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Sunlight, DNA Damage and Skin Cancer: A New Perspective

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If the depletion of the ozone layer is more than a passing perturbation, then the problem of solar carcinogenesis due to increased ambient levels of ultraviolet light is going to become increasingly important. Some recent results from studies with DNA repair-defective individuals have opened a new perspective on the way in which ultraviolet light (UV) gives rise to skin cancer.

Evidence for the involvement of DNA photoproducts in human skin carcinogenesis originally came from the work of Cleaver[1] who showed that patients with xeroderma pigmentosum (XP), who have a propensity for developing light-induced cancers early in life (for reviews see 2, 3), possessed cells that were defective in the excision repair of UV-induced pyrimidine dimers from their DNA. This defect was correlated with hypermutability when XP cells were exposed to UV.[4] Thus one could argue that the early increase in sunlight-induced cancers was a direct consequence of an increase in mutated cells in the skin of XPs. By 1976 the XP model had become one of the cornerstones of the somatic mutation (DNA damage) theory of cancer and was being used to justify the use of short-term mutagenicity tests to detect the potential carcinogenicity of chemicals (cf. 5).

By 1981, however, it had become apparent that there were difficulties with such an interpretation in respect of a number of observations in the literature about XP. An alternative hypothesis was proposed which argued that the crucial effect of sunlight which led to the early appearance of skin cancers was not the excessive induction of mutations but the exacerbation by UV of a defect in immune surveillance which resulted in existing transformed cells being able to grow and express their malignant phenotype.[6] This effect of UV was more akin to promotion than initiation.

It was also pointed out that cells of a patient with Cockayne syndrome were also hypermutable by UV[7] and yet such patients were not known to be prone to skin cancer. This argued that an increase in mutation frequency did not necessarily lead to an increase in cancer. It was, however, based on an observation with one rather poorly growing cell strain and has proved difficult to confirm. From a completely different disease has come evidence that not only supports the above conclusion but extends it. Trichothiodystrophy is a well defined hereditary disease characterised by sulphur-deficient brittle hair usually associated with varying degrees of mental and physical retardation, ichthyosis, dystrophy of the nails, dental caries and cataract. Many patients are also photosensitive and have been shown to be deficient in excision repair.[8] In fact their cells are indistinguishable from those from XP patients of complementation group D and are similarly hypermutable by UV.[9] Trichothiodystrophy patients, however, show no signs of freckling nor do they appear prone to early skin cancer. It may thus be concluded that not only does an elevated frequency of mutations not necessarily lead to an increase in early skin cancer but that a defect in excision repair is also insufficient to lead to early skin cancer.

Why then do XP patients who are not given adequate protection against UV succumb to skin cancer? The suggestion that a defect in immune surveillance is the reason for the early appearance of tumors is not proven, but the evidence is increasingly compelling.

Evidence for an immunological abnormality in XP patients was first reported independently by Dupuy and Lafforet[10] and Salamon *et al.*,[11] who were both unable to obtain sensitization to dinitrochlorobenzene in a skin test. Simultaneously Berkel and Kiran[12] reported a low *in vitro* response to phytohemagglutinin (PHA) in 4 out of 11 XP patients and in 2 out of 11 parents. They did not, however, find that delayed hypersensitivity responses were generally depressed in skin tests for a variety of recall antigens. Interestingly, they noted that rejection of skin grafts was delayed in their XP patients, an observation that is consistent with a subsequent report[13] of a remarkably well-tolerated at-random corneal transplant carried out in an XP patient. This patient had reduced *in vivo* antigen-specific humoral and cell-mediated responses and *in vitro* the cell-mediated immune response was in the lower part of the normal range. In a study with 9 XP individuals, reduced response to PHA in autologous serum and reduced delayed hypersensitivity response in skin tests with recall antigens were reported by Wynsenbeek *et al.*,[14] who also noted a reduction in the number of OKT-4 cells.

One of the problems that must be faced in dealing with immune responses of XP individuals (and in principle even with normal individuals) is that any effects observed may be either constitutive or due to sunlight-induced effects on lymphocytes passing through the skin or on Langerhans cells temporarily resident there. Two recent studies illustrate this. Morison *et al.*[15] studied 12 XP patients who were subject to high levels of ambient insolation and showed evidence of skin damage. Although no detectable difference was found between these XP patients and control subjects in the numbers of

Langerhans cells in skin biopsies, the majority of patients failed to develop an allergic response to the contact-sensitizing agent dinitrochlorobenzene. Moreover, when the group of XP patients was subdivided according to the extent of their cutaneous disease there appeared to be an inverse relation between disease severity and the development of contact allergy. These results may be contrasted with those of Norris *et al.*,[16] who studied five XP children from a region of much lower insolation, all of whom had been protected from exposure to the sun since early childhood. Normal proliferative T-cell responses to antigens and PHA were observed, together with normal numbers of circulating T-cells and normal T helper/suppressor ratios. It therefore seems likely that XP individuals may be particularly sensitive to sunlight impairment of cell-mediated immunity.

A new observation to emerge from the same study[16] was a marked reduction (around 60%) in natural killer (NK) cell toxicity in XP patients measured against the human erythromyeloid leukemia cell line K562 by means of a 4 hour ^{51}Cr-release assay. In the two patients with the greatest decrease in NK-cell activity, the percentage of CD16 (OK-NK)-positive circulating NK cells was normal, indicating a functional rather than a numerical deficiency. Although NK activity in normal individuals has been shown to be slightly inhibited after solarium exposure, it seems to be more likely, given the very low exposure of these children, that impaired NK function is a constitutive characteristic of XP patients rather than a consequence of exposure to light.

One may best summarize our present understanding as follows. Light-exposed XP patients are susceptible to elevated levels of skin cancer because their defect in DNA repair results in an increased frequency of initiated (mutated) skin cells. These are able to grow into tumorous colonies early in life, probably because of failure of the immune system to restrict their growth. This failure may be two-fold: a constitutive defect (probably in NK cell function) exacerbated by a UV-dependent impairment (probably of cell-mediated immunity and possibly also of residual natural killer cell function). If this view is correct, then patients with Cockayne syndrome and repair-defective trichothiodystrophy, who show high UV mutability but not high photocarcinogenicity, should have normal NK function. Norris *et al.*[17] have confirmed this expectation in 2 Cockayne syndrome patients and one trichothiodystrophy patient.

Asymptomatic Cancer-prone Individuals

The DNA repair defect observed in some trichothiodystrophy patients is probably nothing to do with the disease itself, since the basis of that is already known. It has been proposed that the genes for trichothiodystrophy and group D DNA repair deficiency are closely linked and are sometimes eliminated by a deletion covering them both.[18] If this were true, it would imply that individuals carrying only the repair defect should also exist, showing neither the early skin damage typical of xeroderma pigmentosum nor the symptoms of trichothiodystrophy. Such individuals would probably be more or less asymptomatic (at least in early life) but would be expected to have somatic cells that are UV-sensitive and hypermutable by UV. Such individuals might be expected to have an increased risk of skin cancer, particularly in later life as the immune system begins to deteriorate. They might even present as late onset mild cases of XP. It would seem to be worthwhile screening for such repair-defective individuals among otherwise normal patients presenting more than once with skin cancer. Those trichothiodystrophy patients with the repair defect would presumably be similarly at risk of late-onset effects but would not normally live long enough for them to be expressed.

The (presumed constitutive) immune defect in natural killer cell function present in classic XP patients may also be due to a further independently mutated gene, and this may also exist in individuals independently of the DNA repair defect. Such individuals might also be at some increased risk of skin cancer, but this would probably be expressed as single rather than multiple neoplasms (since the mutation frequency would be normal), and the skin cancers might appear earlier than in normal individuals.

The UV-dependent impairment of some function that controls incipient tumorous cells may well be similar in most individuals. The fact that trichothiodystrophy patients with the DNA repair defect show no evidence of skin cancers suggests (but no more) that UV impairment of tumor control might not be a result of DNA damage. It also suggests (since cells are believed to be killed by UV through DNA damage) that it is not simply a case of UV killing a large proportion of skin cells and stimulating the survivors to divide and repopulate the skin. While this may indeed occur and would stimulate both mutation fixation and clonal expansion, it is not sufficient to explain the loss of control of incipient tumors. Impairment of a specific system such as immune surveillance seems to be a necessary postulate. It would be interesting to look at the UV sensitivity of the delayed hypersensitivity reaction in repair-defective trichothiodystrophy individuals. A UV sensitivity similar to normal would indicate that DNA damage is not responsible for the impairment.

Some Speculative Conclusions about Risk

Ultraviolet light induces mutations in skin cells, some of which may be assumed to confer potential carcinogenicity. The induction is a stochastic process but the dose response may not be linear. Both saturation of

repair systems and stimulation of cell division due to the killing of neighboring cells could lead to greater mutagenesis at high doses and particularly at high dose rates. Mutations, once formed, could persist in stem cells for a very long time. Ultraviolet light may also impair immune surveillance. This may be a non-stochastic and decidedly non-linear effect, perhaps even thresholded. The combination of these two effects would produce a very non-linear dose response, with high exposures and fluence rates proportionately much more effective (and therefore presumably much more carcinogenic) than low, and with acute exposures more effective than chronic. To avoid early skin cancer the advice must therefore be to avoid high peak exposures, since these should dominate the risk assessment. In contrast, for cancers appearing in later years as the immune system loses it effectiveness, the total lifetime cumulative exposure would be expected to be relatively more important, although the effect of peak exposures might still be most important.

This brief review has, of course, involved a great oversimplification in that the various types of skin cancer have not been differentiated. It is most likely that melanomas, basal cell carcinomas, and squamous cell carcinomas (all of which are believed to be elevated in XP patients) have different susceptibilities to the various processes discussed above. At present, however, the data are inadequate for a differential analysis. This mini-review has also not covered any of the excellent work on UV-induced alterations in immune response in rodents, and the reader is referred to the reviews by Kripke.[19, 20]

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