

Effects of ultraviolet light on human serum 25-hydroxyvitamin D and systemic immune function

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Background: Many immune-mediated diseases are associated with low levels of vitamin D and sunlight. UV light or supplementation with vitamin D can increase regulatory T-cell activity and prevent animal models of autoimmune disease. Increasing population vitamin D levels may therefore alleviate the burden of human immune-mediated disease.

Objective: To determine the responses of circulating 25-hydroxyvitamin D [25(OH)D] levels, regulatory T-cell numbers, and immune function to UV light exposure in patients being treated for skin disease.

Methods: Twenty-four subjects with skin disease from the North of Scotland were recruited between December and March. At baseline, and after 2 and 4 weeks of narrowband UV light exposure, we measured peripheral blood 25(OH)D level, numbers of regulatory T cells (CD4⁺CD25^{hi}FoxP3⁺), and T-cell proliferative and cytokine responses to anti-CD3/CD28 stimulation.

Results: Median (interquartile range) narrowband UV-B received during the study was 39.1 (30.9) as standard erythema dose, comparable to a quarter of the median summer sunlight exposure received locally. This increased the 25(OH)D level from a mean $\pm$ SD of 34 ± 17 nmol/L to 58 ± 16 nmol/L after 2 weeks and 78 ± 19 nmol/L after 4 weeks. The mean proportion of circulating regulatory T cells increased from 0.5% to 1.6% CD3⁺ cells, which significantly correlated with the increased 25(OH)D level. UV treatment was also followed by reduced proliferative and IL-10 responses to anti-CD3/CD28 independent of the 25(OH)D level.

Conclusion: Narrowband UV light reduces systemic immune responsiveness via the induction of regulatory T cells. Light and 25(OH)D levels may affect particular immune functions independently. The levels of serum 25(OH)D over which these effects are apparent should guide future interventions.

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Key words: Narrowband UV-B, vitamin D, regulatory T cells, eczema, psoriasis, FoxP3

Many autoimmune diseases,¹ and in some studies allergic diseases such as eczema² and asthma,³ show increasing prevalence with distance from the equator, suggesting that environmental exposure to UV irradiation may be protective. The main source of serum vitamin D is photolysis of 7-dehydrocholesterol in skin by UV, and the realization that the vitamin D receptor is widely expressed in the immune system and exerts broad anti-inflammatory actions has prompted suggestions that vitamin D deficiency is an important determinant of these diseases.

Regulatory T (Treg) cells are widely recognized as key controllers of immune effector function, including pathogenic responses,^{4,5} and vitamin D appears to influence their differentiation and activity,⁶ with a role to play in allergic disease and asthma.^{6,7} These effects include modulating cell cycle progression and elevating the expression of the Treg cell master transcription factor FoxP3.⁸ Furthermore, 1,25(OH)₂D inhibits the maturation of dendritic antigen-presenting cells and induces tolerogenic plasmacytoid dendritic cells, both actions that may enhance Treg cell numbers.⁹

Numerous association studies have demonstrated that patients with autoimmune diseases have low 25-hydroxyvitamin D [25(OH)D] levels. However, the disease itself may confound this association, with those affected being less able to pursue outdoor activities.¹⁰ Fewer prospective studies have been performed, but they suggest that low vitamin D levels predate the development of immune disease.¹⁰ Even fewer intervention studies have been performed, and these have either been small or notable for lack of clinical effects.¹¹ Multiple sclerosis (MS) is the prototypical immune-mediated disease in which prevalence correlates with lower vitamin D levels. Recently, a genetic polymorphism predisposing to vitamin D insufficiency was shown to associate with MS and type 1 diabetes.¹² In patients with MS, 1,25(OH)₂D treatment of patients' T cells *ex vivo* increased the number of Treg cells¹³ and supplementation led to reduced auto-antigen responses in an autoimmune encephalitis model,¹⁴ but in an intervention study, high-dose vitamin D₃ did not have an effect on peripheral Treg cell numbers.¹⁵ 1,25(OH)₂D has broad immunosuppressive effects in animal and *in vitro* studies,^{9,16} but the doses used have tended to be supraphysiological.¹⁵

Phototherapy is widely used for inflammatory and immune-mediated dermatoses including psoriasis, atopic dermatitis, and polymorphic light eruption. Narrowband UV-B therapy (311 nm) has become the preferred mode of administration and has immunosuppressive effects. Several recent studies confirm that narrowband UV-B has positive effects on vitamin D status,¹⁷⁻¹⁹

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Abbreviations used

25(OH)D: 25-Hydroxyvitamin D
 ITA: Individual topology angle
 MS: Multiple sclerosis
 SED: Standard erythema dose
 Treg: Regulatory T
 UK: United Kingdom

but other poorly understood mechanisms may also be involved in UV-induced immunosuppression.^{20,21} In patients being treated with sunlight, PBMCs demonstrated reduced cytokine secretion and skin biopsies showed a relative increase in dermal FoxP3 cells.²² Photochemotherapy, in which photosensitizing psoralen medication forms DNA adducts when combined with UV-A exposure (psoralen with UV-A), has also been shown to induce CD4⁺CD25⁺FoxP3⁺ Treg cells in peripheral blood.^{23,24}

Although of great potential therapeutic interest, the links between human UV exposure, vitamin D levels, and immune function remain to be clearly established. In particular, there is an urgent need to test the hypothesis that UV irradiation of human skin is an effective method of inducing Treg cells via the synthesis of vitamin D. The aim of this study was therefore to investigate the effect of narrowband UV-B on vitamin D status, peripheral blood Treg cell numbers, and immune function in patients attending a dermatology outpatients clinic.

METHODS

This study was approved by the North of Scotland Research Ethics Committee (NOSRES), reference 10/S0802/73. In order to maximize the effects of any intervention, subjects were recruited between December 2010 and March 2011, when vitamin D levels are at their lowest.

Adults aged 16 years and older undergoing phototherapy treatment as part of their routine care were exposed to known amounts of narrowband UV-B 3 times a week by using the Waldman 7001 cabinet fitted with TL-01 tubes and exposing the whole body surface area according to a standard escalating protocol based on the patient's measured minimum erythema dose. Using the Commission Internationale de L'Eclairage action spectrum equations, a UV dose of 0.1637 J/cm² is equivalent to 1 standard erythema dose (SED),²⁵ defined as 100 J/m² of erythemogenic UV irradiation.²⁶

Patients taking supplementary vitamin D, cod liver oil, or topical vitamin D analogues were excluded. Age, height, weight, and minimal erythema dose were assessed at the start of the study.

Assessment of vitamin D status, sunlight exposure, and skin color

Serial samples of peripheral blood were taken for serum 25(OH)D, immunophenotyping of Treg cells, and functional assays, initially at a baseline visit prior to starting narrowband UV-B treatment and repeated after 2 weeks and 4 weeks of phototherapy. 25(OH)D was determined as the sum of the concentrations of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3, measured by dual tandem mass spectrometry (Department of Clinical Chemistry, University of Liverpool, Liverpool, United Kingdom [UK]) by using the National Institute of Standards and Technology aligned internal standards (Chromsystems, London, UK). The laboratory is a member of the vitamin D external quality assessment scheme. At each visit, subjects completed questionnaires assessing their dietary vitamin D intake. They gave details on sun exposure (time spent outside each day and body surface exposed, for summer, autumn, and spring, and details of any holidays abroad in the preceding year). Skin color (as individual topology angle [ITA]) was measured at each visit, at the left and right cheekbones, and the inner forearm,

by using a handheld chromameter (CM-2600d Spectrophotometer; Konica Minolta Photo Imaging, London [UK] Ltd). Calibration against a standard white tile was carried out before each set of measurements. Measurements were recorded for L* (dark-light), a* (green-red), and b* (blue-yellow) axes of color based on the Commission Internationale de L'Eclairage L*a*b* system.²⁷ These data were then used to define the ITA by using the following equation: $ITA = (\arctangent(L^* - 50)/b^*) \times 180/\pi$.²⁸ A decrease in ITA is an indication of skin tanning, as the higher the ITA, the paler the skin.

Peripheral blood cell preparation and stimulation

Blood (30 mL) was collected into lithium heparin tubes, and PBMCs were isolated by density-gradient centrifugation (Lymphoprep; Nycomed, Asker, Norway). As described previously,²⁹ PBMCs were resuspended in the α modification of Eagle's medium (Gibco, Glasgow, UK) supplemented with 5% autologous serum and cultured in 1-mL volumes at 1.25×10^6 cells/mL at 37°C in a humidified atmosphere of 5% CO₂/95% air. Cultures were either left unstimulated or stimulated with 2.5×10^5 anti-CD3/CD28 beads/mL (Dynabeads Human T-Activator; Invitrogen, Glasgow, UK) for 3 days.

Proliferation assay

To measure proliferation,²⁹ triplicate samples withdrawn from cultures were incubated with 18.5 kBq ³H-thymidine (Amersham Biosciences, Amersham, UK) for 6 hours before being harvested onto glass-fiber filter mats (LKB-Wallac, Turku, Finland) by using a Mach III Harvester 96 (Tomtec). These were then read on a scintillation counter (Microbeta+; LKB-Wallac) to measure the ³H-thymidine incorporation as counts per minute.

ELISA

Duplicate 100- μ L samples of culture supernatant were plated onto flat-bottomed Nunc 96-well Maxisorp plates (SLS, Glasgow, UK) previously coated with either antihuman IFN- γ or IL-10 monoclonal capture antibody (BD Pharmingen, Oxford, UK), diluted to 2 μ g/mL in bicarbonate-coating buffer. The plates were then developed to measure the respective cytokines as described previously.²⁹

Flow cytometry

Immediately after isolation, PBMCs were stained with the following antibodies: anti-CD3-Alexa Fluor 647, anti-CD4-APC-Cy7 (BD Pharmingen, San Diego, Calif), and anti-CD25-Alexa Fluor 700 (BioLegend, San Diego, Calif). For intracellular protein staining, cells were fixed and permeabilized by using the Cytofix/Cytoperm kit (BD Biosciences, San Diego, Calif) as per manufacturer's protocol, before staining with anti-FoxP3-Alexa Fluor 488 and anti-IL-10-PE. Compensation beads (BD Pharmingen) were used for each sample, providing single positive controls for voltage adjustments and compensation calculations. Data from each patient were acquired by using the LSR II (BD Biosciences) and analyzed by using FlowJo v.9.3.1 (Tree Star, Inc, Ashland, Ore). Cell populations were consistently gated and subgated on isotype controls.

Statistical analysis

Statistical analysis was carried out by using IBM SPSS Statistics 19 (release 19.0.0) (IBM SPSS Statistics; <http://www.spss.com/software/statistics/academic/>). As the markers of immune function were not normally distributed, nonparametric tests were used for preliminary analyses. Spearman correlations were used to test for associations between markers of immune function, UV dose, and 25(OH)D. All tests were 2 tailed. Mixed model linear analysis was used to determine the main predictors (specifically UV dose or 25(OH)D) of immune function. This allowed for the repeated measures of both the dependent variables (Treg cells and IL-10) and independent variables (UV dose and 25(OH)D) and for adjustment of the major confounders (age, body mass index, minimal erythema dose, dietary vitamin D). For the mixed model analysis and analyses of Treg cells using parametric tests, IL-10 and

TABLE I. Characteristics of study participants*

	Mean $\pm$ SD	Range (min-max)	Median	Interquartile range
Age (y)	46.1 $\pm$ 14.05	22-77		
Body mass index (kg/cm ²)	29.71 $\pm$ 5.64	23.1-44.0	28.9	25.4-31.7
Minimum erythema dose (J/cm ²)	0.29 $\pm$ 0.084	0.11-0.35	0.35	0.22-0.35
Dietary vitamin D intake (μ g/d)	2.33 $\pm$ 1.75	0.56-7.37	1.63	1.07-3.56
Number of UV-B treatment sessions	10.82 $\pm$ 1.50	6-13	11	10-12
Total dose of UV light (J/cm ²)	6.41 $\pm$ 3.11	0.98-11.87	6.4	1.07-3.56
Categoric data				
Sex: man/woman	13		9	
Fitzpatrick skin type†: I/II/III	3		12	7
Skin condition: Psoriasis/eczema/other	14		3	5
Percentage of population				
	Spring	Summer	Autumn	
Exposing face only/face and hands/face, hands and arms, or legs	0/57/43	0/19/81	5/76/19	
Spending time outside on weekdays: <15 min/15 min-30 min/30 min-2 h/>2 h	14/38/34/14	5/19/57/19	9/29/48/14	
Spending time outside on weekend days: <15 min/15 min-30 min/30 min-2 h/>2 h	0/14/29/57	0/5/29/66	5/9/38/48	

*Details on dietary intake, skin type, sun behavior, and treatment are included.

†Fitzpatrick skin type classifies a person's complexion and his or her tolerance of sunlight. I: white; very fair; always burns, never tans. II: white; fair; usually burns, tans with difficulty. III: cream white; fair; sometimes mild burn, gradually tans.

Treg cells were natural logarithm transformed, with values below the detection limit for Treg cells being assigned 0.1% CD3⁺ cells prior to transformation. With paired data on 22 subjects, we determined *post hoc* that the study had 80% power to detect a change of 9.0 nmol/L in serum 25(OH)D and a change in Treg cells of 1.2% with a significance level of .05 (using SD of 14.3 nmol/L for 25(OH)D and 1.92% for Treg cells for change from baseline to 2 weeks).

RESULTS

Patients

A total of 24 participants were recruited. Two subjects, however, withdrew consent from the study after their first visit, one because of an adverse reaction to the phototherapy and the other because of commencing anticoagulant therapy. Of the remaining 22 participants, all attended each of the 3 scheduled visits of the study, except one who could not attend the second of the appointments. No subject had previously taken vitamin D supplements or vitamin D-containing fish oil supplements. Demographic details of the participants are given in Table I. Fourteen patients had psoriasis, 3 atopic eczema, and 1 each had pruritis, nodular prurigo, polymorphic light eruption, solar urticaria, and granuloma annulare. In the first 2 weeks, a single session gave between 0.11 and 0.69 J/cm² (0.7-4.2 SED). In the second 2 weeks of the study, higher doses (up to 12.9 SED in a single session) were given. Half-way through the study, patients had received in total between 8.6 and 64.7 SED (median [interquartile range] 11.8 [6.2] SED) and over the complete study 7.1 to 72.5 SED (median [interquartile range] 39.1 [30.9] SED) (Fig 1).

Vitamin D status

As expected from latitude and season of the study, patients had low circulating serum 25(OH)D levels at the start of the study, with 27% of patients having 25(OH)D concentrations of less than 25 nmol/L and 86% of patients having 25(OH)D concentrations of less than 50 nmol/L.³⁰ Exposure to UV light caused a substantial and rapid increase in 25(OH)D concentrations. The mean difference $\pm$ SD in 25(OH)D concentrations between baseline and 2 weeks was 23.0 $\pm$ 14.3 nmol/L and between 2 weeks and 4 weeks

was 21.7 $\pm$ 15.3 nmol/L. The overall change (baseline to 4 weeks) was 43.9 $\pm$ 19.2 nmol/L. The mean $\pm$ SD increased from 34 $\pm$ 17 nmol/L to 58 $\pm$ 16 nmol/L at the second visit and to 78 $\pm$ 19 nmol/L at the third visit (Fig 1, A). At the second visit, none of the patients had 25(OH)D concentrations of less than 25 nmol/L and 30% had 25(OH)D concentrations below 50 nmol/L. The majority (60%) had circulating 25(OH)D levels between 50 and 75 nmol/L and 10% above 75 nmol/L. At the final visit, only 5% of the patients had 25(OH)D levels below 50 nmol/L, 40% between 50 and 75 nmol/L, and 55% had 25(OH)D levels of more than 75 nmol/L. Progressively larger doses of UV light had to be administered in order to cause similar increments in 25(OH)D levels (Fig 1, B). Skin color was also noted to darken with successive visits (Fig 1, C and D). Consistent with earlier work,^{17,19,31} the increase in 25(OH)D levels was seen in the 25(OH)D3 rather than 25(OH)D2 fractions (mean $\pm$ SD for baseline, 2 weeks, and 4 weeks, respectively, was 32.4 $\pm$ 16.8, 56.4 $\pm$ 16.3, and 77.1 $\pm$ 19.6 nmol/L for 25(OH)D3 and 2.0 $\pm$ 3.2, 1.5 $\pm$ 2.3, and 1.1 $\pm$ 2.0 nmol/L for 25(OH)D2. Between 60% and 70% of the subjects had undetectable levels of circulating 25(OH)D2. All subsequent analyses used total 25(OH)D, the sum of D2 and D3 fractions.

Preliminary analysis of markers of immune function in relation to UV dose and vitamin D status

At baseline, there were positive associations between 25(OH)D levels and markers of immune function, which were significant only for numbers of Log_nCD4⁺CD25^{hi}FoxP3⁺ Treg cells (Table II). With UV treatment, the positive relationship between 25(OH)D and Treg cells was consistent over the first half of the study and there were negative nonsignificant associations of 25(OH)D with proliferation, IFN- γ , and IL-10 responses. The negative association between UV and IL-10 was statistically significant for the latter half of the study.

Treg cells

The mean proportion of CD3⁺ cells bearing a CD4⁺CD25^{hi}FoxP3⁺ regulatory phenotype was relatively low

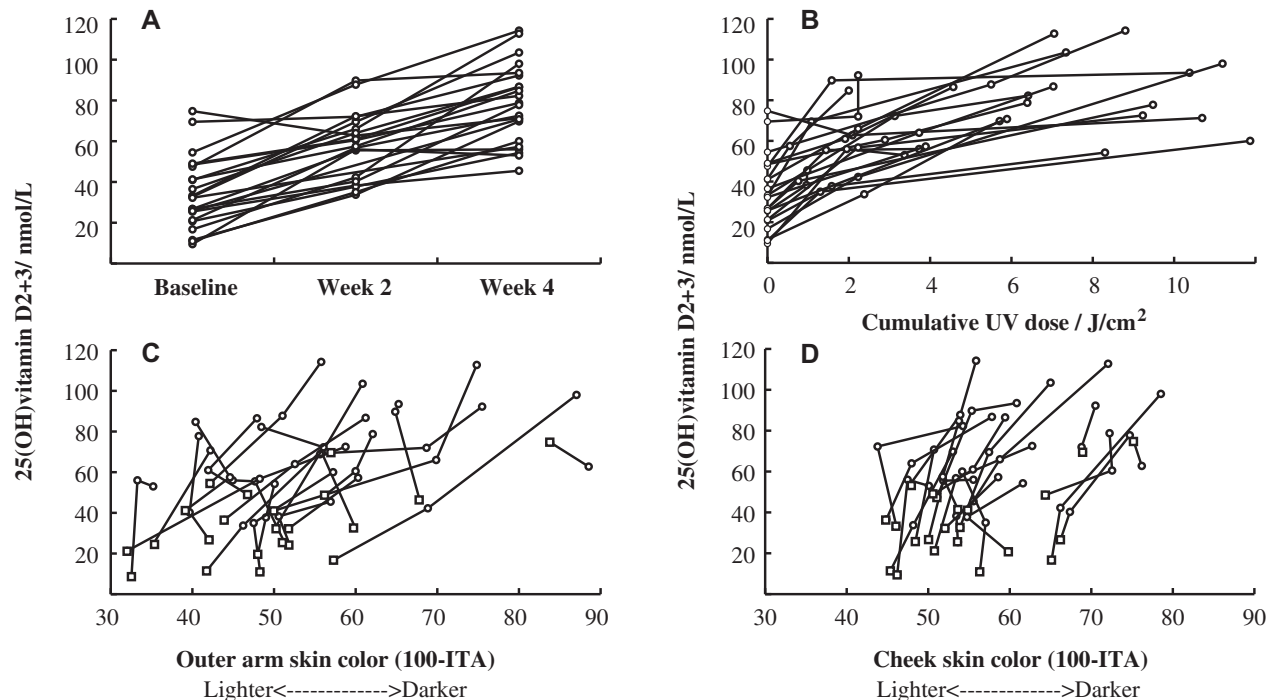


FIG 1. Total 25(OH)D serum concentrations and skin color responses in each individual to narrowband UV-B. 25(OH)D data are split by visit (**A**) and cumulative UV dose (**B**). Total 25(OH) D is the sum of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2. Skin colors are shown for outer arm (**C**) and cheek (**D**). Skin color is measured by ITA. Square points indicate baseline values, circular points indicate data from weeks 2 and 4.

at baseline, 0.5%. After exposure to UV-B, change in Treg cells was 0.81 (1.92%) from baseline to 2 weeks; 0.15 (2.42%) from 2 to 4 weeks; and 0.94 (2.25%) overall (baseline to 4 weeks). The mean proportion of Treg cells increased to 1.4% ($P = .004$ paired t test on logarithmically transformed data) and then to 1.6% at the second and third visits, respectively. In view of the evidence^{6,32} that 25(OH)D levels might influence the differentiation of Treg cells, we analyzed their relationships further. An association between transformed baseline Treg cell proportions and 25(OH)D levels at the first visit was noted ($R^2 = 0.41$) (Fig 2, A). This association between Treg cell proportion and 25(OH)D levels was less strong by visit 2 ($R^2 = 0.17$) and disappeared by visit 3, $R^2 < 0.01$ (Fig 2, B and C), suggesting that low 25(OH)D concentrations limit Treg cell numbers. In addition to these associations at single time points, we analyzed whether longitudinal increases in Treg cell proportions were associated with increases in 25(OH)D levels. Fig 2, D, shows a striking association between increase in 25(OH)D levels and increase in Treg cells when visit 1 is compared with visit 2 ($R^2 = 0.37$). In contrast, this association is lost when comparing visits 2 and 3 (Fig 2, E; $R^2 = 0.04$). Fig 2, D, suggests a trend for a steep dose-response relationship of Treg cells with 25(OH)D at lower concentrations that decreases progressively with increasing 25(OH)D concentrations, with no effect apparent over approximately 50 nmol/L^{-1} . Further exploration of this association by using mixed model linear analysis showed (Table III) that 25(OH)D significantly and positively predicted the increase in Treg cells ($P < .001$). This early effect was robust to the inclusion of possible confounders into the model: dietary vitamin D was found to be an additional

independent predictor of Treg cells as was the time of visit (significant at 2 weeks posttreatment) (Table III).

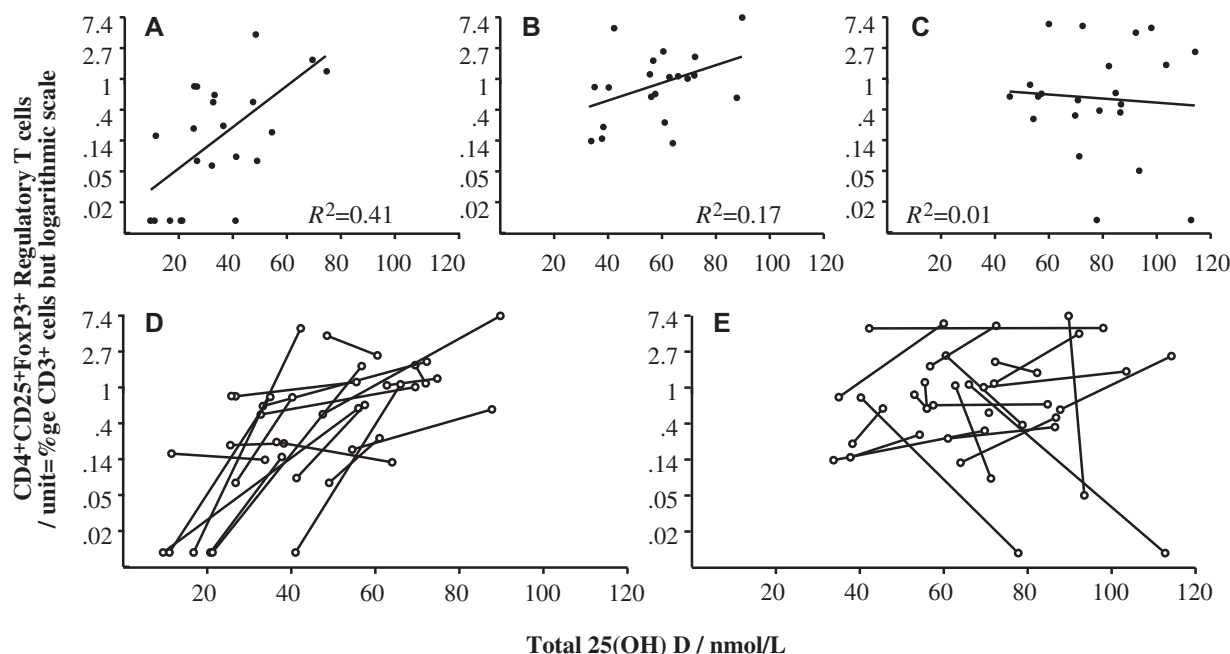
Proliferation and cytokine secretion in response to stimulation with anti-CD3/CD28

If the relative deficiency of Treg cells associated with low circulating 25(OH)D concentrations has functional significance, then low levels of the vitamin might be associated with a concomitant increase in effector T-cell responsiveness. In order to test this, we used activation with anti-CD3/CD28 beads, which stimulate T cells, including Treg cells, nonspecifically, and measured the resultant proliferation and secretion of cytokines IFN- γ and IL-10 (Fig 3). There was a fall in proliferation during the UV treatment period (Fig 3), which was significant between visits 1 and 3 (Wilcoxon's signed rank test $P = .048$). However, proliferation was not significantly correlated with 25(OH)D and was therefore not entered into the mixed model. IFN- γ secretion also fell between visits 1 and 2, but this change did not reach statistical significance. In contrast, log-transformed IL-10 secretion fell significantly between visits 1 and 2 ($P < .001$; paired t test) and between visits 1 and 3 ($P < .001$) but not between visits 2 and 3. Spearman correlation was significant for IL-10 with narrowband UV-B dose ($P = .009$) but not with 25(OH)D levels. Further exploration of this effect by using mixed model linear analysis showed (Table III) that the cumulative dose of narrowband UV-B (and not 25(OH)D) significantly and negatively predicted the IL-10 level ($P = .029$). This finding was also robust to the inclusion of possible confounders into the model, increasing the estimated size of the effect (beta) and observed significance ($P = .016$) (Table III).

TABLE II. The relationship between immune function, vitamin D status, and UV-B dose

Measurement	n	Median (IQR)	Relationship with 25(OH) D		Relationship with UV-B dose	
			Spearman correlation coefficient	Spearman correlation coefficient		
Baseline						
Treg cells (%CD3)	22	0.17 (0.63)	+0.62	0.002	N/A	N/A
Proliferation cpm × 10 ³	20	32433 (37130)	+0.28	0.22	N/A	N/A
IFN-γ (ng/mL)	21	1870 (7913)	+0.27	0.22	N/A	N/A
IL-10 (pg/mL)	22	612 (1856)	+0.20	0.38	N/A	N/A
Early effects (week 2 – baseline difference)						
Treg cells (%CD3)	21	0.38 (0.98)	+0.67	0.001	−0.09	0.68
Proliferation cpm × 10 ³	19	−11022 (27490)	−0.43	0.07	+0.09	0.72
IFN-γ (ng/mL)	20	−115 (4573)	−0.19	0.43	−0.03	0.82
IL-10 (pg/mL)	22	−223 (1352)	−0.18	0.45	−0.15	0.50
Late effects (week 4 – week 2 difference)						
Treg cells (%CD3)	21	0.13 (1.18)	+0.05	0.83	−0.095	0.68
Proliferation cpm × 10 ³	19	−5212 (27382)	−0.12	0.62	+0.16	0.52
IFN-γ (ng/mL)	21	+128 (1500)	−0.23	0.33	−0.23	0.31
IL-10 (pg/mL)	22	−19 (351)	−0.20	0.39	−0.54	0.009
Overall (week 4 – baseline difference)						
Treg cells (%CD3)	22	0.32 (1.49)	+0.31	0.15	+0.14	0.54
Proliferation cpm × 10 ³	20	−11950 (28940)	−0.20	0.39	−0.09	0.70
IFN-γ (ng/mL)	21	−7.4 (6920)	−0.33	0.14	−0.20	0.39
IL-10 (pg/mL)	22	−110 (1361)	−0.06	0.77	−0.04	0.87

IQR, Interquartile range; N/A, not applicable.

**FIG 2.** Correlations between total 25(OH) D levels and the proportion of CD4⁺CD25⁺ FoxP3⁺ Treg cells. **A**, baseline, **B**, week 2, and **C**, week 4. A significant correlation observed at baseline is weaker at 2 weeks and lost at 4 weeks. Longitudinal data show that Treg cells increased between week 0 and week 2 (**D**) ($P < .02$ Spearman) but not thereafter (**E**).

DISCUSSION

We report a study on systemic immune function when patients are exposed to narrowband UV-B. The main findings were that both peripheral blood 25(OH)D and Treg cell numbers increased,

both parameters being highly correlated at low (<50 nmol/L) concentrations of the vitamin. Markers of immune activation were also inhibited, with that of IL-10 being more closely related to UV dose than 25(OH)D concentrations.

TABLE III. Mixed model analysis to determine predictors of Treg cells and IL-10

Independent variable	Estimate (β)	95% CI	P value
Dependent variable: Treg cells*			
Constant	-2.98	-3.95 to -2.02	<.001
25(OH)D total (nmol/L)	+0.04	+0.02 to +0.06	<.001
UV dose	-0.11	-1.30 to +0.06	.19
Adjusted model			
Constant	-4.61	-6.67 to -2.54	<.001
25(OH)D total (nmol/L)	+0.04	+0.03 to +0.06	<.001
UV dose	-0.06	-0.29 to +0.17	.60
Visit			
Week 2	+1.15	+0.11 to +2.20	.03
Week 4	+0.09	-1.72 to +1.91	.92
Age (y)	0.01	-0.01 to +0.04	.13
Body mass index (kg/m ²)	0.04	-0.01 to +0.09	.08
MED	-1.95	-5.25 to -1.35	.23
Dietary vitamin D (μ g/d)	-0.29	-0.45 to -0.12	.002
Dependent variable: IL-10*			
Constant	+6.27	+5.36 to +7.19	<.001
25(OH)D total (nmol/L)	-0.01	-0.02 to +0.01	.51
UV dose (J/cm ²)	-0.14	-0.27 to -0.01	.03
Adjusted model			
Constant	+5.96	+2.47 to +9.44	.002
25(OH)D total (nmol/L)	-0.01	-0.03 to +0.01	.51
UV dose (J/cm ²)	-0.19	-0.34 to -0.04	.02
Visit			
Week 2†	-0.71	-1.44 to +0.01	.05
Week 4†	+0.24	-1.07 to +1.55	.71
Age (y)	-0.03	-0.07 to +0.01	.09
Body mass index (kg/m ²)	+0.03	-0.05 to +0.12	.42
MED	+1.56	-4.27 to +7.40	.58
Dietary vitamin D (μ g/d)	+0.20	-0.09 to +0.48	.16

In this mixed model linear analysis, the dependent variable (either Treg cells or IL-10) is included as a variable that is repeated according to visit ("repeated measures"). There are 3 time points: baseline, 2 weeks, and 4 weeks. The independent time-varying variables tested included UV dose and 25(OH)D with dietary vitamin D as confounder. Age, body mass index, and MED were included as fixed variables. This analysis allows for the adjustment of baseline measurements and confounders.

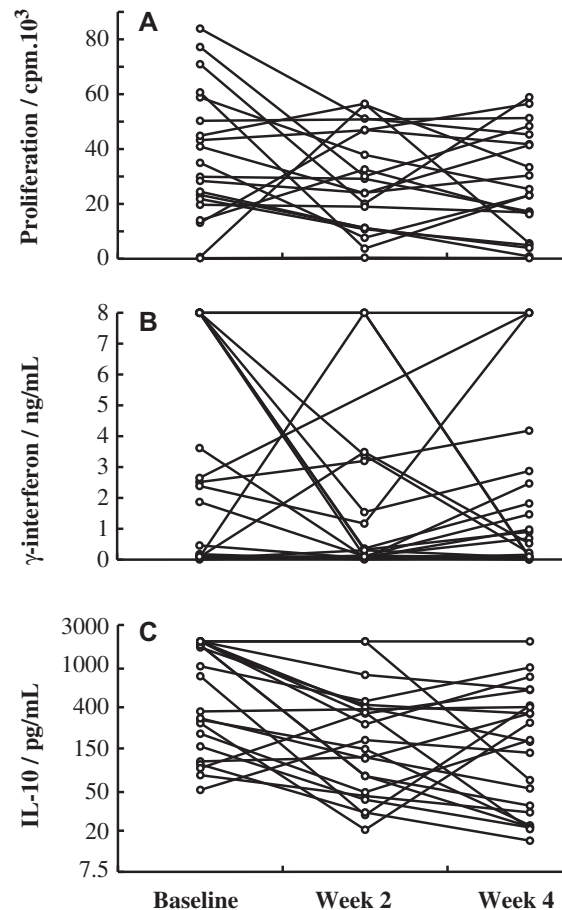
MED, Minimal erythema dose.

*Natural logarithm transformed.

†Baseline visit as comparator.

The geographic location and careful seasonal timing of our study caused our subjects to have low levels of 25(OH)D at the baseline visit, although defining what constitutes deficiency is controversial.³⁰ We showed that 25(OH)D increases rapidly with narrowband UV-B, in agreement with previous studies^{17,18,33-35} We also noted that the increments of 25(OH)D became successively smaller with successive visits, an effect that has been noted before and is partly due to UV light also having a catabolic effect on the vitamin.³⁶ In addition, the majority of our subjects started with lower levels of CD3⁺CD4⁺CD25^{hi}FoxP3⁺ Treg cells than those previously reported,^{37,38} although proportions in different studies are difficult to compare due to variation in methodology. Others have not found a correlation between 25(OH)D levels and Treg cells,³⁹ but our study was performed by using patients with low baseline levels of the vitamin, and the data suggest that the association is greatest under these conditions.

This study is the first to report narrowband UV-B increasing Treg cell numbers in human peripheral blood. However, the result is

**FIG 3.** Relationships between immune responsiveness as narrowband UV-B treatment progresses from baseline to 4 weeks. A significant decrease in proliferative response ($P < .05$) (A) and trend toward IFN- γ production (B) was seen. A significant decrease in IL-10 production ($P < .001$) (C) was observed between weeks 0 and 2.

supported by the recent demonstration²² of a relative increase in dermal FoxP3⁺ cells in patients with psoriasis exposed to controlled natural sunlight. In contrast, a study of 9 patients with atopic dermatitis treated with narrowband UV-B failed to show a significant change in FoxP3⁺ cells⁴⁰ in lesional skin. Our data suggest that the effects of narrowband UV-B on Treg cells were mediated via the restoration of 25(OH)D levels, a view supported by the emerging evidence of the roles of vitamin D in immune function. Oral supplementation with vitamin D and an analogue alfacalcidol increased Treg cell number in 2 human studies^{38,41} and function but not numbers in another,¹⁵ but again the effects we describe may be more prominent due to low baseline levels. Vitamin D can promote a Treg-cell response *in vitro*,^{9,13,42,43} and immunization of mice through skin pretreated with the analogue calcipotriol led to the generation of FoxP3⁺ antigen-specific Treg cells, mimicking the effect of UV-induced tolerance.⁴⁴ Furthermore, knockout experiments indicate that UV-induced tolerance in the mouse is dependent on vitamin D receptor.⁴⁴

One question is whether the local production of vitamin D in the skin has more potent, or different, immune effects from dietary supplementation. Formal comparison of the 2 approaches is now required, but there is already evidence of important local properties. Thus, topical 1,25(OH)₂D calcitriol and analogues are

an effective treatment for psoriasis, and priming of epidermal or dermal dendritic cells with vitamin D induces FoxP3⁺ Treg cells or IL-10-secreting Tr1 regulatory cells, respectively.⁹

Although some of the immune changes we observed could be attributed to 25(OH)D synthesis, it also remains possible that narrowband UV-B has additional, direct effects. For example, UV-induced immunosuppression, much studied in mice, is a complex and systemic phenomenon⁴⁵⁻⁴⁷ involving dendritic and effector cells.^{20,48} Becklund et al⁴⁹ demonstrated that the experimental autoimmune encephalomyelitis model of MS was inhibited much more effectively by UV light than by oral supplementation with vitamin D, which was effective only at toxic doses. In mouse models of contact hypersensitivity, UV-B induces CD4⁺CTLA4⁺ IL-10-secreting Treg cells that inhibit sensitization via lymph nodes, and elicitation when injected into the skin.⁵⁰ These UV-induced Treg cells are thought to be induced by the migration of Langerhans cells harboring UV-damaged DNA.^{46,47} Such direct effects of UV might explain the inhibition of IL-10 being predicted by the UV dose and not by circulating 25(OH)D levels. This reduction in IL-10 responsiveness is not inconsistent with the increases in Treg cell numbers we observed, since human CD4⁺CD25^{hi}FoxP3⁺ regulatory cells do not mediate suppression via this cytokine, at least *in vitro*.^{51,52} IL-10 is a pleiotropic cytokine produced from a number of sources, including T helper 2 (T_H2) effector cells. Although IL-10 production was more closely associated with the UV dose in our study, it may be relevant that vitamin D has also been reported to promote a shift from T_H1 to T_H2 cells and increase IL-10 secretion.¹⁶

Considering the clinical application of our results, it should be noted that the UV doses in J/cm² we delivered to the entire skin surface in a session lasting a few minutes are higher than casual exposure of unprotected skin to terrestrial daylight, particularly in Scotland. In the first 2 weeks, a single session of 0.7 to 4.2 SED is equivalent to a few days of summer sunlight received by free-living women in Aberdeen.^{53,54} Patients had received a median of 11.8 SED midway and a median of 39.1 SED over the complete study.

For healthy older women living in Aberdeen, the median solar radiation for the full year was 176 SED between 2009 and 2010 (unpublished) and 134 SED between 2006 and 2007⁵⁴ (most of it received in the summer months), which is similar to the 166 SED estimated in Danish indoor workers.⁵⁵ In the Aberdeen study, median sunlight exposure was 144 SED during spring-summer 2009, and 95% women had received 39 SED or more, so that during summer most people would be exposed to UV doses similar to the median total UV dose in our study. Whether lower doses comparable to natural exposure are also effective remains to be determined.^{54,55}

In summary, we have shown that exposure of human skin to narrowband UV-B can be responsible for systemic immune effects, which are most prominent when 25(OH)D concentrations are initially low. These regulatory effects have wider implications than the treatment of skin disease and are relevant to the influence of latitude, lifestyle, diet, and therapy in the prevention of immune-mediated disease. To carry this work forward, we propose a randomized trial of the differential effects of UV-B exposure and equivalent dietary vitamin D supplementation on Treg cells and immunity.

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Key messages

- Narrowband UV therapy of human patients increases circulating regulatory T-cell numbers and reduces immune responsiveness.
- It is likely that vitamin D status is a key determinant of regulatory T-cell numbers.
- These findings have important implications for future interventions based on the roles of phototherapy and/or vitamin D supplementation in the prevention or treatment of autoimmune and inflammatory diseases.

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