations The results of these studies are summarized in Table IV. Both groups receiving radiation from solarium lamps had a decrease in the Leu-11 population, which was statistically significant for the group receiving the placebo. The main difference between the 2 groups receiving radiation was the significant increase in the T4/T8 ratio and the $T8^+$ cells in the group applying the sunscreen agent. There was a significant drop in the total T-cell number measured with the OKT11 M.Ab in the 3 groups irrespective of whether they received radiation or not, or sunscreen agent or placebo. The groups receiving radiation plus the placebo sunscreen base had an increase in $T3^+$ cells. Leu-15^+ cells showed a significant decrease in all 3 groups. The significance of the latter changes is not known.

DISCUSSION

The main finding from these studies as noted previously [20] was that NK activity was depressed following exposure to radiation from solarium lamps. This was most marked against the melanoma target cells and was maximal 1 and 7 days after cessation of the exposure. These results were complemented by a reduction in the number of NK cells in blood identified with the Leu-11 M.Ab against the labile Fc receptor [29] in the groups receiving the exposure to solarium lamps, although this was of marginal significance in the group using the sunscreen lotion. This may suggest that the reduction in NK activity was due to a depression in NK cell numbers rather than their functional activity as suggested by previous in vitro studies [36]. However, correlation between NK function and cell numbers was not absolute as shown by return of NK function to normal by 21 days, even though the number of Leu-11^+ cells remained depressed at this time. Al-

Table IV. Sequential Studies on Blood Lymphocyte Subpopulations[a]

Subpopulation	Subjects	Days after Treatment: Pre	1	7	14	21	F[b]	*p*
$T3^+$ T cells	C12[c]	75.4 ± 3.0	68.2 ± 3.4	74.9 ± 3.2	70.0 ± 5.2	77.1 ± 3.3	1.15	NS
	B11	64.0 ± 5.6	64.3 ± 3.6	72.9 ± 2.4	75.7 ± 2.0	79.2 ± 2.2	3.51	0.05
	S11	64.7 ± 4.6	62.3 ± 4.6	70.3 ± 3.0	60.9 ± 6.0	74.9 ± 2.9	1.78	NS
$T11^+$ T cells	C12	75.2 ± 3.7	63.0 ± 4.1	72.6 ± 3.3	59.0 ± 4.1	76.9 ± 3.3	4.26	0.05
	B11	70.5 ± 4.9	62.8 ± 2.9	67.6 ± 5.0	48.4 ± 4.4	76.3 ± 4.5	5.23	0.01
	S11	69.7 ± 3.6	56.8 ± 4.3	66.5 ± 2.1	54.1 ± 4.5	77.0 ± 3.4	6.62	0.01
T4 helper T	C12	43.2 ± 3.6	43.3 ± 2.6	48.4 ± 2.3	46.2 ± 3.2	44.9 ± 2.7	0.56	NS
	B11	42.3 ± 4.4	40.9 ± 3.0	51.2 ± 3.2	40.2 ± 3.8	46.7 ± 2.7	1.78	NS
	S11	40.2 ± 2.5	42.1 ± 2.2	44.2 ± 2.8	36.6 ± 2.7	44.2 ± 2.8	1.51	NS
T8 supp/cyt T	C12	31.4 ± 3.6	29.9 ± 3.2	31.5 ± 1.9	30.0 ± 3.9	33.1 ± 3.4	0.16	NS
	B11	23.0 ± 2.5	25.2 ± 2.2	27.0 ± 3.1	23.4 ± 3.5	31.6 ± 2.8	1.47	NS
	S11	29.2 ± 1.5	27.6 ± 2.0	30.3 ± 1.1	35.8 ± 2.8	35.5 ± 2.5	3.25	0.05
T4/T8	C12	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.4 ± 0.2	1.6 ± 0.2	0.12	NS
	B11	2.0 ± 0.3	1.8 ± 0.3	2.3 ± 0.4	2.0 ± 0.2	1.6 ± 0.2	0.80	NS
	S11	1.4 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	2.57	0.05
Leu-11^+ NK cells	C12	13.1 ± 1.8	10.3 ± 1.1	12.5 ± 2.5	8.3 ± 0.8	12.2 ± 1.8	1.28	NS
	B11	11.3 ± 1.6	8.8 ± 0.9	14.8 ± 3.1	7.5 ± 1.1	8.6 ± 1.2	2.70	0.05
	S11	15.0 ± 2.0	12.0 ± 1.5	14.2 ± 1.8	9.9 ± 1.1	10.5 ± 1.1	2.12	NS
Leu-15	C12	6.7 ± 1.9	4.2 ± 0.4	5.7 ± 0.8	6.0 ± 1.0	17.9 ± 2.3	13.5	0.01
	B11	7.1 ± 1.5	3.8 ± 0.5	6.7 ± 0.2	4.9 ± 1.3	12.1 ± 1.3	7.15	0.01
	S11	7.3 ± 1.2	4.6 ± 0.5	7.3 ± 1.3	5.0 ± 0.7	15.4 ± 2.3	10.95	0.01
B cells	C12	17.1 ± 2.4	15.3 ± 1.9	18.9 ± 3.8	11.8 ± 1.2	17.9 ± 2.3	1.26	NS
	B11	15.1 ± 2.2	12.0 ± 1.7	13.5 ± 2.0	10.1 ± 1.0	12.1 ± 1.3	1.27	NS
	S11	19.6 ± 2.8	12.4 ± 1.3	14.7 ± 2.0	15.5 ± 3.4	15.4 ± 2.3	1.12	NS

[a]Values indicated are means ± 1 SD.
[b]F and *p* values were determined by one-way analysis of variance.
[c]C = control nonexposed group, B = exposed group receiving sunscreen base, S = exposed group receiving sunscreen.
NS = not significant.

though the degree of depression in NK activity was not marked, it may be an indicator of more marked depression with higher doses of UVR. It was shown in previous studies that depression of NK activity appeared to show straightforward dose-response relationships and was evident after six 30-min exposures on consecutive days [37]. Repeated exposure over many hours to solar radiation may therefore have more marked effects.

Other findings of interest were depression of immunoglobulin production in vitro in PWM-stimulated cultures after exposure to radiation from solarium lamps. This was particularly evident in the subjects using the sunscreen lotion prior to each exposure and was associated with a decrease in the T4/T8 ratio and an increase in the percentage of $T8^+$ cells. Whether this depression of Ig production was due to induction of suppressor cells or inhibition of helper cells was not clear from these studies. Functional suppressor activity as determined by the increase in Ig production after depletion of $T8^+$ suppressor cells was unchanged in the group receiving the sunscreen agent but did show an increase in the group receiving the base used in the sunscreen preparation. The number of subjects in this aspect of the study was limited but, if confirmed, may suggest that solarium radiation may have inhibitory effects on the helper T-cell population which reduces Ig production as well as effects on the suppressor T-cell population as noted in previous studies [20,21].

It was previously reported that DTH reactivity to DNCB was also depressed in subjects exposed to solarium radiation. This could not be confirmed in the present study, as DTH responses appeared depressed in each of the 3 groups irrespective of whether they received radiation from solarium lamps or not. This unexpected result may have reflected poor absorption of DNCB into the skin due to the residual base of the sunscreen lotion in the skin 1 day after the last application, or depression of DTH responses due to a chemical factor in the base. The former explanation was supported by recall antigen skin test results, which were within the norm in the nonexposed group but which appeared depressed in those exposed to radiation. The points of the heads of the multitest kit containing the antigen may have facilitated entry of the antigen into the skin past any of the sunscreen base absorbed to the keratin layer. No differences were detected between the exposed groups irrespective of whether sunscreen or base was applied.

It would appear from these results that application of the sunscreen agent used in these studies did not prevent changes in NK activity induced by solarium exposure. The results also suggest that some other UV-induced changes such as depression of DTH responses to recall antigens, depression of Ig production in vitro, or changes in the percentage of T8 cells and the T4/T8 ratio may not be prevented by the use of this particular sunscreen. It may be inferred from this either that the sunscreen agent is ineffective in screening UVB from the radiation transmitted to the skin or that wavelengths outside those filtered by the sunscreen may induce these changes in the immune system. The former explanation appears unlikely in that octyl dimethyl PABA is widely used as an effective agent to prevent UVR-induced erythemal responses. In the formulation used it has a sun protection factor against UVB of 15 [26] and was used in the recommended quantity (2 $\mu l/cm^2$ or 20 ml/m^2) immediately prior to exposure. It would therefore appear possible that the effects observed are very sensitive to UVB or that they are induced by wavelengths outside those filtered by the agent. Studies using cut-off filters such as Mylar sheets should assist in differentiating between these two possibilities.

The present results appear analogous to those of Lynch et al [24] who reported that PABA (5% in 70% alcohol) application to the skin of mice did not protect against the UV-induced changes in Langerhans cells or the suppression of DTH responses to DNFB. This group also reported that PABA did not protect against the tumor susceptible state [23]. In both instances it was thought that UVA was responsible in that the immunologic effects could not be filtered out through glass. These results seem at some variance with those of previous workers who reported that the peak wavelength for inducing systemic suppression of DTH responses was 270 nm [17] and that the tumor-susceptible state was mediated mainly by wavelengths below 315 nm [1]. It was also reported that PABA was effective in preventing the solar radiation–induced tumor-susceptible state in mice [38].

These results may be reconciled by assuming that a larger dose of UVA may have equivalent biologic effects. Information with respect to the wavelengths, mediating effects on the immune system in humans is limited. Studies by Schacter et al [39] revealed that NK activity in vitro could be inhibited by radiation over a wide range of wavelengths from 260 to 450 nm, although the dose required was much greater for the longer wavelengths. The mechanism of the effects in vitro were reported to be limited to the postbinding stage of lysis [36]. UVA may penetrate to the microcirculation in the skin so that it is possible that effects of solarium radiation on NK activity in vivo may result from similar direct effects on NK cells as they pass through the skin rather than by release of chemical factors as proposed for UVB in mice [17]. These results suggest that further examination of the action spectrum responsible for induction of these effects on the immune system in humans is needed.

The failure of sunscreen agents to protect against certain immunologic effects of solarium lamp exposure suggests that caution should be exercised in use of such agents until their effectiveness in preventing systemic effects of UVR on the immune system is more clearly established. It is possible that by preventing erythemal responses their use encourages longer exposure to solar radiation and hence unwittingly increases the risk of damage to aspects of the immune system responsible for protection against skin cancers such as melanoma. The reports by other workers that certain sunscreen agents may act as tumor promoters [40] provides further reasons for care in the evaluation of these agents.

We are grateful to the Hematology Department, Royal Newcastle Hospital for hematologic studies on the subjects and to Professor R. Burton for assistance with flow cytometry.

REFERENCES

1. De Fabo EC, Kripke MC: Wavelength dependence and dose-rate independence of UV radiation-induced immunologic unresponsiveness of mice to a UV-inducted fibrosarcoma. Photochem Photobiol 32:183–188, 1980
2. Fisher MC, Kripke ML: Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. Proc Natl Acad Sci USA 74:1688–1692, 1977
3. Fisher MS, Kripke ML: Suppressor T lymphocytes control the development of primary skin cancers in ultraviolet-irradiated mice. Science 216:1133–1134, 1982
4. Kripke ML, Fisher MS: Immunologic parameters of ultraviolet carcinogenesis. J Natl Cancer Inst 57:211–215, 1976
5. Jessup JM, Hanna N, Palaszynski E, Kripke ML: Mechanisms of depressed reactivity to dinitrochlorobenzene and ultraviolet-induced tumors during ultraviolet carcinogenesis in Balb/c mice. Cell Immunol 38:105–115, 1978
6. Noonan FP, De Fabo EC, Kripke ML: Suppression of contact hypersensitivity by UV radiation and its relationship to UV induced suppression of tumor immunity. Photochem Photobiol 34:683–689, 1981
7. Noonan FP, De Fabo EC, Kripke ML: Suppression of contact hypersensitivity by ultraviolet radiation: an experimental model. Springer Semin Immunopathol 4:293–304, 1981
8. Fisher MS, Kripke ML: Further studies on the tumor specific suppressor cells induced by ultraviolet radiation. J Immunol 121:1139–1144, 1978
9. Kripke ML: Immunologic mechanisms in UV radiation carcinogenesis. Adv Cancer Res 34:69–106, 1981
10. Aberer W, Schuler G, Stingl G, Hönigsmann H, Wolff K: Ultra-

violet light depletes surface markers of Langerhans cells. J Invest Dermatol 76:202–210, 1981

11. Elmets CA, Bergstresser PR, Tigelaar RE, Wood PJ, Streilein JW: Analysis of the mechanisms of unresponsiveness produced by haptens painted on skin exposed to low dose ultraviolet radiation. J Exp Med 158:781–794, 1983
12. Greene MI, Kripke MS, Benacerraf B: Impairment of antigen-presenting cell function by ultraviolet radiation. Proc Natl Acad Sci USA 76:6591–6595, 1979
13. Gurish MF, Lynch DH, Yowell R, Daynes RA: Abrogation of epidermal antigen-presenting cell function by ultraviolet radiation administered *in vivo*. Transplantation 36:304–309, 1983
14. Letvin NL, Greene MI, Benacerraf B, Germain RN: Immunologic effects of whole-body ultraviolet irradiation. Selective defect in splenic adherent cell function *in vitro*. Proc Natl Acad Sci USA 77:2881–2885, 1980
15. Perry LL, Greene MI: Antigen presentation by epidermal Langerhans cells: loss of function following ultraviolet (UV) irradiation *in vivo*. Clin Immunol Immunopathol 24:204–219, 1982
16. Toews GB, Bergstresser PR, Streilein JW, Sullivan S: Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. J Immunol 124:445–453, 1980
17. De Fabo EC, Noonan FP: Mechanism of immune suppression by ultraviolet irradiation *in vivo*. I. Evidence for the existence of a unique photoreceptor in skin and its role in photoimmunology. J Exp Med 157:84–98, 1983
18. Strauss GH, Bridges BA, Greaves M, Hall-Smith P, Price M, Vella-Briffa D: Inhibition of delayed hypersensitivity reaction in skin (DNCB test) by 8 methoxypsoralen photochemotherapy. Lancet 2:556–559, 1980
19. Friedmann PS: Disappearance of epidermal Langerhans cells during PUVA therapy. Br J Dermatol 105:219–221, 1981
20. Hersey P, Bradley M, Hasic E, Haran G, Edwards A, McCarthy WH: Immunological effects of solarium exposure. Lancet 1:545–548, 1983
21. Hersey P, Haran G, Hasic E, Edwards S: Alteration of T cell subsets and induction of suppressor T cell activity in normal subjects after exposure to sunlight. J Immunol 31:171–174, 1983
22. Snyder DS, May M: Ability of PABA to protect mammalian skin from ultraviolet light-induced skin tumors and actinic damage. J Invest Dermatol 65:543–546, 1975
23. Gurish MF, Roberts LK, Krueger GG, Daynes RA: The effect of various sunscreen agents on skin damage and the induction of tumor susceptibility in mice subjected to ultraviolet irradiation. J Invest Dermatol 76:246–251, 1981
24. Lynch DH, Gurish MF, Daynes RA: Relationship between epidermal Langerhans cell density ATPase activity and the induction of contact hypersensitivity. J Immunol 126:1892–1897, 1981
25. Wilkinson FJ: Recommended UV exposure limits for tanning equipment and spectral irradiances of solarium lamps, sunlamps and daylight. Australas Phys Eng Sci Med 6:26–34, 1983
26. Pathak MA: Sunscreens: topical and systemic approaches for protection of human skin against harmful effects of solar radiation. J Am Acad Dermatol 7:285–312, 1982
27. Pross HF, Baines MG, Rubin P, Shragge P, Patterson MS: Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells. IX. The quantitation of natural killer cell activity. J Clin Immunol 1:51–63, 1981
28. Reinherz E, Schlossman S: Human T lymphocyte differentation. Immunology Today 3:239–240, 1982
29. Lanier LL, Myle A, Phillips TH, Warner NL, Babcock GF: Subpopulations of human natural killer cells defined by expression of the Leu-7 (HNK-1) and Leu-11 (NK-15) antigens. J Immunol 131:1789–1796, 1983
30. Burton RC, Ferguson P, Grey M, Hall J, Hayes M, Smart YC: Effects of age, gender and cigarette smoking on human immunoregulatory T cell subsets: establishment of normal ranges and comparison with patients with colorectal cancer and multiple sclerosis. Diagn Immunol 1:216–223, 1983
31. Hoffman RA, Kung PC, Hansen WP, Goldstein G: Simple and rapid measurement of human T lymphocytes and their subclasses in peripheral blood. Proc Natl Acad Sci USA 77:4914–4917, 1980
32. Hersey P, MacDonald M, Hall C, Spurling A, Edwards A, Coates A, McCarthy WH: Immunological effects of recombinant interferon alpha-2A in patients with disseminated melanoma. Cancer 57:1292–1300, 1986
33. Rabin H, Hopkins RF, Ruscetti FW, Neubauer RH, Brown RL, Kawakami TG: Spontaneous release of a factor with properties of T cell growth factor from a continuous line of primate tumor T cells. J Immunol 127:1852–1856, 1981
34. Engleman EG, Benike CJ, Grumet FC, Evans RL: Activation of human T lymphocyte subsets: helper and suppressor/cytotoxic T cells recognize and respond to distinct histocompatibility antigens. J Immunol 127:2124–2129, 1981
35. Anderson CT, Roumiantzeff M, Kniker WT: The multitest system for assay of delayed cutaneous hypersensitivity (DCH) to ubiquitous antigens. J Allergy Clin Immunol 61:167–172, 1978
36. Weitzen ML, Bonavida B: Mechanism of inhibition of human natural killer activity by ultraviolet radiation. J Immunol 133:3128–3132, 1984
37. Hersey PH: Effects of electromagnetic radiation on the immune system in mice and man. EOS J Immunol Immunopharmacol, in press
38. Morison WL, Kelley SP: Sunlight suppressing rejection of 280- to 320-nm UV-radiation-induced skin tumors in mice. JNCI 74:525–527, 1985
39. Schacter B, Lederman MM, Levine MJ, Ellner JJ: Ultraviolet radiation inhibits natural killer cell activity and lymphocyte proliferation. J Immunol 130:2484–2487, 1983
40. Gallagher CH, Greenoak GE, Reeve VE, Canfield PJ, Baker RSU, Bonin AM: Ultraviolet carcinogenesis in the hairless mouse skin. Influence of the sunscreen 2-ethylhexyl-P-methoxycinnamate. Aust J Exp Biol Med Sci 62:577–588, 1984