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Frequencies of *hprt*⁻ mutations and *bcl-2* translocations in circulating human lymphocytes are correlated with United Kingdom sunlight records

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Between 1983 and 1995 we have monitored human populations for evidence of exposure to environmental mutagens, taking blood samples to measure *hprt*⁻ mutant frequency in T cells and more recently *bcl-2* t(14:18) translocation frequency in B cells. We have now analysed data from 785 assays on 448 blood samples from 308 normal subjects and find that there is a highly significant statistical correlation between *hprt*⁻ mutant frequency and the sunlight record for the 3 weeks prior to taking the blood sample. We discuss the weaknesses in retrospective studies of this nature and the possibility of spurious epidemiological correlations that may result. More controlled experiments can be envisaged that would give a firmer basis to the statistical associations observed. *hprt*⁻ mutations in T cells show little evidence of a UV fingerprint, so that the correlation may be due to immunomodulation rather than mutation. We also find a correlation between the sunlight record and *bcl-2* translocation. This translocation is found at a low frequency in the B cells of many normal subjects and is the commonest translocation observed in non-Hodgkin's lymphoma. Our results strengthen the case for a link between sunlight and this increasingly common cancer.

Introduction

Since the early 1980s we have been monitoring blood samples from members of the UK population for evidence of exposure to mutagenic agents in the environment. We have measured the frequency of mutations at the X-linked *hprt* locus in T cells (Cole *et al.*, 1988, 1997). Patients with the serious sex-linked recessive disease Lesch–Nyhan syndrome (Lesch and Nyhan, 1964) carry mutations in this gene, but carriers show no obvious adverse effects and a small proportion of mutant T cells can be found in all adults we have tested. More recently we have also determined the frequency of B cells carrying the *bcl-2* t(14:18) translocation (Liu *et al.*, 1994, 1997; Cole *et al.*, 1996). This translocation leads to overexpression of the anti-apoptotic oncogene *bcl-2* in B cells and is the characteristic chromosomal alteration of the commonest form of non-Hodgkin's lymphoma (Jaffe *et al.*, 1992), follicular B cell lymphoma. The translocation, however, is also found at a low frequency in B cells from the majority of adult donors (Liu *et al.*, 1997).

In common with other laboratories (Robinson *et al.*, 1994), we have found that the frequency of mutant T cells increases with age and is higher in smokers. On the other hand, no effects of oil exposure following the wreck of the tanker *Braer* (Cole *et al.*, 1997) or occupational radiation exposure (Cole *et al.*, 1995) were observed and a possible effect of radon levels in homes was not confirmed (Bridges *et al.*, 1991; Cole *et al.*, 1996). For B cells the *bcl-2* translocation frequency has been shown to be elevated in heavy (Bell *et al.*, 1995) but not light (Liu *et al.*, 1997) smokers and is increased with age (Liu *et al.*, 1994). The frequencies of these two end-points of mutation and translocation were seen to be correlated (Liu *et al.*, 1997).

During our studies it has become apparent to us that *hprt*⁻ mutant frequency is influenced by some additional factor, related to the date when the sample is obtained (Cole *et al.*, 1997). Our analysis of the data suggests that this factor is sunlight.

Sunlight is a complete carcinogen in the skin (IARC, 1992). Its action as an initiating agent can be attributed to its ability to induce mutagenic lesions such as dipyrimidine photoproducts in DNA (Brash *et al.*, 1991; Dumaz *et al.*, 1994; Ziegler *et al.*, 1994; Daya-Grosjean *et al.*, 1995; Brash, 1997; The p53 Database, 1998). Its promoting action is believed to arise from its ability to modulate the immune response (Kripke, 1994; Shimizu and Streilein, 1994). Exposure of the cellular components of blood to sunlight will be low because of the screening effects of the skin. Nevertheless, there are claims, based on epidemiological arguments, that sunlight could account for the inexorable increase in non-Hodgkin's lymphoma in the UK and Western Europe (Cartwright *et al.*, 1994; Adami *et al.*, 1995; Bentham, 1996; Cliff and Mortimer, 1999), the seasonality of diagnosis of acute lymphocytic leukemia (Badrinath *et al.*, 1997) and the association of latitude with type I diabetes (Green *et al.*, 1993) or multiple sclerosis (McMichael and Hall, 1997). Such effects could arise through sunlight-induced release by skin cells of soluble cytokines such as interleukin-6 (IL-6) (Devos *et al.*, 1994; Petit-Frère *et al.*, 1998), which would then act indirectly on haemopoietic tissues.

The UK is particularly suitable for the detection of short-term effects of sunlight, because in addition to seasonal variation, the vagaries of the climate lead to substantial variation from week to week and between successive years in the amount of the highly damaging UVB (280–315 nm) component of sunlight reaching ground level. This allows the effects of sunlight to be distinguished from non-specific seasonal effects. Furthermore, people wear fewer clothes and spend more time out of doors in fine weather (Diffey, 1998). Regulation of body temperature leads to increased blood flow through the skin, so that fine weather can lead to a potent multiplier effect on exposure to UVB. Moller *et al.* (1998) have presented evidence of possible sun-associated seasonal variation in strand breakage in lymphocytes from blood

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samples. In the present paper we present evidence that variation in UVB exposure may affect the frequency of mutations and translocations in circulating blood cells. Our observations may be relevant to epidemiological evidence for (Cartwright *et al.*, 1994; Adami *et al.*, 1995; Bentham, 1996) and against (Hartge *et al.*, 1996) a link between sunlight and non-Hodgkin's lymphoma.

Materials and methods

Measurement of hprt⁻ mutant frequency

The majority of the material analysed here has been reported in earlier studies (Cole *et al.*, 1995, 1996, 1997; Liu *et al.*, 1997). To obtain an estimate of *hprt⁻* mutant frequency, a blood sample is taken following informed consent, the mononuclear cell (MNC) fraction is isolated, cryopreserved and subsequently cultured under conditions allowing T cells to grow to form colonies. Mutations inactivating the *hprt* gene allow a cell to grow to form a colony in the presence of the toxic purine analogue 6-thioguanine (Albertini *et al.*, 1988; Cole *et al.*, 1988). Data on *hprt⁻* mutant frequencies were available from 785 assays based on 448 samples from 308 subjects collected between May 1983 and October 1995. Some of these assays were derived from data collection campaigns carried out for specific reasons in three areas of the country: Somerset (120 assays) (Cole *et al.*, 1996), Cumbria (83 assays) (Cole *et al.*, 1995) and the Shetland Islands (164 assays) (Cole *et al.*, 1997). However, the majority (418 assays) came from sampling of individuals mainly from the Brighton area. The subjects included in the present analysis were normal individuals, either from control groups for different studies or from test groups where there was no evidence that hypothetical exposure to an environmental mutagen had influenced mutant frequency. As well as data on *hprt⁻* mutant frequency, information was available on the age, sex and smoking status of the subjects, on the cloning efficiency of T cells in the assay, on the date when the blood sample was taken and on the date when the assay was performed. The dates were used to link the mutant frequencies with estimates of solar UVB.

Measurement of bcl-2 translocation frequency

For estimation of *bcl-2* translocation frequencies, DNA was extracted at the MRC Cell Mutation Unit from the MNC fraction by conventional methods (Sambrook *et al.*, 1989) and either sent to the US laboratory or (the majority) analysed at Sussex. A nested PCR approach (Liu *et al.*, 1994) was used to detect the *bcl-2* translocation in 2 μg aliquots of these DNA samples. Translocation frequency was estimated from the proportion of aliquots giving no product and assuming that 1 μg DNA from the MNCs was equivalent to 150 000 cells. Where possible, the percentage of B cells in the MNC fraction was estimated by flow cytometry (Liu *et al.*, 1997) but where insufficient cells were available for this to be done, it was assumed to be 10%, a figure based on the mean of our determinations on 43 normal adult blood samples.

Estimation of UVB levels prior to taking blood samples

We have made an indirect estimate of UVB levels at and before the time at which the blood samples were taken, using daily data from Meteorological Office measurements (British Atmospheric Data Centre, 1997) of sunshine hours for the station nearest to the place where the blood sample was obtained. Since there are no long-run measurements of solar UVB radiation for the UK, our study had to be based on estimates. Mo and Green (1974) give tables of erythemally weighted solar UVB under clear sky conditions for each month of the year by latitude, from which daily values were calculated by interpolation. The actual amount received at the surface will be less than this, in particular because of variations in cloud cover. Mo and Green state that an approximation of the effect of cloud cover on UVB flux is given by:

$$F = 1 - 0.056\ C$$

where F is UVB flux as a proportion of clear sky values and C is cloud cover in tenths. Cloud cover was estimated from daily data on sunshine hours by subtracting the actual hours of sunshine from the maximum possible and using the relevant Meteorological Office measurements (British Atmospheric Data Centre, 1997). Combining the sunshine records with Mo and Green's data on monthly UVB under clear sky conditions and using their approximation for the effects of cloud cover generated an estimate of erythemally weighted UVB (in daily J/m^2) for each day of the study period. These daily estimates were then averaged for weekly periods of up to 8 weeks preceding the dates of each sample.

Statistical analysis

The main analysis was based on data from 785 *hprt⁻* assays. The mutant frequency data are highly skewed and following the practice in earlier studies the logarithm of mutant frequency was used in the analysis (Robinson *et al.*, 1994). If solar UVB radiation does act as a mutagen on circulating lymphocytes, *hprt⁻* mutant frequency would be expected to be higher in the summer, when UVB levels are greatest. However, a number of other possible influences, such as diet or exposure to infections, may also vary seasonally (Ames, 1983; Simic and Bergtold, 1991; Ohshima and Bartsch, 1994) and may affect the exposure of lymphocytes to reactive oxygen and nitrogen species. The pattern of mutant frequency over the year may therefore represent the combined effects of several factors. Any summer excess of *hprt⁻* mutant frequency associated with UVB levels could be obscured by a winter excess associated with the seasonal pattern of common infections or reduced antioxidant status of the diet. To control for seasonal factors the monthly means for the whole data set were subtracted from each observation of log *hprt⁻* mutant frequency. Similarly, the monthly means of UVB for 1983–1995 were subtracted from each UVB estimate. This allows the analysis to focus on whether when UVB is unusually high or low for that month of the year there is also an unusually high or low *hprt⁻* mutant frequency.

The *hprt* data were analysed separately for the summer (April to September) and winter periods. In winter UVB levels (and hence variations in UVB levels) are minimal. Multiple regression analysis was used to examine the relationship between adjusted log *hprt⁻* mutant frequency and UVB level, controlling for the other variables on which information was available, i.e. cloning efficiency, log age, sex and smoking status. UVB levels for individual weeks and for cumulative periods up to 8 weeks before the date of the sample were tested. Data were also tested for a link with the UVB estimate for the day on which the assay to determine mutant frequency was undertaken.

Data on *bcl-2* translocations were adjusted for seasonal factors in the same way as described for *hprt⁻*. An analysis was performed only for the summer period because we had few winter samples. Again, frequencies were log transformed, but after a constant of 0.5 had been added to each observation to allow for 0 values. Multiple regression analysis was then used to analyse association between log *bcl-2* translocation frequency and UVB, controlling for log age, sex and smoking status. Cloning efficiency is not relevant to the *bcl-2* analysis and smoking was not significant, therefore, these terms were dropped from the analysis. The marginally significant effects of log age and sex were retained.

Copies of the complete data set are available from the corresponding author.

Mutation spectra

Data on mutation spectra for the *hprt* gene were obtained from the MutaBase Software Human HPRT Database, Release 6 (Cariello *et al.*, 1997), including results for T cells from normal and xeroderma pigmentosum (XP) subjects. Additional data on lymphocytes from three XP subjects (46 mutants) were obtained by A.M.W. and are not yet in the Database. Data for *p53* mutation in skin tumours (Brash *et al.*, 1991; Dumaz *et al.*, 1994; Ziegler *et al.*, 1994; Daya-Grosjean *et al.*, 1995; Brash, 1997) were obtained from The p53 Database (1998).

Results

The outcome of a multiple regression analysis of our *hprt⁻* data is displayed in Table I. Analysis was performed separately on samples obtained in the summer or winter. Factors which we have shown previously to influence estimates of *hprt⁻* mutant frequency, T cell cloning efficiency, log age and smoking habit (Robinson *et al.*, 1994), were similar for summer and winter. For the summer period there was a strong correlation between mutant frequency and estimated UVB levels in the 3 weeks prior to sampling ($P < 0.001$). Associations with shorter and longer periods up to 8 weeks were statistically significant, but weaker. Taking the exponents of the regression parameters, the results in Table I for the summer period suggest that *hprt⁻* mutant frequency increases by ~70% for each doubling of age, is ~16% lower for females than males, is ~36% higher for smokers than for non-smokers, decreases by ~1% for each 1% increase in cloning efficiency and increases by ~0.1% for each 1 J increase in daily average UVB. This suggests that the highest level of UVB recorded in the study (530 J/m^2 above average) is associated with a 78% increase in *hprt⁻* mutant frequency. Removing UVB from the model produces a highly significant decrease in explanatory power ($P < 0.001$) with the coefficient of determination falling

Table I. Multiple regression analysis of factors influencing *hprt*⁻ mutant frequency and *bcl-2* translocation frequency

Predictor	Coefficient	SE	T	*P*
hprt⁻, summer (345 assays)[a]				
Constant	–2.2402	0.3116	7.19	<0.001
Log age	0.7638	0.0820	9.31	<0.001
Sex[b]	–0.1783	0.0669	2.66	0.008
Smoking[c]	0.3045	0.0843	3.61	<0.001
Cloning efficiency (%)	–0.01083	0.00146	7.41	<0.001
3 weeks UVB	0.00108	0.000241	4.51	<0.001
hprt⁻, winter (419 assays)[d]				
Constant	–2.1355	0.1846	11.57	<0.001
Log age	0.6947	0.0485	14.31	<0.001
Sex[b]	0.06105	0.06305	0.97	0.33
Smoking[c]	0.3214	0.0627	5.12	<0.001
Cloning efficiency (%)	–0.009358	0.001224	7.65	<0.001
3 weeks UVB	–0.002617	0.002692	0.97	0.33
bcl-2, summer (95 assays)[e]				
Constant	–4.272	2.046	2.09	0.040
Log age	1.2581	0.5526	2.28	0.025
Sex[b]	–0.7514	0.3490	2.15	0.034
3 weeks UVB	0.003119	0.0009858	3.16	0.002

[a]Four cases with missing values.
[b]Sex is coded as a binary variable with females = 1 and males = 0.
[c]Smoking is coded as a binary variable with smoking = 1 and non-smoking = 0. The multiple coefficients of determination (r^2) for *hprt*⁻ summer, *hprt*⁻ winter and *bcl-2* were 0.333, 0.414 and 0.173.
[d]Seventeen cases with missing values.
[e]Smoking habit and proportion of B cells were not significant and were omitted as terms from the *bcl-2* analysis.

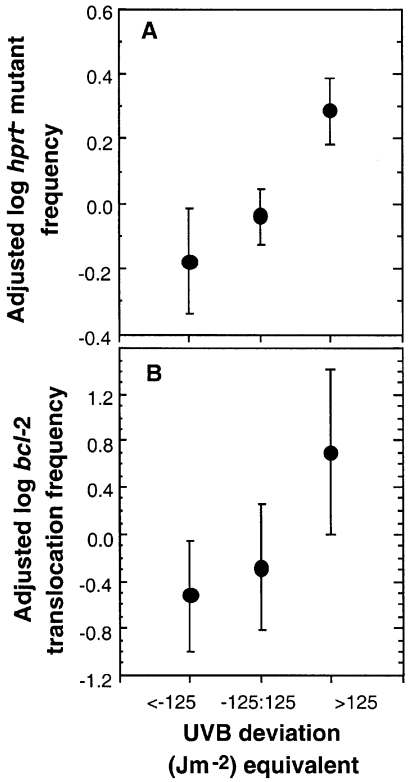

Fig. 1. (A) Adjusted log *hprt*⁻ mutant frequencies for blood samples obtained following periods of low (84 assays, more than 125 J/m^2/day below the seasonally adjusted UVB level for the period), average (184 assays, –125 to +125 J/m^2) and high (77 assays, >125 J/m^2 above) daily UVB levels for the 3 weeks before sampling. (**B**) Similar results for *bcl-2* translocation: low UVB (41 assays), average UVB (22 assays), high UVB (32 assays). In each case values presented are means and 95% confidence intervals.

to 0.293. There was no correlation for the winter data, when UVB levels are generally low. Because the range of UVB values in winter is small, a trivial negative effect has been magnified to an apparently large effect when expressed per J/m^2, but with an even larger standard error.

In a study such as this it is important to assess whether any alternative explanation of our results is plausible. An obvious environmental factor that might be confounded with sunlight is ambient temperature. Information on daily maximum temperatures is also available from the meteorological database (British Atmospheric Data Centre, 1997). As expected, sunlight and temperature are highly correlated (Pearson coefficient 0.761 for the summer period). A correlation of mutant frequency with temperature was indeed observed, but did not provide as good a fit to the data as estimated UVB level and when both temperature and UVB levels are included in the model only the latter remains significant. We have also considered the possibility that weather might affect laboratory conditions and that the association might be with the date when the experiment to determine mutant frequency was performed. This rarely coincided with the date of taking the blood sample, and was often months or even years later. There was no correlation of mutant frequency with the sunlight record for the date when the assay was performed.

The analysis was repeated with data obtained for *bcl-2* translocation frequency but restricted to the summer period. The analysis of this data set is less satisfactory for two reasons. Firstly, since this assay became available much later than the *hprt* system, fewer samples were available for analysis. Secondly, the distribution of the translocation frequencies includes a substantial number of 0 values (Liu *et al.*, 1997). Because of the 0 values, a constant of 0.5 was added to each value before transformation to logarithms, although this procedure does not eliminate difficulties in the estimation of variance. Nevertheless, it can be seen from Table I that there is a significant association ($P = 0.002$) between *bcl-2* translocation frequency and UVB levels in the 3 weeks prior to sampling. To ensure that the apparent significance of the sunlight effect was real, a number of alternative analyses have been performed. These include logistic regression and χ^2 tests of low and high sunlight groups for the proportion of subjects or assays without translocations. Each approach indicates a significant sunlight effect.

To give an indication of the size of the UVB effect on mutation and translocation, we divided the summer data set into three groups based on mean daily UVB relative to normal in the 3 weeks before sampling [<–125, –125 to +125, >+125 J/m^2; 125 J/m^2 is equivalent to ~50% of a minimal erythemal dose (IARC, 1992)]. Figure 1A shows that after adjusting for the other factors in the regression model there was a clear dose–response pattern with mean *hprt*⁻ mutant frequency for the high UVB group (77 assays), being 1.59 times greater than for the low group (84 assays) and 1.35 times greater than for the mid group (184 assays). This can be compared with the regression model estimate of an excess of 1.36-fold in frequency for smokers relative to non-smokers. The data for *bcl-2* translocations in Figure 1B also show a dose–response pattern with values for the higher exposure group (32 assays) being 3.40 times greater than for the low group (41 assays) and 2.67 times those for the mid group (22 assays). The 95% confidence intervals for *bcl-2* (Figure 1B), however, are substantially wider than for *hprt* (Figure 1A).

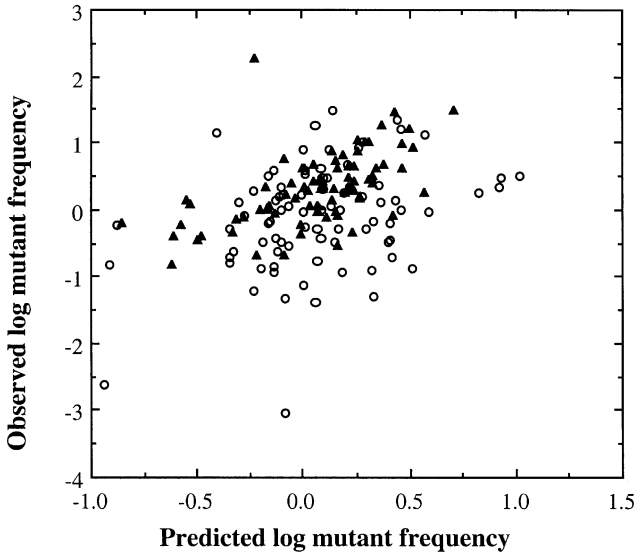

Fig. 2. Log *hprt*⁻ mutant frequencies for individual assays for samples obtained following periods of low (○) or high (▲) daily UVB levels for the 3 week period before sampling. Values have been seasonally adjusted, by normalizing against the average mutant frequency for blood samples for that month, so that values are distributed about log(1), i.e. 0. The observed values are then plotted against expected values, which allow for the effect of cloning efficiency and for the age, sex, and smoking habit of the donor.

Figure 2 shows the observed *hprt*⁻ mutant frequencies, plotted for individual assays, corresponding to periods of high sunlight, plotted against expected values for the observed cloning efficiency for donors of that age and smoking status. It can be seen that there is a complete overlap of values for samples from the high and low sunlight groups. However, as would be anticipated from the analysis in Table I, there is a clear tendency for values for the high sunlight group to be found towards the upper part of the spread of observed results. The plot is similar in appearance to that obtained for the effect of smoking in Sussex subjects (Robinson *et al.*, 1994) and, indeed, the strength of the sunlight and smoking effects are similar (Table I).

Sunlight is a carcinogen and will act as a DNA damaging agent and mutagen. The mutagenicity of the UVB component of sunlight is revealed by the spectrum of mutations in the *p53* gene found in both premalignant skin lesions and non-melanoma skin cancer. The major photoproducts induced by UVB and UVC irradiation are formed between adjacent pyrimidines on the same DNA strand and the *p53* mutations found in skin cancers from normal subjects occur almost exclusively at dipyrimidine sites (Brash *et al.*, 1991; Dumaz *et al.*, 1994; Ziegler *et al.*, 1994; Daya-Grosjean *et al.*, 1995; Brash, 1997; The p53 Database, 1998) (Table II). In addition, the CC→TT tandem mutation, an even more specific UV fingerprint, is found at elevated frequency in these skin cancers. In the DNA repair-deficient, sun-sensitive, cancer-prone syndrome xeroderma pigmentosum (XP), cells are deficient in the repair of UVB-induced DNA damage and the frequency of skin tumours is highly elevated (Kraemer *et al.*, 1987; Arlett and Lehmann, 1996). Again, the *p53* genes in these tumours show an elevated incidence of both mutations at dipyrimidine sites and tandem mutations.

Data are available on mutation spectra for the *hprt* gene in a number of human cell types (Cariello *et al.*, 1997), including results for T cells from normal and XP subjects. The influence of UVB on the DNA damage induced in these cells can be assessed by comparing the mutation spectra in cells from the normal and XP subjects. As the latter are unable to repair UVB-induced DNA damage and have an elevated *hprt* mutant frequency (Cole *et al.*, 1992), the XP cells should show an increased incidence of both transitions at dipyrimidine sites and tandem mutations, when compared with normal subjects (Table II). However, there is only a slight but not statistically significant increase in the incidence of transitions at dipyrimidine sites in the XP subjects and no increase in the incidence of tandem mutations. Thus, mutation spectra analysis offers little evidence of direct induction of mutants in T cells by the UVB component of sunlight. However, it should be acknowledged that the solar exposure history of both normal and XP subjects whose mutants have been analysed in these studies is not known.

Discussion

Although spurious epidemiological correlations are commonplace, the strength of the association that we have found, the length of the study and the size of the data set are unusual. How likely is our result to be an artefact? One criticism could be that our use of log transformed frequencies could underestimate the true level of variation, especially with the *bcl-2* translocation. The alternative analyses which we have attempted, however, confirm our conclusion. A more serious criticism arises from the way the data were obtained. Samples were not obtained uniformly over the 12 year period, but when a specific study was undertaken, a large number of samples were obtained over a limited time, so that specific periods of weather were over-represented. Nevertheless, a significant effect of sunlight was still seen when over-represented periods were omitted and when analysis was confined to samples from the Sussex area. Another point adding confidence to our conclusion is that detailed analysis indicates that the effect of sunlight is cumulative. *Hprt*⁻ mutant frequency is associated with UVB levels 0–7 days (parameter estimate 0.0004741 ± 0.0001339), 8–14 days (parameter estimate 0.0006450 ± 0.0001825) and 15–21 days (parameter estimate 0.0003660 ± 0.0001433) before taking the blood sample, but the cumulative effect for 0–21 days is nearly additive (parameter estimate 0.001108 ± 0.000241), despite the correlation that must exist between weather on one day and the next.

Real or artefactual, the consequences of this analysis have important implications for study design in all types of population monitoring. The possibility that sunlight, which is normally not controlled for, may have a profound influence on the outcome, counsels caution in ascribing effects to any particular component of the environment, either naturally occurring or artificial. It is clearly important to consider sample size, in order to reduce the risk of confounding a test parameter with a chance environmental effect and to ensure that genuine effects achieve high statistical significance. One major difficulty which always applies is the retrospective nature of investigations such as ours. It is, however, difficult to envisage any rational prospective study (such as on phototherapy patients or holiday-makers) which would be likely to achieve funding.

If the effect is real, what is the mechanism? While circulating T cells may have some exposure to sunlight, this is unlikely in cells which give rise to *bcl-2* translocations, since these events are generated in B cell precursors in the bone marrow.

Table II. Spontaneous/background mutation spectra from the human HPRT and p53 databases

Mutation type	*hprt*		*p53*	
	Primary T lymphocytes from 124 normal donors[a]	Primary T lymphocytes from 5 XP donors (groups A, C, F and V)[a]	Mutations in skin cancers from normal donors[d]	Mutations in skin cancers from XP donors[d]
Total	256	68	141	38
Base pair substitutions[b,c]	127 (50%)	39 (57%)	122 (86%)	29 (76%)
F/I/D[b]	113 (44%)	16 (24%)	7 (5%)	2 (5%)
Tandem	2 (2%)	2 (5%)	21 (17%)	18 (62%)
Others[b]	16 (6%)	13 (19%)	12 (9%)	7 (18%)
Mutations at dipyrimidine sites (%)	67%	79%	91%	100%
No. of G→A or C→T (%)	53 of 125 (42%)	13 of 38 (34%)	81 of 122 (57%)	20 of 29 (69%)
No. of G or C mutations (%)	85 of 125 (68%)	23 of 38 (60%)	110 of 122 (90%)	24 of 29 (83%)

[a]Data from Cariello *et al.* (1997). Mutations from three of the subjects (46 mutants) were analysed by A.M.W. at the SEAC Toxicology Unit, Colworth, Unilever Research and are not yet in the database.
[b]F/I/D, frameshifts, insertions and deletions; others includes mutants with more than one mutation and those where the mutation has not been defined.
[c]The total number of mutations for base pair substitutions includes tandem mutations. Tandem mutations are excluded from the no. of G→A or C→T (%) and no. of G or C mutations unless the 2 bp substitutions comprising the tandem mutations are identical.
[d]Data from the The p53 Database (1998).

Furthermore, expression of *de novo hprt*⁻ mutations in T cells takes ~7 days in mouse lymphoma (Cole and Arlett, 1984) or hamster (Arlett, 1977) cells and almost certainly longer in human lymphocytes *in vivo*. It is, therefore, necessary to consider an indirect mechanism for the significant UVB effects we have described. We may be observing the equivalent of the UVB systemic immunosuppressive effect in the mouse (Kripke, 1990; Shimizu and Streilein, 1994). In humans evidence of immunosuppression comes from the increased incidence of skin cancer in immunosuppressed transplant patients (Liddington *et al.*, 1989) and the demonstration of UVB-induced down-regulation of the key signalling molecule ICAM-1 (Ahrens *et al.*, 1997). How then might solar-directed immunomodulation be responsible for perturbation of the *hprt*⁻ mutant frequency in circulating T cells or *bcl-2* translocation in B cells? A possible explanation is that different subsets of T cells have different mutant frequencies (Baars *et al.*, 1995). Thus, if an agent such as IL-6 is able to encourage the multiplication or increase the circulation of a particular class of T cells, the effect will be an apparent change in mutant frequency. The relative lifespans of different classes of T cell in culture are problematical and complex (Freitas and Rocha, 1993; Green *et al.*, 1995) so that it is not easy to predict the effect of such a change or how long an apparent shift in frequency might persist. Similarly, modification of the immune response might lead to selective amplification of B cells in the circulation bearing the *bcl-2* translocation. Indeed, in experiments with transgenic mice, IL-6 has been shown to be required for the development of B cell neoplasms (Hilbert *et al.*, 1995).

While the perturbation of *hprt*⁻ mutant frequency is considered indicative of the damage occurring in the overall genome, it is unlikely to have any direct consequences for human health. The potential outcome of an increase in the frequency of *bcl-2* translocation, however, has more serious implications. The *bcl-2* t(14:18) translocation is the characteristic chromosomal alteration of the commonest form of non-Hodgkin's lymphoma (Jaffe *et al.*, 1992). If cells carrying the translocation can be regarded as premalignant, any exposure which increases the proportion of such cells in the circulation will similarly increase the number of targets available for any further steps in the progress to disease. It is of interest that one of the major causes of death following organ transplantation and immunosuppression is non-Hodgkin's lymphoma (Opelz and Henderson, 1993), although these tumours have a large Epstein-Barr virus involvement (Hanto, 1995). Furthermore, a role for sunlight in the aetiology of non-Hodgkin's lymphoma has been suggested by some epidemiological studies (Cartwright *et al.*, 1994; Adami *et al.*, 1995; Bentham, 1996; Cliff and Mortimer, 1999), although this is controversial (Hartge *et al.*, 1996). Perhaps because of the nature of the UK climate, our results lend support to the claims of a link and may provide some clue as to a possible mechanism.

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