The effects on human health from stratospheric ozone depletion and its interactions with climate change†

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Ozone depletion leads to an increase in the ultraviolet-B (UV-B) component (280–315 nm) of solar ultraviolet radiation (UVR) reaching the surface of the Earth with important consequences for human health. Solar UVR has many harmful and some beneficial effects on individuals and, in this review, information mainly published since the previous report in 2003 (F. R. de Gruijl, J. Longstreth, M. Norval, A. P. Cullen, H. Slaper, M. L. Kripke, Y. Takizawa and J. C. van der Leun, *Photochem. Photobiol. Sci*., 2003, **2**, pp. 16–28) is discussed. The eye is exposed directly to sunlight and this can result in acute or long-term damage. Studying how UV-B interacts with the surface and internal structures of the eye has led to a further understanding of the location and pathogenesis of a number of ocular diseases, including pterygium and cataract. The skin is also exposed directly to solar UVR, and the development of skin cancer is the main adverse health outcome of excessive UVR exposure. Skin cancer is the most common form of malignancy amongst fair-skinned people, and its incidence has increased markedly in recent decades. Projections consistently indicate a further doubling in the next ten years. It is recognised that genetic factors in addition to those controlling pigment variation can modulate the response of an individual to UVR. Several of the genetic factors affecting susceptibility to the development of squamous cell carcinoma, basal cell carcinoma and melanoma have been identified. Exposure to solar UVR down-regulates immune responses, in the skin and systemically, by a combination of mechanisms including the generation of particularly potent subsets of T regulatory cells. Such immunosuppression is known to be a crucial factor in the generation of skin cancers. Apart from a detrimental effect on infections caused by some members of the herpesvirus and papillomavirus families, the impact of UV-induced immunosuppression on other microbial diseases and vaccination efficacy is not clear. One important beneficial effect of solar UV-B is its contribution to the cutaneous synthesis of vitamin D, recognised to be a crucial hormone for bone health and for other aspects of general health. There is accumulating evidence that UVR exposure, either directly or *via* stimulation of vitamin D production, has protective effects on the development of some autoimmune diseases, including multiple sclerosis and type 1 diabetes. Adequate vitamin D may also be protective for the development of several internal cancers and infections. Difficulties associated with balancing the positive effects of vitamin D with the negative effects of too much exposure to solar UV-B are considered. Various strategies that can be adopted by the individual to protect against excessive exposure of the eye or the skin to sunlight are suggested. Finally, possible interactions between ozone depletion and climate warming are outlined briefly, as well as how these might influence human behaviour with regard to sun exposure.

Introduction

There are many harmful and some beneficial effects of solar ultraviolet radiation (UVR) on human health. Skin cancer and cataract are examples of the former category while the synthesis of vitamin D is one example of the latter category. With ozone depletion and the consequent increase in terrestrial UV-B, these effects may be enhanced. Various models predict increases in the number of skin cancers and cataracts that can be attributed to ozone depletion over the baseline that occurred before ozone

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depletion.[1,2] However, as stated previously,[1] human choice in determining where, when, how and for how long an individual is exposed to solar radiation is a major, if not the principal, factor that establishes the health outcomes. Assuming the same human exposure habits, ozone depletion with resulting increase in UV-B will increase the numbers of skin cancers and cataracts, while a positive effect could be a general improvement in vitamin D status.

In this report, discussion will centre first on interactions between solar UVR and the eye and, secondly, on interactions between solar UVR and the skin, concentrating on the risks of, and trends in, the incidence of skin cancers and the genetic factors involved in their development. A section on the immune effects of UVR comes next, followed by another on the UV-induced synthesis of vitamin D and its relationship with a range of diseases. Finally, strategies for responding to the problem of ozone depletion are considered, especially those that protect the individual. In most instances, only new information available since the previous full report in 2003 is included although in certain instances reference is made to earlier key publications. It should be noted that the topic of air pollution relating to ozone depletion is addressed in ref. 3, this includes reference to aspects concerning human health.

The eye

The eye and the skin are the only organs of the body that are exposed to solar UVR. The effects of sunlight on the eye may be acute (usually after a latent period of several hours), long-term after an acute exposure, or long-term following chronic exposure to levels of UVR below those required for acute effects (Table 1). In our last report we focused on cataract, the UV-B related eye disease with the most serious public health implications.[1] This section of the report concentrates first on how UV-B reaches and interacts with the surface and internal structures of the eye, and then provides an update on chronic effects that may impair vision.

Interaction of solar UV-B with target tissues in the eye

At low solar zenith angles (high solar elevation angles), the UV-B photons most likely to fall on the cornea and other ocular tissues are those from indirect sunlight, *i.e.*, those scattered by atmospheric components or reflected from surfaces. In contrast to its effects on the skin, direct sunlight plays a minor role in UV-B-related eye disorders due to a natural aversion to looking directly at the sun, and shadowing by the brows when the sun is high. Under conditions of cloud cover (with lower light levels), the natural defence mechanisms of the eye, for example squinting, are relaxed, permitting greater exposure of the outer surface and internal structures of the eye, such as the lens. At the same time, scattering and reflection by clouds increases the diffuse radiation incident on the eye.[4,5] UV reflectance values vary considerably for different natural terrains and manufactured materials. Grass and other green vegetation are natural strong absorbers of UV-B and reflect this waveband poorly (2–3%), whereas fresh snow is an excellent reflector (more than 90%). These variations can result in significant errors in estimating UV-B exposure based solely on location, as was commonly done in early epidemiologic studies of the role of sunlight in eye disease.

Peripheral light focusing by the eye. A factor that must be considered when assessing exposure of the internal structures of the eye to UV-B is that the various zones of the cornea direct the radiation to different locations within the eye. Coroneo *et al.*[6] suggested that light and UVR incoming from the side are focused on specific areas of the cornea, resulting in a twenty-fold increase in exposure that may be important in the induction of pterygia and cataract. They also proposed that UVR was similarly concentrated in the lower nasal quadrant of the crystalline lens, the location where age-related cortical cataract is commonly first detected. Human[7] and mannequin[8] studies have confirmed that incoming temporal UVR from behind the coronal plane (100 to 135° to the sagittal plane, see Fig. 1) was focused into the anterior chamber angle. This is modified by corneal shape, anterior chamber depth, and location of the eye within the bony orbit, squinting, eyelashes, prominence of cheekbones and presence of lid skin folds on the temporal side of the eye.

Transmittance of the ocular media. In order for UV-B incident on the surface of the eye to reach the crystalline lens, it must first pass through the cornea and the aqueous humour. Although the aqueous humour absorbs little environmental UV-B, the cornea has a significant role in preventing UV-B from reaching the lens, with some parts of the cornea being more effective than others. Kolozsvari *et al.*[9] have shown that UV-B absorption is about twice as high in the anterior layers (epithelium and Bowman layer) of the human cornea as in the posterior layers. Their data indicate that the whole cornea begins to transmit at 280 nm (<0.01%), increases to 1% at 295 nm and approaches 5% at 300 nm. Although the actual amount of UV-B transmitted is low, it should be noted that

Table 1 Potential acute and chronic effects of exposure to UV-B on the eye and adjacent tissues

Tissue	Acute effect	Chronic effect
Lid and peri-ocular skin	Sunburn: erythema (redness), blistering, exfoliation (peeling) Tanning	Freckling Lentigines (age spots) Hypomelanosis (vitiligo) Non-melanoma skin cancer Actinic keratosis Cutaneous melanoma
Conjunctiva	Photoconjunctivitis Chemosis (swelling)	Pinguecula (local degeneration) Dyskeratosis (abnormal epithelial cell differentiation) Intraepithelial neoplasia
Cornea	Photokeratitis Endothelial damage (swelling) Reactivation of latent herpes viruses	Climatic droplet keratopathy (epithelial degeneration) Pterygium (see text) Endothelial changes
Lens	Anterior subcapsular opacities	Age-related cataract (see text)

UV-B at 300 nm is about 600 times more biologically effective at damaging ocular tissue than UV-A at 325 nm.

At birth the human lens is colourless and allows both UV-B and UV-A to pass through to the retina. As the lens ages, there are significant changes in the lens proteins, including a decrease in their solubility, that result in increased, wavelength independent, scatter and consequent degradation of vision (clinically called nuclear sclerosis). Frequently there is also a yellowing which can eliminate the passage of UVR and limit the passage of light in the violet-blue end of the visible spectrum.

Chronic effects of UVR on the eye

Pterygium. This wing-shaped, inflammatory, proliferative and invasive growth occurs on the conjunctiva and cornea of the human eye (Fig. 2). It is induced, in part, by intracellular damage caused by UV-B exposure[10] and most commonly occurs in the superficial layers of the nasal cornea. Pterygia grow towards the centre of the cornea and can severely impair vision. In their early stages, they appear as small opacities at the nasal edge of the cornea and then spread to become a fleshy raised area. A number of causal factors, other than UVR, have been proposed as important to pterygium development including mechanical irritation, heredity, heat, cold, and wind. None of these adequately explains the predominately nasal location of pterygia. This preferred location has been explained, however, on the basis of the peripheral light focusing effect discussed above.[11]

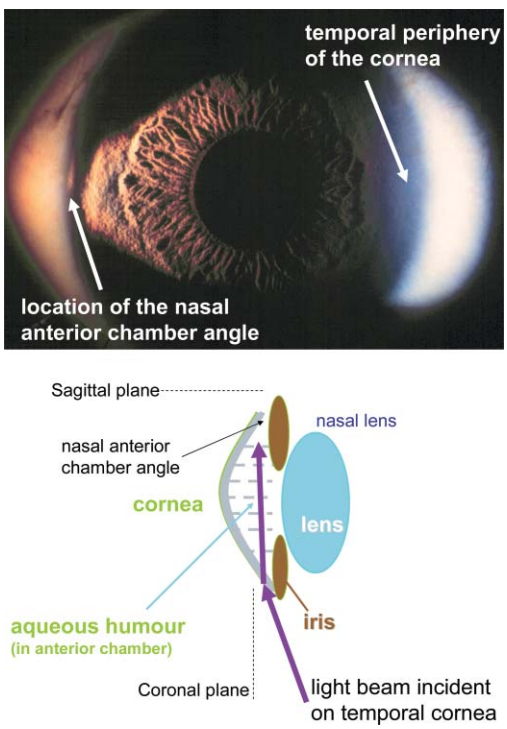

Fig. 1 Peripheral light focusing. Top: Photograph showing how a beam directed towards the temporal side of the cornea is focused into the anterior chamber angle. Bottom: A beam of light (or UVR), from behind the coronal plane, directed onto the temporal periphery of the cornea is refracted and focused into the nasal angle of the anterior chamber of the eye, as shown by arrows. If the incident beam originates in front of the coronal plane, the focus shifts into the nasal part of the lens.

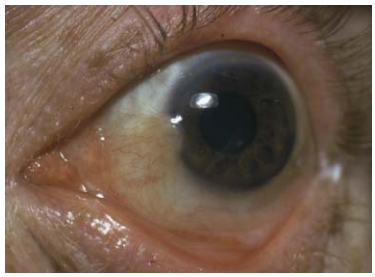

Fig. 2 An early pterygium.

Pterygia are more prevalent and progress more rapidly in individuals living in regions near to the equator or at very high altitudes. Al Bdour and Latayfeh[12] reported a strong correlation between pterygia and environmental UVR in Australian aborigines. In the more temperate climate of the northeastern US, a significant relationship was found between the cumulative dose of solar UV-A and UV-B and the prevalence of pterygium.[13] The higher prevalence of pterygium in outdoor occupations has been attributed to exposure to excessive amounts of sunlight. In a population-based sample of residents of the Australian state of Victoria who were aged 40 years and older, statistical modelling revealed that 43.6% of the risk of pterygium could be attributed to cumulative dose of sunlight.[14] This result was the same when cumulative dose of ocular UV-B was substituted in the model for cumulative dose of sunlight. Pterygium continues to be considered a significant public health problem in rural areas and occurs primarily as a result of ocular sun exposure.[14] In a study conducted in Perth, Western Australia,[15] there were strong positive associations between pterygium and measures of potential and actual sun exposure. The strongest associations were seen for the estimated daily ocular solar radiation dose at any age, which in those in the highest quartile of exposure resulted in about a 7-fold greater risk. Although other agents may contribute to pterygium development,[16–18] in most epidemiological studies the common factor is UVR exposure, thereby indicating that UVR can be considered a causal agent. Thus, the implication for prevention of pterygium is that ocular protection from sunlight is beneficial at all ages.

Cataract. Three main types of age-related cataract can be distinguished, based on their location: cortical cataract involving the anterior (and posterior) cortices of the lens; posterior subcapsular cataract at the extreme posterior cortex lining the lens capsule[1] and nuclear (sclerotic) cataract at the nucleus of the lens. However by the time the individual requires surgery, mixed categories are most commonly present. Cortical cataract arises from localised changes occurring in the cortex of the lens, where opaque radial spokes begin to develop on the periphery and extend towards the centre, eventually affecting vision.[19,20] The second form of cataract, posterior subcapsular cataract, is thought to develop when the lens epithelial cells migrate to form a plaque of opacities and cysts at the posterior surface of the lens. These lesions are particularly detrimental to vision when the pupil constricts due to sunlight or other bright sources, or during near tasks. The third form of cataract, nuclear cataract, occurs as the crystalline lens of the eye ages and the nucleus loses its transparency, becoming more opalescent and sometimes turning yellowish to brown in hue.[21,22]

A number of publications has reviewed the epidemiologic information linking UVR exposure to cataract.[23–25] Although

earlier reviews concluded that the range and variability of the study designs precluded definitive conclusions, most of the more recent analyses suggest a role for UV-B in some types of age-related cataract, particularly cortical cataract. A frequently cited early estimate of risk from personal ocular exposure to solar UV-B is that of Taylor *et al.*[26] in the Chesapeake Bay Waterman Study. Watermen in the highest quartile had a threefold increased risk for cortical cataract. It is important to note that the subjects in this cohort were only exposed to levels of solar UV encountered in mixed, often overcast, climate at intermediate latitude. The same group[27] that conducted the Chesapeake Bay Waterman Study also conducted a population-based epidemiologic study in Salisbury, Maryland. The increased risk of 10% of developing cortical cataract associated with UV exposure in this study (the Salisbury Eye Evaluation [SEE] project[28]) was more modest, but the population was considered more representative of the US as a whole. West and her colleagues subsequently used these data from the SEE project as the basis from which to develop risk estimates for the entire US population under conditions of ozone depletion.[27] These risk estimates, which were calculated for fixed levels of ozone depletion ranging between 5 and 20%, indicated that the number of cortical cataract cases seen by 2050 would increase between 1.3 and 6.9% respectively, with associated health costs for the US of between about $0.6 and $3 billion respectively. There are, in addition, important social costs associated with cataract development.

A recent review found that there was insufficient evidence to conclude that UVR exposure played a causal role in the development of posterior subcapsular cataract.[29] However, a recent study in Japan[30] showed that the severity of nuclear cataract increased with UV-B exposure. Furthermore, lifetime cumulative UV-B exposure and particularly exposure in the teenage years correlated with the presence of nuclear cataract in females. Another report indicated that the association between nuclear cataract and occupational sun exposure was significant for exposure between the ages of 20 and 29 years.[31] Supporting evidence for such a difference in a period of age susceptibility is provided by an animal study in which the same dose of UVR induced more severe cataracts in young than in older animals.[32]

The skin

Sunburn is the effect most frequently experienced by the human population due to excessive solar UV-B exposure. It is an inflammatory reaction to a toxic assault on the skin. Although human skin is adapted to the ambient UVR, the sunburn reaction demonstrates that excessive exposures can stretch defensive mechanisms to the limit, or even exceed them (pain and blister formation). Despite the remembered discomfort from past episodes, about a third of US residents report at least one sunburn per year.[33] Fair-skinned people are most susceptible to sunburn, and they correspondingly run a higher risk of long term adverse effects, such as skin cancer. In the following sections, the relationship between various types of skin cancer and solar UVR is outlined.

Skin cancer types and trends

Skin cancer is the most common form of cancer among fair-skinned populations and its incidence has increased markedly over the last century. Many skin cancers are detected early, at a stage where they can be easily and effectively treated. This limits morbidity and mortality. In addition, for the majority of skin cancers, the 'non-melanoma' skin cancers (NMSC) consisting of basal cell carcinomas (BCC) and squamous cell carcinomas (SCC), the malignant potential is low which also reduces death from these diseases. This is not the case for the most malignant form of skin cancer, melanoma, that arises from pigment cells (melanocytes), and is responsible for most of the deaths from the skin cancer. Projections show an approximate doubling in all types of skin cancer from 2000 to 2015 in the Netherlands, but this is also due, in large part, to ageing of the population.[34]

Melanoma

As found in earlier epidemiological studies, cutaneous malignant melanomas are related to sun exposure in early life, to episodes of severe sunburn, and to the number of moles (nevi), which in turn is related to sun exposure in early life.[35] In the last decade, much progress has been made in identifying genetic changes in melanoma cells, and the functional importance of these genetic changes for melanoma development has been demonstrated in genetically modified mice. However, the precise mechanisms underlying nevus formation and progression to melanoma, and the role of solar UVR in this process, remain to be resolved.[36,37]

Epidemiology of melanoma. Although rates of increase in melanoma incidence appear to be levelling off in countries with the highest number of cases,[38–40] the absolute incidence is continuing to rise. Mortality, however, has risen much less or has even stabilized especially in females, and in younger age cohorts, although not in older males in countries such as the USA, Scotland and Australia. The major increase in incidence recently has been attributed to the thin melanomas that have high survival rates.[41] A thin melanoma is defined as being less than or equal to one mm thick. This predominance of the early stages of melanoma could be due to greater awareness in the general population regarding the dangers of suspicious-looking moles. Prompt diagnosis and treatment may then limit any increase in mortality.[42,43] Melanomas with an attached nevus from which they apparently originated are on average thinner, of the more superficial spreading type and occur more often in irregularly exposed skin than melanomas that show no remnants of a precursor nevus.[44] Patients with the nevus-associated melanomas are younger and have more nevi.

Strouse *et al.*[45] found that the incidence of melanoma in children in the USA is rising rapidly but survival is improving. They showed that the incidence rate of melanoma was positively correlated with environmental UVR exposure. The chance of surviving a melanoma decreases with age and is lower for boys compared with girls. It is also lower if the primary tumour occurs on body sites other than the extremities and the torso (*i.e.*, locations other than those exposed intermittently to the sun). The latter finding is in agreement with a study of adults by Berwick *et al.*[46] who found that survival from melanoma was higher in individuals with a history of increased intermittent sun exposure and episodes of sunburn. However, these authors also found improved survival with a history of increased skin awareness and increased solar elastosis (*i.e.*, a skin 'aged' by chronic sun exposure).

Earlier reports regarding a seasonal variation in the diagnosis of melanoma were confirmed in recent European and Australian studies. These revealed maximum incidence for thin melanomas

on extremities in the summer in females.[47,48] This effect may be attributable to enhanced skin awareness in the hotter months or to stimulation of melanoma growth after (over-)exposure to the sun. Boniol *et al.*[48] found that survival from melanomas diagnosed in the summer was higher, as might be expected from the higher number of thin melanomas diagnosed at that time of the year, but, after correction for tumour thickness, the effect was still significant. The authors therefore suggest that patients in whom melanomas are diagnosed after recent sun exposure may show better survival.

Trends and changes in skin cancer incidence over recent decades clearly indicate the importance of human behaviour, particularly in relation to exposure to solar UVR.[38] For example, Gandini *et al.*[49] undertook a meta-analysis of 57 observational studies which showed that intermittent sun exposure and sunburn history played considerable roles as risk factors for melanoma, and Agredano *et al.*[50] found a very strong relationship between increasing access to air travel to leisure destinations and increasing melanoma incidence. However, case-control studies generally find that genetic factors carry more risk than behavioural aspects, such as moderating UV exposure.[51] It should be noted, however, that the genetic factors can be determined more accurately than personal UV exposure; the latter is assessed by very poor surrogates (*e.g.* recalled number of sunburns in youth or lifetime hours of sun exposure). This inaccuracy in determining past UV exposure will tend to lead to lower estimates of relative risk. Moreover, an individual's behaviour with regard to UV exposure can be altered to reduce risk, very much in contrast to an individual's genetic background.

Latitudinal and temporal trends in skin cancer, notably in melanomas, underline the major importance of UV exposure as an environmental risk factor. The integrity of the stratospheric ozone layer, as the prime atmospheric UV filter, therefore remains crucial in protection against melanoma.

Genetic risk factors for melanoma. There are well-established genetic factors conferring susceptibility to melanoma—notably inherited mutations in the cell-cycle control gene, *p16INK4a*, and in the "hair-colour" gene which codes for the melanocortin 1 receptor (MC1R). The MC1R gene contributes to the control of pigmentation in hair and skin[52] and is an important risk factor for all types of skin cancer, including melanoma.[53] Other additional genes are related to melanoma risk, *e.g.*, the OCA2 gene which also controls skin and eye colour,[54] and an as yet unknown gene located on chromosome 1.[55]

UVR causes DNA damage which can give rise to gene mutations which in turn can contribute to skin cancer formation (see below). Hence, repair of this damage is of crucial importance. The solar UV-B induced DNA damage (mainly cyclobutane pyrimidine dimers, CPDs) is removed by a 'cut-and-paste' type of DNA repair ('nucleotide excision repair'). A complete dysfunction in one of the enzymes in this repair system results in a dramatic increase in risk of skin cancer, including melanoma. More subtle genetic variations (polymorphisms) in the repair enzymes can modify the efficacy of DNA repair, and thus affect skin cancer risk. Certain genetic variations in repair enzymes were indeed found to be associated with melanoma risk.[56–62]

UVR can generate reactive oxygen species and thus inflict damage to cell components, particularly DNA. Although melanin pigment is generally protective, it may also contribute to oxidative damage under certain conditions,[63] especially its red variety, pheomelanin.[64] Certain inherited or acquired traits that increase oxidative stress appear to be associated with melanoma and its precursor lesion, dysplastic nevus.[65–67] Genetic variation in a protein (APE1) involved in the repair of oxidized DNA modifies melanoma risk.[68]

These inherited predispositions to develop melanoma will help to identify high risk groups who may be particularly susceptible to increases in ambient UV-B radiation.

Oncogenic alterations in melanomas. In terms of molecular mechanisms, melanomas from chronically exposed, intermittently exposed and unexposed skin sites have different molecular signatures.[69] Notably, the melanomas from intermittently exposed skin have a high frequency of activating mutations in a critical signalling molecule, B-RAF. MC1R variants, which are associated with enhanced risk of melanoma, are strongly associated with B-RAF mutations.[52] 10 to 20% of melanomas from chronically exposed skin bear mutations in N-RAS, a protein preceding B-RAF in the signal cascade for cell proliferation.[70] Mutations in B-RAF and N-RAS genes are already present in some cells in nevi,[71] but nevus cells do not proliferate and are kept in a 'senescent state'.[72,73] The mutations in the B-RAF oncogene are not typical of UV-B radiation, but could be due to UV-induced oxidative damage.

The epidemiological finding that melanomas associated with intermittent sun exposure[46] show better survival may be linked to the specific molecular changes found in these tumours.

Animal experiments on UVR and melanoma. Because of the well-established role of UVR in NMSC and the known mutagenic and carcinogenic properties of UV-B radiation, it seems most likely that UV-B wavelengths are also contributing to the development of melanoma. However, human melanomas show no gene mutations that are typical of UV-B radiation. Animal models may serve to elucidate whether, and if so, how UV-B radiation contributes to the development of melanoma.

Experiments with transgenic mice confirmed the epidemiological finding that the neonatal period can be critical to the development of melanomas later in life.[74,75] More specifically, a study in transgenic mice showed neonatal UV-B exposure to be highly effective.[76] These melanomas, which closely mimic the human disease, could not be evoked by neonatal UV-A exposure.[76] The latter finding is in accordance with earlier experiments in opposums,[77] but differs from the results obtained with small *Xiphophorus* fish.[78] In the fish, both UV-B and UV-A neonatal exposure proved to be very effective in causing melanomas, and the variation in effectiveness with wavelength was recently found to closely follow the variation in the UV induction of oxidant radicals from melanin in the skin of the fish.[79] In the initial experiments with neonatal UV exposure, the HGF transgenic mice were albino, but recent experiments showed that these mice crossed into a pigmented background were also susceptible to melanoma induction by neonatal UV-B exposure (Noonan and De Fabo, personal communication). Further experimentation with this model may shed more light on the wavelength dependency of melanoma induction in mammals. In another model, melanomas were induced by massive doses of UV-B radiation delivered to

repair-deficient transgenic mice.[80] However, the severe skin trauma inflicted may have caused non-specific tumour promotion.[81]

In support of the epidemiological finding that intermittent sunburning exposures increase the risk of melanoma, experiments in hairless mice have shown this type of exposure regimen to be considerably more effective in inducing nevi (potential precursors of melanoma) than a regimen in which the exposure was more evenly spread over time.[82] Thus, sunburning UV-B exposure of adults may indeed also contribute to melanoma development by stimulating the proliferation of melanocytes.[83]

Immunity and melanoma. There are indications that immune mechanisms against melanomas are present in humans as demonstrated by the occasional spontaneous regression of some pigmented skin lesions. Further, immune responses against melanoma antigens are readily detectable in patients and immunotherapy is actively used for melanoma treatment.[84,85] As found earlier for BCC, melanoma risk appears to be related to the density of mast cells in unexposed skin[86] so that the higher the number of mast cells, the greater the chance of developing melanoma. Interestingly, children with eczema (atopic dermatitis) develop fewer melanocytic nevi than children without eczema,[87] and the therapeutic effect of UV exposure on the eczema might be related to a possible effect of the radiation on the cytokine network in the skin, the products of which then stimulate melanocytic growth.

While there is substantial evidence for a role for UV-induced immunosuppression in NMSC, it is not known as yet if this mechanism is a factor in melanoma progression; this is currently an area of intense investigation.

Non-melanoma skin carcinomas (NMSCs)

In epidemiological studies prior to 1980, the skin carcinomas, SCC and BCC, were not considered separately, and were commonly found in people who had accumulated excessive hours of solar (UV) exposure. In more recent studies, important differences between SCC and BCC have emerged. SCC is associated mainly with chronic and life-long accumulated sun exposure[88] whereas BCC, similar to melanoma, is more closely associated with early-life and intermittent exposures resulting in episodes of severe sunburn. In addition, while SCCs occur on body sites most regularly exposed to the sun such as the face, BCCs are also found frequently on sites exposed intermittently to sunlight. Also, the genetic alterations identified in SCC and BCC show important differences.

Epidemiology of NMSC. Studies continue to show increases in the incidence of both SCC and BCC,[34,89,90] with disproportionately high increases in BCC in young females on the lower limbs.[91,92] Sunbathing is associated with a fivefold rise in the risk of BCC on the trunk.[58]

Although BCC is locally invasive, it is usually a slow growing and not very aggressive tumour; superficial BCC on the trunk is often misdiagnosed and confused with eczematous skin lesions. Detailed skin examination of subjects in a Queensland community established that the incidence of BCC on sites other than head, neck, hands and arms was threefold higher than actually treated[93]; a smaller study in Spain produced a similar result.[94] Hence, the large majority of the BCC on irregularly exposed sites appear to remain 'sub-clinical', *i.e.*, cause no great discomfort, are never presented to a physician and remain essentially undetected.

Death due to NMSC in the USA has declined, and when it occurs, is often related to an excessively long delay before seeking medical care.[95]

Genetic risk factors for NMSC. UV-B radiation inflicts highly characteristic DNA damage (mainly CPDs), and the repair of this damage in human skin diminishes with age.[96] This type of DNA damage causes specific 'point mutations' which are found in the *P53* tumor suppressor gene in NMSC (see below). However, NMSC also show frequent crude chromosomal aberrations.[97] Such aberrations are already abundantly present in the benign precursor lesions of SCC, the actinic keratoses (AKs). Complete double strand breaks (DSB) in the DNA cause these gross chromosomal losses and duplications. Interestingly, variants in genes involved in the repair of DSB in DNA appear to be related to NMSC risk, but not to melanoma risk.[98] The association between NMSC and DSB repair ties in nicely with the recent finding that UV-B-exposed blood cells from patients with skin carcinomas are more prone to develop chromatid breaks than equivalent cells from melanoma patients and control subjects.[18]

Genetic variations in specific antioxidant proteins are associated with NMSC risk.[99] Variants of a repair enzyme, involved in excision of oxidized bases in DNA, affect SCC risk, but not the risk of BCC or melanoma.[98] Hence, there appear to be considerable differences in how oxidative DNA damage (such as induced by UVR) and its repair are related to the various types of skin cancer.

Oncogenic alterations in NMSC. Although considerably less efficient than UV-B, long-wave UV-A radiation can cause the same type of DNA damage as UV-B radiation, and thus give rise to 'UV-B-like' mutations in the *P53* tumor suppressor gene. However, oxidative damage contributes substantially at these longer wavelengths and causes different *P53* mutations from those induced by UV-B.[100,101] Microscopic clusters of cell clones with strong expression of mutant-p53 protein in sun-exposed skin carry the same types of 'UV-B-like' *P53* mutations as skin carcinomas.[101,102] Hence, all of these common microscopic clusters of cells with mutant-p53 in human skin could be potential precursors of skin carcinomas. In Swedish studies, microdissection of skin carcinomas showed consistent mutations in the *P53* gene throughout the tumour masses, *i.e.*, most tumours appeared to be a clonal expansion from a founder cell with a particular 'UV-B-like' *P53* mutation.[102,103] This conclusion is in agreement with earlier studies that found dominant 'UV-B-like' mutations in SCCs and BCCs.[104,105] In contrast to these findings, a recent Australian study reported *P53* mutations to be very diverse, heterogeneous and disjunctive in SCCs and adjacent skin, *i.e.*, every microdissected part of a tumour showed different *P53* mutations without any suggestion of a founder mutation or any clear overall indication of UVR as the cause.[106] By arguably separating out UV-B-like, UV-A-like, oxidative and 'other' *P53* mutations, the authors found the UV-B-like mutations to be located in the shallow parts of the tumours and the UV-A-like mutations in the deeper parts. This issue clearly needs to be investigated further.

Although both SCC and the precursor AK frequently carry various chromosomal aberrations, the loss of a particular part of chromosome 18 appears to be related to the progression from AK to SCC.[97] The presence of multiple copies of parts of chromosomes

may explain the amplification of the *H-RAS* oncogene frequently found in SCC.[107]

As reported in our previous review[1] and confirmed recently,[108] nearly all BCCs display activation of the Hedgehog proliferative pathway, mostly through a defect in the PTCH protein in the cell membrane by mutations or loss of the coding gene. Some of these mutations are 'UV-B-like'. Further research has shown that certain variations in the *PTCH* gene may predispose towards BCCs,[109] and that UV-B radiation can suppress *PTCH* function and thus potentially stimulate BCC development.[110]

Hence, the oncogenic alterations found in NMSC are attributable largely to UVR, and in some cases more specifically to UV-B radiation.

Animal experiments on UV and NMSC. Experiments in transgenic mice have identified the type of UV-B-induced damage (CPDs) that causes SCC and more immediate effects such as sunburn and thickening of the outer viable layer of skin (the epidermis).[111] Clones of cells with mutations in the *P53* gene—such as those found in human skin—have been induced in well-controlled experiments in which mice were exposed to UV-B radiation.[112] Here these p53-mutant clones were tightly linked to the subsequent occurrences of SCC. In mice, UV-B-induced DNA damage gives rise to DSBs and strong signals for DSB repair.[113] Thus, UV-B radiation may induce the chromosomal aberrations present in human NMSC.

Immunity and NMSC. Organ transplant recipients (OTR) have a dramatically increased risk of developing SCC and, until recently, this was considered to be solely the result of taking immunosuppressive medication to prevent rejection of the transplant. Evidence is now accumulating to indicate that conventional immunosuppressive drugs can also adversely affect UV-induced DNA damage and repair in skin cells[114–116] and thus they may increase the risk of SCC. Immunosuppressed patients other than OTR may be affected similarly.[117] A new generation of immunosuppressive drugs with a different mode of action may substantially reduce the risk of SCC.[118] Hence, the increased incidence of SCC in relation to immunosuppressive drugs may be due in large part to detrimental effects on UV defensive mechanisms in the skin, rather than to immunosuppression per se.

Immune effects of solar UVR

Mechanisms of UV-induced immunosuppression

When the skin is exposed to UVR, a complex cascade of events begins that ends in the suppression of certain types of immune responses, mainly those involving cell-mediated immunity. The main interactions affected are between three types of immunologically active white blood cells: antigen-presenting cells (APCs), T-helper (Th) lymphocytes and T-regulatory (T_{reg}) lymphocytes. The degree of suppression and the forms of cell-mediated immunity affected can vary depending on the quality, quantity and timing of the UVR, the frequency of the exposures, and the extent and location of the body surface irradiated.

One distinction commonly made is between local and systemic immunosuppression. Local immunosuppression occurs when an antigen (a "non-self" molecule that the host recognises as foreign and makes an immune response to) is applied directly to the irradiated body site soon after the UV exposure, resulting in a down-regulation of immunity to that antigen. In systemic immunosuppression, following UV exposure of one part of the body, the antigen is applied to a distant unirradiated body site, again leading to systemic down-regulation of immunity to that antigen. Certain steps of the two pathways differ such as whether the APCs have been directly exposed to the UVR or not.

The process for local immunosuppression is outlined in Fig. 3, and details of both local and systemic mechanisms can be found in several excellent reviews.[119–122] In brief, at least three photoreceptors located at or near the skin surface are involved—DNA, *trans*-urocanic acid and membrane components. On absorption of photons, the respective structural changes include formation of thymine dimers in DNA, isomerisation of *trans* to *cis*-urocanic acid, and lipid peroxidation in membrane components. These alterations initiate the pathway and stimulate the local production of the large range of immune mediators shown in Fig. 3. Such molecules have profound effects on various cell populations in the irradiated site and possibly elsewhere in the body. In particular, there are changes in the numbers and function of the APCs which lead to alterations in particular T lymphocyte subsets. For example, inhibition in the release of certain (type 1) cytokines from T helper 1 (Th1) cells occurs. This is significant as the type 1 cytokines are very important in the responses to simple chemicals, such as nickel, and in the immunological control of tumours and intracellular infections, such as those caused by viruses. At the same time, T_{reg} cells are stimulated to release immune mediators that are involved in the control of other T-cell subsets. Upon activation by a specific antigen, these T_{reg} cells are capable of down-regulating immunity by the production of immunosuppressive cytokines. There is much interest currently in trying to characterise populations of T_{reg} cells, particularly as they may have therapeutic value in the treatment of autoimmune and other diseases.[123]

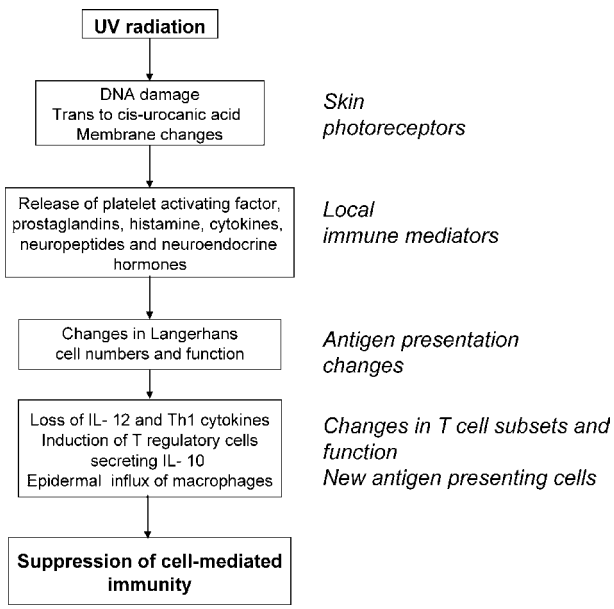

Fig. 3 Outline of pathway leading to local immunosuppression (antigen applied to the irradiated skin site) following UV irradiation.

The majority of experimental systems to date have involved a single or limited number of exposures to UVR, containing the UV-B waveband predominantly, and in doses sufficient to cause sunburn (erythema) followed by application of the test antigen. Under natural conditions, people are exposed to solar UVR in which the UVB represents less than 6% of the total UV spectrum[124] and they frequently receive suberythemal doses on a daily basis, especially during the summer months. Many respond to this chronic low level exposure by tanning and by skin thickening. These responses, which provide some protection against the burning effect of UVR, might lead to photoadaptation so that protection against UV-induced immunosuppression could also develop. This possibility has been tested recently in both mice and humans. For most immune responses, photoadaptation did not occur so that the immunosuppression continued throughout a period of repeated daily exposures to suberythemal solar simulated radiation.[125,126]

The impact of UVR on infectious diseases and vaccination of human subjects

The 2003 UNEP report[1] summarized the evidence available at that time demonstrating that solar UVR exposure could adversely affect the pathogenesis of various infections. Information on this topic reported since 2003 is outlined below. The two cases in which UVR exposure definitely causes a detrimental change in the pathogenesis are herpes simplex virus (HSV) which causes cold sores and human papillomavirus (HPV) which commonly causes warts. In both cases, UV appears to have dual effects—both on the immune response and on the virus itself, and these mechanisms are outlined below. The apparent inability of UVR to alter the course of other human infections could be because the causative agents themselves do not contain any UV responsive elements, or that the human immune system is sufficiently robust so that if one aspect of it is suppressed, another can compensate. It should be noted, however, that only a limited number of human infections have been investigated thus far in the context of solar UVR.

Herpes simplex virus (HSV). Several epidemiological and experimental studies have indicated that exposure to solar UVR exposure is a common stimulus for the reactivation of HSV type 1 from latency in the nerve tissue. The virus then travels down the sensory nerve and replicates in the skin to induce a recurrent lesion (cold sore) at the same site as the initial infection had occurred. A large-scale study of 3678 infected patients, 2656 of whom suffered HSV recurrent lesions, was undertaken recently in a Prefecture of Japan to further evaluate the role of solar UVR exposure.[127] The self-reported cause of the recurrence of cold sores was the sun in 10.4% of individuals. In the summer months, this rose to 19.7% overall, and to 40% in subjects younger than 30 years. One mechanism likely to be important here is the suppression of local immune responses as a result of UV exposure: the virus arriving at the cutaneous site from the nerve will have time to replicate and induce the clinical symptoms before effective immunological control is regained.[128,129] Such a scenario has been shown to operate in mice infected cutaneously with HSV and then UV irradiated.[130] New studies indicate that a second mechanism involving a more direct interaction between HSV and UVR is probably required to reactivate the virus, in addition to the immune effects of the UVR. For example, UV-induced damage to nerve endings can lead to changes that result in the activation of HSV promoters, and hence to the reactivation of the virus from latency.[131]

Human papillomavirus (HPV). It has been recognised for several years that, in immunosuppressed subjects and those with epidermodysplasia verruciformis (EV; a rare genetic disease in which the APCs are defective), infections with certain cutaneous HPV types (EV-HPV) are associated with the development of SCCs but only on body sites most exposed naturally to sunlight, such as the face and backs of the hands. New information has now provided evidence that immunocompetent individuals can be similarly affected, *i.e.*, UVR exposure and infection with certain cutaneous HPV types can act as co-factors in the development of not only SCCs but also of BCCs (reviewed in ref. 132). The interactions here are complex but, in brief, the HPV is able to stimulate cell proliferation and inhibit UV-induced programmed cell death (apoptosis) in the epidermis. These properties, together with the local immunosuppression and the additional genetic changes induced by the UVR exposure, may lead to tumour progression. Furthermore, on the basis of a lifetime-retrospective questionnaire on sun exposure, it has been suggested that sunburn episodes in the past lead to an increase in the risk of infection with particular HPV types in healthy subjects.[133]

The conjunctiva of the eye represents a further site where an association between HPV, SCC and sun exposure is probable. Conjunctival SCCs from subjects in Uganda, where the sunlight exposure is very high, were analysed for particular *P53* mutations (CC → TT) as a molecular signature of mutagenesis by solar UVR.[134] The prevalence of CC → TT transition (56%) was the highest observed in any of the cancer types evaluated and matched that of skin cancers in xeroderma pigmentosum (XP) patients (see The impact of UVR on tumour immunity section below). In addition EV HPV types were found in 86% of cases of SCCs of the conjunctiva.[135] It was suggested that these results confirm the causal role of solar UVR exposure in SCC of the conjunctiva and lead to the conclusion that the HPV infection could act as a co-factor in the mutagenesis process.

Recently an unexpected interaction between HPV types and solar UVR exposure has been revealed. Hrushesky and colleagues in the Netherlands observed a seasonal fluctuation in the frequency of cervical smears that were positive for the anogenital HPV types: it was twice as high in the summer months with a peak in August.[136,137] There was a positive correlation between the monthly HPV detection rate and the monthly solar UVR exposure. Hrushesky *et al.* speculate that UV-induced systemic immunosuppression could be the main reason for the increase in active HPV infections in the cervix in the summer months. This finding could be of importance as the high-risk anogenital HPV types are recognised to be the primary cause of carcinoma of the cervix, a tumour that is estimated to kill about 500 000 women annually worldwide.

Vaccination. To date, only one large-scale experimental study, carried out in the Netherlands, has evaluated whether solar UVR exposure can affect the generation of immune responses to vaccines.[138] In brief, subjects were vaccinated with recombinant hepatitis B surface antigen following whole-body UV irradiation on five consecutive days in half of the individuals. While natural killer cell activity and contact hypersensitivity responses were suppressed in the irradiated subjects compared with the unirradiated

subjects, there was no difference between the two groups in the hepatitis B-specific T cell or antibody responses. However, when the subjects were genotyped to characterise their cytokine polymorphisms (which can affect cytokine production or activity), it was found that individuals with a particular interleukin-1β polymorphism showed suppressed antibody responses to hepatitis B virus, if exposed to UVR prior to the vaccination.[139] Furthermore when skin samples were assessed for *cis*-urocanic acid concentration (which acts as major photoreceptor for UVR in the skin and can initiate the cascade resulting in immunosuppression), UV-irradiated subjects with higher *cis*-urocanic acid levels had suppressed T cell responses to the vaccine.[140] These results indicate that there are genetic and other differences in the way in which an individual might respond to vaccination in the context of UVR exposure. Therefore, it may not be appropriate to put all the irradiated or unirradiated subjects into single groups in order to make valid comparisons regarding UV-induced effects on immune responses during vaccination.

Three further studies are of interest. Sharma *et al.*[141] investigated an outbreak of measles in children in an Indian city and found that one-third of the cases had occurred in individuals who had been vaccinated previously against measles and who should have been protected as a result. They suggested that the virus-specific immunity could have waned due to solar UV-induced suppressive effects although experimental evidence is required to substantiate this idea. Snopov *et al.*[142] studied plasma cytokine levels following measles and poliovirus vaccination in infants in St Petersburg, Russia, some of whom had received ten daily suberythemal whole-body exposures to UV lamps (emitting predominantly UV-B) prior to the vaccination. This procedure was thought to improve the general health of such children. A shift towards a Th2 cytokine response occurred in the infants who had been UV exposed, but without the development of any clinical symptoms; antibody titres were not measured. Finally, Ghoreishi and Dutz[143] demonstrated recently that if mice were immunised with a protein applied directly to UV-irradiated skin together with an adjuvant (the trancutaneous route), immune responses to that protein were not generated. This outcome was mediated by T_{reg} cells that function through the production of the immunosuppressive cytokine, interleukin-10. In the future, the trancutaneous route may become preferred to subcutaneous inoculation as it avoids the use of needles; thus this result is of considerable interest.

In conclusion, there is limited evidence that UVR exposure can reduce the efficacy of vaccination, at least in some individuals. Clearly, this issue requires further investigation, particularly with regard to the identification of UV-susceptible groups within a population.

The impact of UVR on tumour immunity

There is considerable evidence that UV-induced immunosuppression contributes significantly to the progression of both melanoma and non-melanoma skin cancers.[1] Recently Jans *et al.*[111] demonstrated that prevention of the formation of the most common UV lesion in the skin, CPDs, also prevented the vast majority of the acute responses in UV-exposed skin and increased the resistance to UV-induced tumour development. Furthermore, Kuchel *et al.*[144] found that CPD development is the initiating event for suppression of memory immune responses in human subjects. This study looked at the effect of UVR exposure on the memory immune response in individuals who were allergic to nickel. This means that they had already shown a cell-mediated immune response to nickel in the past and would therefore have an immunological memory of nickel. When irradiated with solar-simulated UVR and then challenged on the skin with nickel, the normal cell-mediated response (seen as reddening and inflammation of the skin) was suppressed. However, if liposomes containing a DNA repair enzyme were applied immediately after the exposure, the cell-mediated response was not suppressed.

Subjects with the genetic disease XP, in whom there are mutations affecting DNA repair, show enhanced UV-induced acute inflammation and a high incidence of UV-induced skin cancers, up to 5000 times that of the general population.[145] Application of liposomes containing a DNA repair enzyme to the exposed skin leads to a decrease in the rate of newly occurring actinic keratoses (precursors of SCC) and skin cancers compared with the placebo.[146] Mice with different genetic defects in nucleotide excision repair (used as animal models of XP disease) have been investigated to determine further the effect of DNA repair on UV-induced local immunosuppression.[147] In another example, transgenic mice with a defect in one form of nucleotide excision repair have been used to demonstrate that tumour cells, derived from a murine UV-B-induced SCC, first develop into tumours following subcutaneous injection, and then are subsequently rejected in exactly the same fashion as in the wild-type.[148] However, if the transgenic and wild-type mice were UV-B exposed prior to the tumour cell inoculation, the tumours were rejected in 40% of the transgenic mice, as compared to 96% rejection in the wild-type mice. It was concluded that this immune-mediated impairment in tumour rejection, induced by the lack of repair of the DNA damage following the UV exposure, could contribute significantly to skin cancer development in XP patients. This work on XP represents further compelling evidence that the immunosuppression caused by UVR can be a crucial factor in the generation of skin cancers.

Vitamin D

Although exposure to solar UVR has many adverse health effects in human populations, one very beneficial effect is its contribution to vitamin D status. The vitamin D status of an individual is based on measuring the serum or plasma concentration of 25-hydroxyvitamin D [25(OH)D]. The active form of vitamin D, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] is synthesised in the final step of the metabolic pathway. The levels of 1,25$(OH)_2$D are maintained even when 25(OH)D levels become sub-optimal. Currently the serum levels of 25(OH)D considered excessive, sufficient, insufficient and deficient are >250, 50–250, 25–50 and <25 nmol L^{-1}, respectively.[149–151] These values are the topic of continuing discussion. For example, a recent report indicates that the most advantageous serum concentration of 25(OH)D for a number of health endpoints begins at 75 nmol L^{-1} with the optimum between 90–100 nmol L^{-1}.[152] For most people, more than 90% of their vitamin D requirement is acquired from exposure to solar UVR. An action spectrum for vitamin D formation in human skin indicates that synthesis occurs most effectively following exposure to the UV-B waveband.[153] As solar UV-B is reduced to almost zero in the winter months at latitudes above

50° North or South,[154] vitamin D status can vary greatly with season and location. Various surveys have provided evidence that many individuals, even those living in countries with high solar UVR,[155] may have inadequate vitamin D status.[156–160] Because of its ability to absorb UV-B, melanin in the skin can also decrease vitamin D status although, with sufficient UV-B, adequate vitamin D status can be achieved.[161] As an example of the effect that skin pigmentation can have on vitamin D production, 42% of black American women were considered 25(OH)D-deficient compared with 4.2% of white women in a recent survey.[162]

Vitamin D was identified almost one hundred years ago, and the link between sunlight exposure and childhood rickets proposed about four hundred years ago. Vitamin D is a very important hormone for many aspects of general health. It plays a major role in the growth, development and maintenance of bone, with deficits leading to low bone mineral density resulting in an increased risk of osteoporosis and fractures in adults and rickets in children. However adequate vitamin D status is now implicated in the prevention of an increasing list of non-skeletal disorders including several internal cancers and autoimmune diseases, and hypertension. $1,25(OH)_2D$ most commonly acts as a factor that stimulates cell differentiation and cell death.

Immune effects

Following the discovery of vitamin D receptors (VDRs) on several populations of immune cells, it is now known that vitamin D status can affect the immune system by suppressing T-cell proliferation, down-regulating antigen presentation, stimulating the generation of T_{reg} cells and Th2 cells, and activating macrophage function (reviewed in Mathieu *et al.*[163]). Indeed, once it became known that $1,25(OH)_2D_3$ can be synthesised in the skin following UVR, it has been suggested as a mediator of UV-induced immunosuppression. One illustration of this aspect is its inhibitory effects on the ability of Langerhans cells (which form a dendritic cell network in the outermost layers of the skin and survey the skin for any foreign challenges) to present antigens.[164]

Cancer

The most persuasive evidence to date suggesting a protective role for vitamin D in human disease relates to some internal cancers. Most information is available for colon, breast, prostate and ovarian tumours. Recently Garland *et al.*[165] undertook a review of relevant epidemiological studies and concluded that 20 out of 30 studies on colon cancer, 9 out of 13 on breast cancer, 13 out of 26 on prostate cancer and 5 out of 7 on ovarian cancer reported a significant benefit of vitamin D, its serum metabolites, sunlight exposure or another marker of vitamin D status on cancer risk or mortality. The other studies demonstrated a favourable trend (of borderline significance) or no association with vitamin D or its markers. A second recent review found a significant inverse correlation between sunlight exposure and the incidence or mortality of prostate, ovary and colon cancers with the data on non-Hodgkin lymphoma giving conflicting results.[166] Vitamin D might provide a protective effect by controlling cell proliferation, inducing terminal differentiation of tumour cells and inhibiting angiogenesis. There are many VDR polymorphisms, and only particular genotypes of VDR in combination with low 25(OH)D levels may correlate with the increased risk of cancer or metastasis. Notably one large longitudinal case-control study in the Nordic countries found that the risk of prostate cancer was greatest in two groups: those men with a low serum 25(OH)D (below 19 nmol L^{-1}) and those with a high serum 25(OH)D (above 80 nmol L^{-1}).[167] In addition, the results of a very recent large randomised double-blind placebo-controlled trial taking place in post-menopausal women showed that the incidence of colorectal cancer in the 18 176 individuals assigned to receive calcium carbonate plus vitamin D_3 (400 IU) daily was no different from the 18 106 individuals assigned to the placebo group.[168] This finding has been criticised as the daily dose of vitamin D taken by the subjects was lower than that recommended by some experts.[169,170] Furthermore a meta-analysis of 44 observational studies of either prospective (cohort) or retrospective (case-control) design concluded that individuals taking >1000 IU day^{-1} oral vitamin D or with >82 nmol L^{-1} serum 25(OH)D had 50% lower incidence of colorectal cancer compared with reference values.[171] In addition to vitamin D status, consideration of calcium status may be of crucial importance in the prevention of internal cancers, as has been demonstrated for colorectal adenomas.[172]

The majority of the epidemiological studies linking low UV exposures to higher incidence of internal cancers used latitude as a surrogate for exposure rather than measuring personal UV dose. However, several reports have now tried to include a personal estimation of sun exposure. In a case-control Australian survey, the risk of non-Hodgkin lymphoma fell with increasing solar irradiation, as assessed *via* a self-administered questionnaire and telephone interview.[173] As already noted in a previous section, Berwick *et al.*[46] reported that, following the diagnosis of early stage cutaneous melanoma, sun exposure was associated with increased survival rates over an average of a 5 year period. The irradiation was assessed by personal interview and a review of histopathological parameters, such as solar elastosis. Rukin *et al.*[174] assessed various parameters regarding past sun exposure, *via* a questionnaire, that might affect susceptibility to prostate cancer: in men with very low UV exposure, polymorphisms in particular subregions of the VDR gene were associated with risk. The difficulties of accurately estimating past personal UV exposure have already been indicated in this report. The finding of a significant protective effect is thus of some importance. Furthermore the inclusion of objective measures of past sun exposure such as solar elastosis provides further weight to this conclusion. No studies in animals have attempted to assess a protective role for solar UV exposure in internal cancer development, although several such studies have shown that vitamin D has activity against tumour proliferation and metastasis (reviewed in Giovannucci[175]).

Autoimmune diseases

As discussed above for the internal cancers, a protective role for vitamin D status is postulated for some autoimmune diseases, namely multiple sclerosis (MS), diabetes mellitus type 1, rheumatoid arthritis (RA) and inflammatory bowel diseases (IBDs). A brief overview of each is given below.

MS is an autoimmune disease in which an overactive Th1 cytokine response to an unidentified antigen stimulates an immune attack on myelin in the central nervous system. Initial epidemiological studies in human populations using latitude as a surrogate

for solar UV exposure[176] and experimental studies in a mouse model of MS (experimental allergic encephalomyelitis, EAE)[177] support the view that there is a link between poor vitamin D status, due to low sunlight exposure, and MS incidence. New evidence has indicated that increased sun exposure during ages 6–15 years is associated with a decreased risk of MS.[178] In a prospective cohort study of almost 20 000 nurses in the USA, Munger *et al.*[179] revealed that vitamin D supplements (>400 IU day^{-1} *vs.* nil) after the age of 25 was inversely associated with MS onset (40% decrease in risk). Also a record linkage study of skin cancer and MS has revealed that skin cancer incidence is significantly less common in MS patients than in those patients with other autoimmune or neurological diseases.[180] It is postulated that 1,25(OH)$_2$D could act by suppressing Th1 function while concurrently increasing T_{reg} and Th2 activities, thus helping to reduce the risk of MS development. However, in one of the few animal model studies to date in which UVR was incorporated, UV exposure induced progressive disease in some mice that had already developed the relapsing-remitting form of EAE.[181] It was shown that systemic immunosuppression had resulted from the UVR. These findings were explained by suggesting that Th1 responses contribute to disease onset while Th2 responses that are promoted by UVR may be more important in disease progression. It should be noted that in most of the mouse studies of EAE, 1,25(OH)$_2$D$_3$ was added to the diet rather than vitamin D$_3$, the metabolite formed in the skin after UV exposure, and calcium supplementation was also provided. A recent paper reports that dietary vitamin D$_3$ provided protection from the development of EAE in female mice, but not in ovariectomised female mice or in male mice.[182] Thus a complex relationship between vitamin D and female hormones may be indicated.

For type 1 diabetes, epidemiological studies show increased incidence at higher latitude, the converse to skin cancer incidence. Added to this, convincing evidence from models of non-obese diabetic mice demonstrates that vitamin D deficiency in early life accelerates the appearance of the disease.[183] A birth-cohort study in Finland indicated that regular vitamin D intake in early childhood reduced the risk of type 1 diabetes development in later life.[184] Two other reports show the protective effects of vitamin D or cod-liver oil (rich in vitamin D) in type 1 diabetes.[185,186] No studies have attempted to relate individual solar UVR dose with type 1 diabetes in humans or animal models thus far.

Unlike MS and type 1 diabetes, the incidence of RA does not correlate convincingly with latitude.[187] However, a prospective large-scale study has revealed an inverse association between vitamin D intake and RA.[188] As the symptoms of RA are largely due to the overactivity of the Th1 cytokines, especially tumour necrosis factor-α, low levels of 1,25(OH)$_2$D may not be sufficient to suppress this imbalance.

IBDs have an unknown aetiology but are immune-mediated and consist of at least two forms, ulcerative colitis and Crohn disease. A mouse model in which the VDRs are not expressed has been used to illustrate the importance of vitamin D for the maintenance of normal immune responses in the gastro-intestinal tract.[189] In another mouse model, 1,25(OH)$_2$D$_3$ prevented and ameliorated the symptoms of IBD.[190] Therefore it is possible that a vitamin D deficiency may lead to a lack of suppression of the enhanced Th1 cytokine responses that are typical of IBDs in humans. The role of sunlight in IBD has not been examined experimentally, although it is known that IBDs have a complex aetiology involving environmental factors and are most prevalent in higher latitudes where exposure to solar UVR is reduced compared with lower latitudes.

In conclusion, for the human autoimmune diseases, MS, type 1 diabetes, RA and IBDs, there is growing, although still not definitive, evidence to associate low solar UVR exposure and/or vitamin D with occurrence. Recent cohort studies have indicated convincingly that poor vitamin D status can be prospectively associated with the onset of the first three of these diseases (reviewed in Ponsonby *et al.*[191]). However, it is possible that another factor, apart from vitamin D, which is also linked with sun exposure, may be involved in modulating immune responses. Suggested factors include the UV-induced release of the neuropeptides, α-melanocytic-stimulating hormone and calcitonin-gene related peptide, or the light-induced suppression of melatonin levels.[191]

As a further indication of how complicated and confusing the links are between vitamin D deficiency and an increased risk of certain autoimmune diseases, there appear to be certain subsets of patient populations in whom the production of 1,25(OH)$_2$D$_3$ is increased.[192–194] In those with Crohn disease, the elevated 1,25(OH)$_2$D$_3$ is associated with low bone density and active disease which Abreu *et al.*[194] suggest may arise from inflammation occurring in the intestinal tract. In patients with sarcoidosis, elevated vitamin D was seen more frequently in those with extrathoracic involvement, a more serious form of the disease.[193]

Infectious diseases

Few studies to date have considered vitamin D in the context of infectious diseases, although Cantorna *et al.*[190] found that the susceptibility of mice to infection with HSV or the yeast *Candida albicans* was not affected by 1,25(OH)$_2$D$_3$ given in the diet. However, historically vitamin D has been used to treat tuberculosis and there is more recent evidence that 1,25(OH)$_2$D$_3$ can activate antimycobacterial activity in a murine model[195] and in cattle infected with *Mycobacterium bovis.*[196] An explanation of how this mechanism might operate has been provided using a mycobacterial model system. It has been shown that the activation of Toll-like receptors on human macrophages by mycobacterial lipopeptides leads to the up-regulation of the VDRs and vitamin D hydroxylase genes, resulting in the activation of the macrophages and the killing of the intracellular bacteria.[197] Several surveys have shown that, in temperate climates, the incidence of tuberculosis is higher in human subjects with low serum 25(OH)D levels,[198] and a recent study involving foreign-born people living in London concluded that 25(OH)D deficiency correlated with tuberculosis amongst all ethnic groups, except white Europeans and Chinese/South Asians.[199] The lack of solar UVR exposure is likely to contribute to the low levels of vitamin D, but poor dietary intake may be important and particular VDR polymorphisms may provide a genetic risk factor for some ethnic groups. An interesting recent review suggests the hypothesis that the occurrence of epidemic influenza predominantly in the winter months might be explained by the seasonal deficiency in vitamin D, leading to a significant reduction in several anti-viral immune mechanisms.[200]

Safety of response strategies

Response strategies to deal with the problems arising from ozone depletion can be divided into those that are directed at restoring

the appropriate level of ozone in the stratosphere by replacing ozone depleting substances (ODSs) with alternative chemicals, and those that are directed at protecting individuals from the increased solar UV-B arising from ozone depletion. Both strategies may have unintended consequences for human health. The sections below will summarize the safety aspects associated with the development and use of ODS replacement chemicals and then discuss some of the issues associated with various personal protection strategies for the eye and the skin.

ODS replacement chemicals

Much of the safety testing of many of the substitutes for ODSs, for example HCFC-124, HFC-134a and HFC-227, continues to find low toxicity in humans and animals.[201,202] However, there has been an increasing number of reports indicating that use or exposure to HCFC-123, in particular in occupationally exposed populations, can be associated with liver toxicity.[203–205] As the number of chemicals being proposed as replacements for ODSs is steadily increasing (EPA 2004, available at http://www.epa.gov/ozone/snap), it will be important to monitor their use for adverse events. This is particularly true for those chemicals that have seen limited use in the past and for which exposure and toxicity information is limited.

Personal protection strategies

Many of the protective strategies against excessive exposure to sunlight have been developed and advocated by those concerned about the effects of UVR on the skin. The first step towards protection from any toxic agent is to be aware that the hazard exists. The general advice to *seek shade* has become a keynote slogan for those involved in sun safety; this has been an effective addendum to the popular Australian *slip*, *slap*, *slop* campaign (now modified by New Zealand to be *slip*, *slap*, *slop* and *wrap*). The equivalent programme in the USA is called SunWise and it seeks to teach the public, especially children, how to protect themselves from overexposure to the sun (http://www.epa.gov/sunwise/).

Most public health pamphlets now include a reference to the need for hats and sunglasses. Wide brimmed (>10 cm) hats are recommended for head and eye protection and can reduce ocular exposure by up to 50%.[206] Protection from side-angles of UVR is often provided by the hood of a jacket and similar headwear. Although, as discussed above, there have been concerns that under-exposure to UV-B may impair vitamin D status, one recommendation suggests that 10–15 min per day in sunlight in the summer months should be sufficient to maintain adequate vitamin D status for most individuals.[165] This dose relates to white-skinned people living in countries such as north-west Europe and the USA, with exposure on unprotected skin. It should be modified considerably for those living at high or low latitude, for the season of the year and for immigrants with darker skin colour. In addition age, type of clothing, diet, whether the work place is in- or outdoors and the social environment are all important variables in determining how much ambient UVR exposure is optimal. One recent study illustrates the complexity of estimating recommended UV exposure times for the Australian population, and concludes that a single simple message for the general public is not possible.[207]

The skin and eye normally have some defences against oxidative and photo-induced damage. These include pigments such as melanin, antioxidant enzymes such as superoxide dismutase and catalase, and antioxidants such as vitamins C and E, lutein, β-carotene and other carotenoids, and glutathione. Many of these defences begin to diminish after 40 years of age resulting in less protection from radiation-induced damage to various structures of the eye.[208] The use of antioxidants, free radical scavengers and trace minerals, principally *via* the diet, appear to be effective in reducing the immunosuppressive effects of UVR as well as UV-B induced skin carcinogenesis[209]; no evidence was found of a similar effect for cataract or other UV-B-related ocular diseases. However, recent clinical and experimental data suggest that modifying a person's antioxidant status *via* supplementation may require extreme caution as the antioxidant defence system is complex and intricately balanced, and altering it may actually make the carcinogenic impact of UV worse.[209]

Protection specific to the eye. The eye is located in the bony orbit, and the forehead, eyebrows, lids and eyelashes provide considerable protection from overhead solar irradiance.[210] This explains why solar exposure at levels that should produce corneal damage within minutes, if the exposure were directly onto the cornea, does not do so. The need to protect ocular tissues from excessive exposure to UVR using appropriate absorptive glass and plastic materials is generally accepted and well understood.[211,212] Plastic lenses absorb up to about 350 nm and most high refractive index plastic (including polycarbonate) and glass lenses absorb even more UV-A. Thus, even clear spectacle lenses provide protection from UV-B. However, in the case of non-wraparound spectacles there is potential for ambient UVR to enter the eye from the side. This effect can be exacerbated by tinted sunglass lenses, which provoke a wider opening of the eye. This is particularly significant for the potential exposure of the crystalline lens from peripheral rays. Dose estimate factors have been proposed for the efficacy of a wide range of forms of eye protection, *i.e.*, from ordinary glass spectacles to highly protective ski goggles.[211]

Most early contact lens materials, other than fluorosilicone acrylate, provided little protection from UVR. As a result, rigid and soft contact lenses have now been developed which offer various levels of protection from UVR. Consideration of the optical absorption characteristics of a given lens and the related protection factors may be used to predict the protection afforded by a given lens. This has been confirmed by Walsh *et al.*[213] using modelled and measured data under high levels of solar UVR in the summer months in Houston, Texas. Rigid contact lenses provide no protection for the peripheral cornea or from the effects of peripheral light focusing. On the other hand, soft contact lenses that cover the entire cornea will protect the eye from UVR entering from the side or below. Using model eye and mannequin studies, Kwok *et al.*[8] have demonstrated that UVR-blocking soft contact lenses effectively shield against peripheral corneal focusing of obliquely incident UVR in the anterior segment of the eye. They also re-emphasise that many sunglasses do not protect against these rays and that contact lenses would provide protection when sunglasses are not worn. Sliney[211] concluded that UVR-blocking soft contact lenses provide protection from UV-B equivalent to ski-goggles for the cornea and internal eye structures.

Protection specific to the skin. Broad spectrum sunscreens are being used increasingly by the general population to minimise the erythemal effect of high sun exposure. They are generally effective

for that end-point but concerns have been expressed that regular sunscreen usage may impair cutaneous vitamin D synthesis, if the cream is applied at the correct concentration. While some reports indicate that sunscreens significantly decrease the production of 25(OH)D and $1,25(OH)_2D_3$,[214] others found little effect on the levels of these two substances.[215,216] Farrerons *et al.*[217] followed two groups of elderly subjects living in Barcelona, one treated with sunscreen and the other without treatment. The sunscreen users showed a minor decrease in serum 25(OH)D levels in both the summer and winter months compared with the controls, but this reduction was not sufficient to induce secondary hyperparathyroidism. It should be noted that, in practice, sunscreen application is frequently problematic with insufficient quantity being used to achieve the sun protection factor rated, or the spreading being non-uniform resulting in some skin sites getting little or no protection, or to some being washed or towelled off.[218,219]

Efforts have been made to define sunscreens in terms of their ability to protect against UV-induced immunosuppression. The immune protection factor (IPF) has been developed in an attempt to compare one preparation with another.[220] The IPF is defined as the ratio of UV doses influencing a particular immunological end-point in the presence or in the absence of the sunscreen. Using delayed hypersensitivity as an example, several reports indicate that sunscreens that absorb the UV-A waveband offer the most effective immunoprotection (reviewed in ref. 221). Of course, protection against the immune effects of solar UVR might not be beneficial if consideration of protection against selected internal cancers, autoimmune and infectious diseases is taken into consideration. Apart from sunscreens, there is considerable interest currently in identifying dietary constituents that could protect the skin's immune system against UV damage.[222,223]

Some concerns have arisen about unintended consequences from the increased use of sunscreens to protect against UV-B. A number of the UV-B absorbing components in sunscreens have been shown to have weak estrogenic activity so may have adverse consequences for reproductive function in human and animal populations in the environment, lending strength to the recommendation that protection from UV-B should not rely solely on sunscreen use.[224–231] As discussed above, there are many protective strategies for the skin that do not have unintended consequences for the environment. These include staying indoors, wearing clothing that covers sun-exposed areas of the body during conditions of high ambient UVR or seeking shade during the middle hours of the day although this will provide partial protection only.

Possible interactions between climate change and ozone depletion

If the predicted higher ambient temperatures in summer due to global warming are combined with drier weather, people living in mid-latitudes may spend more time outdoors, thus increasing their solar UV exposure. Indeed, it has been shown, at least in schoolchildren in the UK, that climate and ambient temperature influence behaviour and hence sun exposure more than ambient solar UV.[218] While such behavioural adaptation may have benefits in terms of vitamin D synthesis, the impact on skin cancer incidence and other health aspects of solar UVR are predicted to be adverse. There is also the possibility that climate change may result in wetter weather with more individuals staying indoors. Also, there would be regional differences in behavioural responses to warming.

In the previous report (UNEP 2002, published in de Gruijl *et al.*[1]) the possibility that rising temperatures due to global warming might enhance the induction of skin cancer by solar UVR was considered. This suggestion was based on experiments in mice performed many years ago.[232,233] As the process of UV-carcinogenesis is similar in mice and humans, rising temperatures could have a similar impact on skin cancers in humans, but the effect might be quantitatively different. Data on the influence of temperature on UV-carcinogenesis in human populations are not available but it is possible to investigate skin cancer incidence in people of similar skin colour living at different altitudes.[234] An attempt was made to find some indication from existing results: the incidence of NMSC in fair-skinned males and females in 10 different, well distributed regions of continental USA, collected in the Third National Cancer Survey,[235] has already been compared with UV-B measurements in the same region. In the new analysis, temperature data for these regions were added (van der Leun, personal communication). It was discovered that there was a similar trend to that in the mouse experiments towards a higher incidence of NMSC at relatively high temperatures compared with relatively lower temperatures. These preliminary results on human skin cancer reinforce the suggestion that the interaction of temperature and solar UV radiation may become an important health effect due to climate change. In addition it should be noted that, following the work of Sasaki *et al.*,[236] higher ambient temperatures as a result of global climate change may interact with UVR exposure to further increase the risk of nuclear cataract development.

As temperatures increase, changes in the quality and quantity of pest infestations are likely to require the increased use of pesticides. There are recent reports that exposure to certain pesticides can result in immunosuppression, and, in the case of permethrin, that such immunosuppression[237] may be additive to that caused by exposure to UV-B.[238]

Conclusions and gaps in knowledge

In the four years since our last report, considerable progress has taken place regarding the impact of ozone depletion, and hence of increased solar UV-B, on human health. The mechanisms whereby UVR interacts with structures in the eye and causes a variety of ocular diseases are becoming clear, as are details regarding the genetic basis of skin cancers and the pathways leading to UV-induced immunosuppression. The suggested links between solar UVR exposure, vitamin D and protection against a variety of internal cancers, autoimmune diseases and infection require further confirmation. In Table 2, we indicate areas where crucial knowledge is lacking.

Despite the distinct possibility that the ozone layer will repair itself in the coming decades, the general public will still require to maintain vigilance regarding their sunlight exposure. While it remains fashionable, for example, to have tanned skin, to wear minimal clothing in hot weather and to experience holidays in the sun, the risk of overexposure of the white population is high. The projection of a doubling in the incidence of all three types

Table 2 Suggested current gaps in knowledge regarding solar UVR and human health

Subject	Key questions
The eye	What are the pathogenic mechanisms involved in the cataract types?
	What are the wavelength dependencies for cataract development?
	What are the associations between UVR and other environmental factors that contribute to the induction of nuclear cataract in residents of developing countries?
The skin	What is the action spectrum for induction of melanoma?
	Are there any interactions between UV-A and UV-B in the induction of non-melanoma skin cancer and melanoma?
	What are the pathogenic mechanisms underlying infant *vs* adult UVR exposure in skin carcinogenesis?
	What is the mechanism of the interactions between UV-B and UV-A with regard to effects on immunity?
	What is the action spectrum for the synthesis of vitamin D3 in pigmented and unpigmented skin?
	How much solar UVR exposure is required, and how should it be distributed over the year, to maintain adequate vitamin D levels in people of different skin phototypes living at different latitudes?
	Can valid estimates be given to the general public regarding optimal doses of solar UVR for vitamin D synthesis while reducing the risk of developing skin cancer?
	What is the effect of solar UVR in animal models of auto-immunity and internal cancers?
Protective measures	Should the immune protection factors of topical sunscreens be measured and publicised?
	Are there factors in the diet that could give significant protection against the harmful effects of solar UVR?
	Should the UV index be used and analysed in developing countries, and should attempts be made to educate the general public regarding its meaning?
Climate change interactions	What are the combined effects of solar UVR and temperature on the skin and the eye?
	Will future changes in climate lead to people in mid-latitudes spending more time outdoors?

of skin cancer in the next ten years, plus a large increase in the number of cataracts, due partly to an ageing population, mean that health campaigns that stress the harmful effects of solar UVR are required and justified. However, to maintain sufficient vitamin D levels, the protective measures employed by an individual should not go to the extreme of minimal or no solar UVR exposure in the summer months.

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