Effects of ultraviolet irradiation on natural killer cell function in systemic lupus erythematosus

Ola Nived, Ingrid Johansson, Gunnar Sturfelt

In vitro irradiation with long wavelength ultraviolet light (UV-A), in clinically relevant dosages, of a natural killer cell line containing cell preparations from 17 control subjects reduced natural killer cell cytotoxicity with the cell line K562 as target. The spontaneous function of natural killer cells from 12 patients with systemic lupus erythematosus (SLE) correlated inversely with the one hour erythrocyte sedimentation rate, but not with glucocorticoid doses. After UV-A exposure, natural killer cells from patients with SLE exert either increased or decreased cytotoxicity, and the direction of change is inversely correlated with the spontaneous natural killer cell function.

Natural killer cell activity is reduced in patients with systemic lupus erythematosus (SLE) using a standard K562 cell line as a target,¹ lymphoblastoid and myeloid cell lines,² and mammary epithelial cell lines.³ In patients with SLE a reduction of delayed hypersensitivity is also reported,⁴ which is at least partly mediated by natural killer cells. Silfverman and Cathcard¹ proposed that the reduced cytotoxicity is due to membrane bound serum inhibitory factors, probably immune complexes. Talal⁵ suggested that antibody dependent cellular cytotoxicity, regarded as the most important cytotoxicity mechanism in SLE and also known to be reduced in SLE due to immune complexes, was in fact an overlapping function with natural killer cells, mediated by similar, if not identical, cell populations.

Photosensitivity induced by short wavelength ultraviolet (UV-B) irradiation is a well documented symptom of SLE,⁶ and general photoimmunology has attracted a great deal of interest.⁷ In solar simulator studies, with a dominance of long wavelength ultraviolet light (UV-A), increased suppressor T cell activity reducing natural killer cell function has been reported in vivo in normal subjects.^{8,9} It has also been reported that the in vitro exposure of normal natural killer cells to UV-B radiation inhibits their function by a direct non-lethal effect, and that this inhibition occurs selectively at the postbinding stage of target lysis.¹⁰ In this work the in vitro effects of irradiation with UV-A light of natural killer cells from patients with SLE have been studied.

Patients and methods

PATIENTS AND CONTROLS

Twelve patients with SLE fulfilling at least four American Rheumatism Association revised

classification criteria⁶ were included in the study. There were two men and ten women, mean age 43 years (range 14-64). Disease activity at the time of blood sampling was assessed and graded with the SLEDAI index, where scores from 0 to 49 are theoretically possible, although in validation studies the range has been from 0 to 14 (Kalunian *et al*, unpublished data). Treatment with drugs was recorded and routine laboratory tests such as erythrocyte sedimentation rate, white blood cell counts, and complement components C1q, C3, and C4 were performed.

Seventeen healthy hospital staff, five men and 12 women, mean age 37 years (range 18-57), were used as controls. All participants gave informed consent and the SLE study was approved by the ethical committees of the University of Lund.

ISOLATION OF MONONUCLEAR CELLS

Cells were prepared according to Pross and Maroun,¹¹ with the exception of the iron carbonyl addition and the passage over a magnet. Blood samples were collected in heparinised vacuum tubes and 30 ml volumes were layered onto 15 ml of Lymphoprep (Pharmacia, Uppsala, Sweden).¹² The gradients were centrifuged for 30 minutes at 750 g at room temperature and the lymphocyte monocyte band at the interface, containing natural killer cells, was removed. The band was washed twice in RPMI 1640 and resuspended in RPMI 1640 containing 10% fetal calf serum, 50 µg/ml gentamycin, and 2 mM L-glutamine. The mononuclear cells were counted and adjusted to three concentrations (2.5×10^6 , 1.25×10^6 and 0.625×10^6 viable cells/ml).

TARGET CELL PREPARATION

The chronic myelogenous leukaemic, HLA negative, and Epstein-Barr free standard cell line K562 was used as the target for natural killer cell activity.¹³ Cells were cultured in suspension (RPMI 1640 containing 10% fetal calf serum, 50 µg/ml gentamycin and 2 mM L-glutamine) and the cultures were fed three times weekly. For labelling, approximately 10^6 target cells in 0.1 ml of phosphate buffered saline were incubated with 5550 kBq chromium-51 for one hour at 37°C. The labelled cells were washed three times in medium and resuspended at the appropriate concentration (2.5×10^4 cells/ml) to give 2500 cells/well.¹¹

ULTRAVIOLET IRRADIATION

The ultraviolet source was a solar simulator bed (Solana, Motala, Sweden) equipped with 19

Department of
Rheumatology,
University Hospital,
Lund, Sweden
O Nived
I Johansson
G Sturfelt

Correspondence to:
Dr Ola Nived,
Department of
Rheumatology,
University Hospital,
S 221 85, Lund, Sweden.

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fluorescent tubes (Philips TLK 100 W) with an emission spectrum of 295–400 nm and an effect of 136–166 W/m² in the UV-A range and 0.005–0.010 W/m² in the UV-B range (National Institute for Radiation Protection, Stockholm, Sweden), determined at 0 cm distance.

Irradiation of normal cells was studied in a dose response manner and an exposure time of 14 minutes was selected, giving a decrease of cell mediated target lysis from a median of 14.8% (range 4.5–43%) to a median of 1.9% (range 0–9.4%) with an effector to target cell ratio of 100:1. Longer exposure times tested did not have any significant further decrease of target cell lysis. The exposure distance chosen, 50 cm, was the shortest possible distance compatible with the requirement of constant temperature at the time given. The mean UV-A effect at this distance was 98 W/m² (Waldmann PUVA-meter, Villingen-Schwenningen) and thus the UV-A dose was 82.3 kJ/m². The cell viability determined with trypan blue exclusion was within the range 96–100% in all experiments. Control experiments with an ultraviolet filter (Kodak Wratten, gelatin filter No 1A) and ultraviolet longpass filters (Schott glass filters Nos WG320, WG345, GG375, and GG395) were performed.

NATURAL KILLER CELL ASSAY

The natural killer function was assessed by the chromium release assay with the target cells labelled with chromium-51 incubated for four hours in 5% carbon dioxide at 37°C, with three twofold dilutions of effector cells. Each effector to target (ET) ratio was plated in triplicate. The cell mediated lysis (CML) was calculated from the following equation, where CPM_{test} refers to the counts in the supernatant from wells containing effector cells, CPM_{spont} is the spontaneous release without effector cells present, and CPM_{max} is the maximum incorporated counts obtained by lysis with detergent¹¹

$$\%CML = \frac{CPM_{test} - CPM_{spont}}{CPM_{max} - CPM_{spont}} \times 100$$

DATA ANALYSIS

Killing at each effector to target ratio was expressed as the mean percentage of target cells lysed during the incubation period. Lytic units

were calculated using a linear regression analysis that incorporated the percentage of chromium released at each of three effector to target ratios. Results are expressed as the number of lytic units per 10⁷ effector cells (LU₂₀/10⁷); LU₂₀ represents the number of effector cells required to lyse 20% of the target cells (10³). To correct for day to day variations at least one or two of the 17 controls were included in each experiment and the percentage cytotoxicity for each patient was compared with the normal control value. To standardise the comparison between different experiments the results are also presented as relative natural killer cell function (RNK). The RNK value was calculated according to the following equation:¹¹

$$RNK = \text{individual LU on day of assay} / \text{mean LU for all controls}$$

Only non-parametric statistical tests were used; for two independent samples the Mann-Whitney *U* test and for related samples the Wilcoxon matched pairs signed rank test. Correlation was calculated with Spearman's rank test.¹⁴ *p* Levels less than 0.05 were considered significant.

Results

There was no difference in lytic activity between the groups of patients and controls before irradiation of cells, but four individual patients had a lower RNK value than any of the controls. All of these four patients had active disease; one young women (patient No 1) had cutaneous vasculitis, cranial nerve manifestations, and new rash; one man (patient No 2) had new onset proteinuria; another young women (patient No 3) had new rash and fever; and patient No 4, a man, had pronounced polyarthritits (table).

After irradiation lytic activity of natural killer cells was decreased in the controls (*p*<0.005) but not in the patients. Lytic activity was increased in five patients, whereas it was decreased in all controls. Two of these five patients were among the four with a low initial RNK value (patients Nos 1 and 2), whereas the other three patients (patients Nos 5, 6 and 10) were all clinically inactive, but had low complement concentrations (table). The amount of irradiation induced change of natural killer cell function also differed when patients and controls were compared (*p*<0.025, fig 1).

The ultraviolet wavelengths responsible for the decrease of natural killer cell function seen in controls were UV-A, as shown with the longpass ultraviolet filters (fig 2).

Correlations were calculated between the RNK value, before and after irradiation, and the disease activity index, glucocorticoid dosage, erythrocyte sedimentation rate, and levels of C1q, C3, and C4. Neither of the complement components correlated with the RNK values. A trend towards an inverse correlation was found between disease activity and the RNK value before irradiation (table).

One patient (No 9) was sequentially analysed through a flare of disease activity and an inverse

Relative natural killer cell function (RNK) related to SLE disease activity (SLEDAI), erythrocyte sedimentation rate (ESR), and prednisolone dose (P).

Patient No	RNK-0 (0 min)	RNK-14 (14 min)	SLEDAI (14-0)	ESR (mm/h)	P (mg)
1	0.12	0.24	20	62	15
2	0.13	0.24	6	30	5
3	0.21	0.07	7	90	0
4	0.24	0.09	6	40	5
5	0.32	1.18	2	14	2.5
6	0.47	2.37	2	46	0
7	0.59	0.02	4	17	5
8	0.74	0.05	0	29	7.5
9	1.18	0.44	5	26	12.5
10	1.18	1.63	2	24	3.75
11	1.47	0.35	11	5	8.75
12	2.35	1.47	3	16	7.5
Correlation with RNK-0	—	—	r _s =-0.39 NS	r _s =-0.71 p<0.01	NS —

Figure 1 In vitro natural killer (NK) cell function in control subjects and patients with SLE before and after 14 minutes in vitro UV-A irradiation, expressed as individual relative natural killer cell cytotoxicity in (A), and as lytic units (LU) in groups in (B). Bars indicate median, 25th and 75th centiles, and range.

Figure 2 Relative in vitro natural killer (NK) cell cytotoxicity, before and after 14 minutes in vitro UV-A irradiation, in the presence or absence of ultraviolet longpass filters. Filter numbers indicate the longpass cutoff wavelengths. The Wratten filter transmission is 59% at 400 nm, 76% at 410 nm, 82% at 420 nm, and 84.6% at 430 nm.

Figure 3 Sequential relative natural killer (NK) cell in vitro cytotoxicity, before (●) and after (○) 14 minutes in vitro UV-A irradiation, of natural killer cells from a patient with SLE with varying disease activity (disease activity index).

relationship between the RNK value and disease activity was noted (fig 3). The difference in the RNK value, before and after irradiation, in patients with SLE tended to increase if the starting value was low and to decrease if the RNK value before irradiation was in the normal range (table).

Discussion
This study shows that 14 minutes of irradiation with ultraviolet light reduces natural killer cell function in vitro, and that this reduction is mediated by the long wavelength, low energy ultraviolet spectrum, or UV-A. A parallel in vivo observation has been reported by Hersey and coworkers, who found that repeated in vivo

exposures of normal subjects to UV-A induced a reduced natural killer cell activity up to 14 days after exposure.^{8,9} Interestingly, this inhibitory pattern was most pronounced in normal donors, whereas only natural killer cells from some patients with SLE reacted as controls. In contrast, five of 12 patients with SLE increased their RNK value when cells were exposed to UV-A irradiation.

We confirmed the previous observation that the RNK value is reduced in active SLE,¹ at least if an increased erythrocyte sedimentation rate is accepted as an indicator of active disease. Our results suggest that patients with a low RNK value are those who react with an increased RNK value on exposure to ultraviolet irradiation. The fact that the RNK value in our patients as a group did not differ from the controls before irradiation is probably due to the proportion of patients included with inactive disease. No influence of treatment, especially glucocorticoids, could be detected, but the doses were in all instances below those previously reported to reduce the RNK value.¹⁵ One patient treated with azathioprine was among those with a reduced RNK value, which is in accordance with the inhibited maturation of natural killer cells reported in patients with rheumatoid arthritis receiving this drug.¹⁶ The natural killer cells from this patient with SLE also increased their lytic activity after UV-A irradiation.

The UV-A radiation reaching the dermal capillaries of a subject on the earth's surface on a sunny day is about 25 W/m², assuming a tissue transmittance of 10%.¹⁷ Thus our UV-A irradiation dosage corresponds to one hour in the sun under similar conditions. The exposure therefore seems to be clinically appropriate, but caution is needed in interpreting data based on circulating natural killer cells, even if some precursors travel through the circulation before homing to their target tissue.¹⁸

The mechanism behind reduced natural killer cell function in active SLE is not known, but serum factors, perhaps blocking immune complexes,¹ antibodies to lymphocytes,¹⁹ or reduced interleukin 2 mediated signal transduction²⁰ are likely to play a part. A high proportion of immature, circulating natural killer cells are found in patients with SLE and multiple sclerosis,²¹ and natural killer cells in patients with SLE are less responsive to interferon.²² Interferon γ , also produced in natural killer cells, increases the lytic activity of normal natural killer cells, but a negative feedback through increased Fc receptor presentation on the cell surface by interferon γ has also been shown.²³ As interleukin 2 stimulates interferon production, the low levels of interleukin 2 in serum samples from patients with active SLE²⁴ also might influence natural killer cell function.

Hypothetically, a reduction of interferon activity induced by UV-A would lead to reduced spontaneous activation of natural killer cells from normal donors. In the patients with SLE with increased natural killer cells,²⁵ expressing high Fc receptor densities on the cell membrane, and Fc bound immune complexes, all down-regulating the RNK value, a reverse scenario

seems possible. Hypothetically, UV-A might release the blocking immune complexes from Fc binding and thereby increase the RNK value.

Photoactivation of cutaneous SLE has long been known to occur,²⁶ as has the activation of systemic manifestations.^{27,28} The importance of UV-A in this respect is poorly documented, but it has been suggested by an anecdote of exposure to ordinary fluorescent tubes.²⁹ It is too early to say if the increase in the RNK value observed in vitro in some of our patients with SLE has any bearing on the clinical disease; however, the selective in vitro effects of UV-A on natural killer cells in SLE merits further investigation.

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