Carcinogenic Mechanisms Related to Immunosuppressive Therapy

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Introduction

Before 1985, azathioprine and corticosteroids were used to suppress the immune response and prevent allograft rejection in organ transplant recipients. Since 1985, the majority of patients have received cyclosporin in combination with azathoprine and/or corticosteroids. More recently, other immunosuppressive agents have been introduced, including tacrolimus, sirolimus, and mycophenolate mofetil [1–3], which are described elsewhere in this book (see Part I).

Excess skin cancers, and indeed other cancers, in organ transplant recipients have been attributed in very large part to chronic suppression of the immune system by drugs used to prevent allograft rejection [4]. Loss of immunocompetence facilitates the frequency and persistence of viral infection, causal in the development of some transplant-associated cancers, including cervical and anogenital cancer, and in post-transplant lymphoproliferative disorders [4]. In addition, it is believed that such loss may reduce both "immune surveillance" and eradication of precancerous lesions, although the mechanism by which this occurs in the immunocompetent host is not well defined [5]. The important contribution of immunosuppression is further highlighted by the similarities between the range of cancers in organ transplant recipients and among human immunodeficiency virus (HIV)-infected individuals [6]. Kaposi's sarcoma, non-Hodgkin lymphoma, liver cancer, and cervical cancer are common in both groups.

In addition to overall intensity of immunosuppressive load contributing to excess skin cancer risk, there is increasing evidence to suggest that some drugs, principally azathioprine and cyclosporin, may also be directly carcinogenic, whereas others, specifically rapamycin, may have antineoplastic properties.

This chapter reviews the evidence for the contributions of overall reduction in immunosurveillance and specific carcinogenic properties of immunosuppressive drugs in the pathogenesis of post-transplant skin cancer.

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General Effects of Immunosuppression on Carcinogenesis

As most organ transplant recipients receive a combination of two or more immunosuppressive agents, it has been difficult to attribute quantifiable risk to any individual immunosuppressive agent. There are currently no data on skin cancer risk associated with the newer agents such as mycophenolate mofetil and tacrolimus, although some evidence suggests that sirolimus may confer a lower risk than standard therapy [7–9].

There is no satisfactory method to quantify immunosuppressive load, and it has therefore not been possible to establish the association between skin cancer risk and intensity of immunosuppression in the laboratory. Surrogate markers of immunosuppression have been employed, including lymphocyte subset analysis, lymphocyte proliferation assays, immunoglobulin levels, and Langerhans' cell density in the skin. Each method provides circumstantial support for the hypothesis that immunosuppressive load per se contributes to the development of skin cancer [10–12].

There is also clinical evidence to support this. For example, some tumours regress on withdrawal of immunosuppression [13] and skin cancer risk generally increases with increased duration of therapy [14]. In addition, triple immunosuppressive therapy is associated with a higher risk for skin cancer than dual therapy [5, 15]. There may also be a dose effect for individual drugs; low-dose cyclosporin regimens are, for example, associated with a lower cancer risk than standard doses [16]. Other studies show that cardiac transplant patients, who generally receive more intense immunosuppressive therapy, have an age- and sex-matched risk of skin cancer that is threefold higher than that of renal transplant recipients [15]. One report suggests that rejection episodes in the first year post transplant may be predictive for patients at higher risk of skin cancer, possibly because they require higher levels of immunosuppressive therapy to maintain graft function [17].

However, the effect of immunosuppressive dose on skin cancer risk for an individual needs careful interpretation because genetic [18] and pharmacokinetic variability may be important potential confounders. For example, the contribution of genetic variation to susceptibility to skin cancer has been investigated with regard to glutathione *S*-transferase genes. Glutathione *S*-transferases are a group of genes that encode enzymes involved in the detoxification of a variety of potentially mutagenic compounds, including ultraviolet radiation (UVR)-induced oxidative stress. Studies have shown that polymorphism in the glutathione *S*-transferases is associated with nonmelanoma skin cancer (NMSC) in organ transplant recipients in both the UK [19] and Australia [20].

Effects of Individual Immunosuppressive Drugs

Glucocorticoids

Prednisolone is the main glucocorticoid used to prevent allograft rejection in organ transplant recipients. It causes blockade of interleukin (IL)-1, -2, -3, -4, and -6,

tumor necrosis factor (TNF)-α, and interferon-γ by inhibition of cytokine gene transcription [1]. This action occurs through binding of the steroid receptor complex to the glucocorticoid response element in the promoter regions of cytokine genes. Prednisolone also exerts its antiinflammatory effect by inhibiting phospholipase A2 and the arachidonic acid cascade and by inhibition of monocyte migration and the synthesis, release, and action of chemotactic factors, permeability agents, and vasodilators [1]. In one study, long-term exposure to prednisolone alone was associated with an increased risk of skin cancer in non-organ transplant recipients [21], but there is no evidence to suggest any directly mutagenic or carcinogenic effect [22]. In mouse models of UV-induced carcinogenesis, for example, prednisolone does not enhance tumour formation [23].

Cyclosporin

Cyclosporin is a nonpolar cyclic oligopeptide. Its immunosuppressive activity is the result of inhibition of T-cell signalling. It binds cyclophilin, an immunophilin, which prevents dephosphorylation of nuclear factor of activated T cells (NF-AT) by the phosphatase calcineurin. Once translocated to the nucleus, NF-AT is responsible for stimulating IL-2 production and, therefore, the subsequent immune response [1]. Other calcineurin inhibitors used to prevent allograft rejection include tacrolimus.

Cyclosporin used alone is associated with an increased risk of keratinocyte skin cancer; a 5-year cohort study of psoriasis patients treated with cyclosporin showed that this increased risk was particularly enhanced in those on treatment for more than 2 years [24]. Early studies comparing the prevalence of cutaneous malignancy in organ transplant recipients receiving both azathioprine and cyclosporin reported varying results [25, 26]. Bunney et al. reported no difference, at least in the early stages of immunosuppression, in the prevalence of skin cancers between cyclosporin- and azathioprine-treated renal allograft recipients [25], whereas Shuttleworth et al. reported a higher prevalence of cutaneous dysplasia in transplant patients receiving cyclosporin [26]. More recent studies report an earlier onset and increased incidence of skin cancer in organ transplant recipients treated with cyclosporin [5, 15, 27, 28].

Evidence suggests that, in addition to being immunosuppressive, cyclosporin may also be mutagenic [29]. In vivo and in vitro studies have shown that calcineurin inhibitors including cyclosporin are associated with delayed repair of DNA damage and apoptosis in skin exposed to UV [27, 30–34] and increased UV sensitivity in human fibroblasts [35]. In one study, p53 mutations were reported in 15 of 25 (60%) keratinocyte skin cancers from immunosuppressed renal transplant recipients. Most (78%) were UV-specific C-to-T transitions at bipyrimidine sites, and, importantly, 35% of these were tandem mutations (including four UV signature CC-to-TT transitions), a significantly higher frequency than that found in the general immunocompetent population. This finding prompted the authors to propose that these mutations may be linked to inhibition of DNA repair by cyclosporin [35]. Inhibition of repair by cyclosporin may result in more cells with unrepaired

UV-induced DNA lesions in which deamination has time to occur and result in the formation of tandem CC-to-TT mutations. These findings not only confirm the importance of UV light as a major risk factor for skin carcinogenesis in transplant recipients on long-term immunosuppression but also highlight the potential importance of cyclosporin-modulated DNA repair in skin carcinogenesis in this patient group.

Cyclosporin may also promote cancer progression. Hojo et al. [36] reported that addition of cyclosporin to cultured adenocarcinoma cells increased their malignant phenotype, mediated through interaction with transforming growth factor (TGF)-β receptor. Adenocarcinoma cells treated with cyclosporin underwent morphological changes characteristic of invasive cells including membrane ruffling, increased motility, anchorage-independent (invasive) growth, and pseudopodial protrusions. Development of this cyclosporin-induced invasive phenotype appears to be related to TGF-β on the basis of several observations. First, cyclosporin stimulated TGF-β secretion in adenocarcinoma cells. Second, in contrast to IgG monoclonal antibodies, anti-TGF-β monoclonal antibodies prevented the cyclosporin-induced alterations. Third, recombinant TGF-β induced morphological alterations similar to those induced by cyclosporin in adenocarcinoma cells. Cyclosporin also induced phenotypic alterations in other cell types including murine renal cell adenocarcinoma cells, mouse mammary gland epithelial cells, and mink lung epithelial cells. Tumour growth was also enhanced by cyclosporin in immunodeficient SCID-beige mice, which were used to minimize the possibility that cyclosporin-induced suppression of the host immune system contributed to tumour progression. This finding suggests that cyclosporin induces tumour cells to produce TGF-β, which promotes cell invasiveness by a cell-autonomous mechanism independent of the immunosuppressant effect of cyclosporin on the host immune system [36].

Azathioprine

Azathioprine was initially introduced for the control of graft rejection after solid organ transplantation. It has since been used in other conditions including inflammatory arthropathies, such as rheumatoid arthritis, and inflammatory bowel disease (Crohn's disease and ulcerative colitis).

In mouse models, azathioprine, but not prednisolone, enhances the frequency of UV-induced tumours in hairless mice [23]. These findings were confirmed in another study in which 57% of mice treated with azathioprine and UVR (280–370 nm; peak, 310 nm) developed cutaneous squamous cell carcinomas (SCCs), compared with 18% treated with UVR alone, but none treated with prednisolone alone or in combination with UVR, suggesting a potential protective effect for prednisolone [37]. These combined data from murine models provide evidence that azathioprine has carcinogenic as well as immunosuppressive potential. Subsequent

studies have confirmed this and have proposed a mechanism of action, and azathioprine is now a recognised carcinogen [38].

The thiopurines, including azathioprine, are prodrugs requiring metabolic activation to thioguanine nucleotides that are, in turn, precursors for 6-thioguanine (6-TG) incorporation into DNA [39]. Azathioprine first undergoes cleavage to generate 6-mercaptopurine (MP). 6-MP and 6-TG are subsequently metabolised to 6-thiodeoxyguanosine triphosphate (6-TdGTP), which produces DNA-TG, believed to be responsible for most of the characteristic biological effects of delayed cytotoxicity and chromosome damage [40].

Modes of Action of Azathioprine

Incorporation of 6-TG into the DNA of rapidly dividing precursor lymphocytes may contribute to the primary immunosuppressive effect of azathioprine. The subsequent methylation of a small fraction of DNA 6-TG bases to form 6-meTG possibly results in its toxic effect. Because less than 0.1% of 6-TG in DNA is methylated and converted into a lethal lesion, it follows that there is a threshold below which thioguanine bases remain in cellular DNA without overt toxicity. The existence of a toxic threshold is demonstrated by the fact that mismatch repair-proficient cells tolerate significant, albeit lower, levels of DNA 6-TG without being killed. The approximately 0.01% substitution of DNA guanines by 6-TG in circulating lymphocytes of patients undergoing thiopurine therapy for leukaemia [41] or Crohn's disease [42] suggests that there is a similar toxic threshold in vivo. Since patients often receive systemic azathioprine for many years, particularly organ transplant recipients, it is likely that cells in other tissues also accumulate significant steady-state levels of DNA 6-TG. In addition to this, azathioprine metabolites also inhibit de novo purine synthesis. The effect of 6-TG on dNTP synthesis may contribute to immunosuppression as the dNTP pool of T cells is normally increased upon their activation, a requirement for subsequent function [43].

More recently, the discovery that 6-TdGTP can alter signalling pathways in activated T cells resulted in the proposal of an alternative/additional mechanism for the primary immunosuppressive effect of azathioprine [44, 45]. Apoptosis of activated T cells is prevented by the Rac-initiated signalling pathway that activates the apoptosis inhibitor bcl-x_L. Activation of the Rho GTPases Rac1 and Rac2 is stimulated by the Vav protein. 6-TdGTP can bind to Rac proteins instead of GTP and is subsequently hydrolysed to 6-TdGDP. Vav, however, is unable to stimulate exchange of 6-TGDP for GTP or 6-TdGTP, thereby inactivating Rac; this results in inhibition of the downstream signalling pathway and failure to activate bcl-x_L, thus allowing apoptosis to occur. The subsequent removal of activated T cells means that foreign antigens from the allograft in organ transplant recipients are tolerated. This proposed mechanism may explain why T cells are specifically affected by azathioprine, 6-TG, and 6-MP.

Potential Enhancement of UV-Induced Skin Carcinogenesis by Azathioprine

UVA produces DNA damage via endogenous cellular UVA photosensitisers that remain largely unidentified. Azathioprine and/or its metabolites also act as photosensitisers and increase the oxidative DNA damage caused by UVA irradiation. Thiopurines possess distinct photochemical properties, absorbing light in the UVA region in vitro, with 6-TG absorbing maximally at 342 nm. 6-MP generates reactive oxygen species (ROS) when exposed to UVA [46], as does 6-TG [47]. Human cells grown in nontoxic concentrations of 6-TG are sensitised to killing and mutation by low UVA doses within the normal sunlight range [47, 48]. The skin of patients on azathioprine also contains DNA 6-TG and is selectively photosensitive to UVA wavelengths [47]. DNA 6-TG and UVA interact to generate DNA-damaging ROS in cell nuclei [47]. Because oxidative DNA damage to normal DNA bases is implicated in the development of human cancer [49], it is plausible that 6-TG-mediated photochemical oxidation of DNA may contribute to the development of transplant-related skin cancer. In addition, guanine-6-sulfonate, the photochemical oxidative product of UVA/6-TG interaction, is a strong replication block. Bypass of replication-blocking guanine sulfonate by error-prone Y-family DNA polymerases may represent another potential source of mutation and a carcinogenic hazard [47] (Fig. 1).

mTOR Inhibitors/Proliferation Signal Inhibitors (PSI)

The mTOR inhibitors, also known as proliferation signal inhibitors (PSI), are a more recent addition to the immunosuppressive regimes used to prevent allograft rejection in solid organ transplant recipients. The mTOR inhibitor rapamycin (sirolimus), a macrocyclic lactone isolated from a strain of *Streptomyces hygroscopicus*, inhibits the mammalian target of rapamycin (mTOR)-mediated signal transduction pathways, which results in arrest of the cell cycle of various cell types, including T- and B lymphocytes. The mechanism of immunosuppression is described in detail in Part I of this book. mTOR inhibitors are potentially useful for organ transplant recipients as, in addition to their immunosuppressive effect, they also possess anticancer properties (see Part I). The mTOR pathway controls various signalling pathways required by cancer cells, and so inhibition of this pathway may reduce the prevalence of cancer in this high-risk group of patients. Preliminary evidence suggests that conversion of transplant recipients to mTOR inhibitors such as rapamycin or treating patients with rapamycin from the time of transplantation may reduce development of nonmelanoma skin cancer [50]. One study reported remission of nonmelanoma skin cancers in 37 of 53 (70%) renal transplant recipients after converting to mTOR inhibitors [51], and another study concluded that mTOR inhibitors may be useful in the management of post-transplant cutaneous and extracutaneous tumours [52]. Mathew et al. found that transplant recipients receiving rapamycin

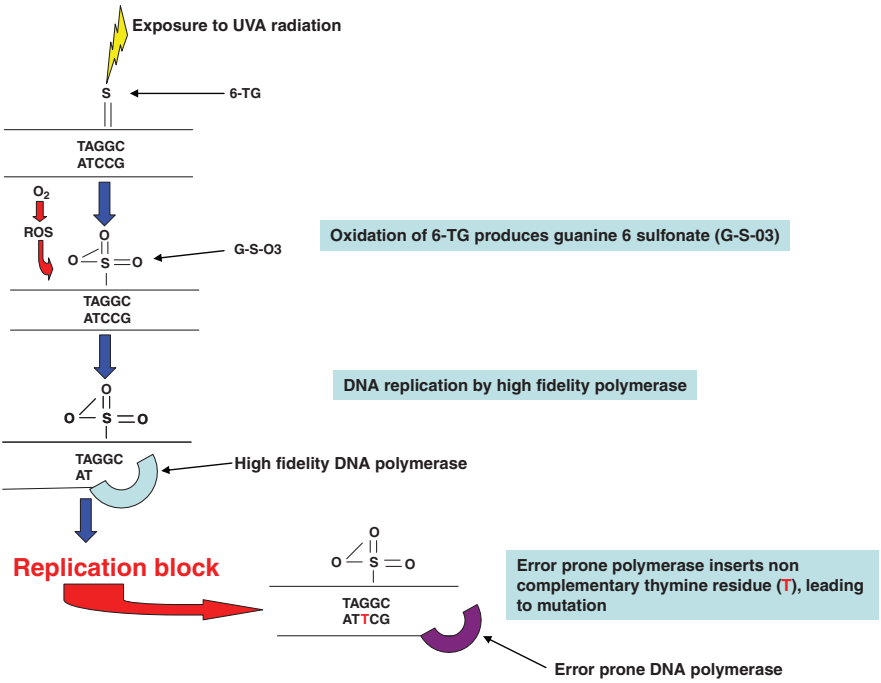

Fig. 1 Generation of mutagenic oxidative DNA damage by the interaction of 6-thioguanine (6-TG) and UVA. 6-TG, a metabolite of azathioprine, is incorporated into the DNA of skin cells of patients receiving azathioprine. UVA radiation photoactivates 6-TG to produce guanine-6-sulfonate (G-S-O3). DNA strands separate, and a high-fidelity DNA polymerase attempts to synthesise a new strand. However, G-S-O3 is a powerful block to high-fidelity replicative DNA polymerases, resulting in recruitment of low-fidelity error-prone polymerases, which facilitate the insertion of a noncomplementary residue leading to mutations [45]

without cyclosporin or rapamycin maintenance therapy after early cyclosporin withdrawal have a lower risk of malignancy in the first 2 years after renal transplantation [9]. Although these studies appear promising, further clarification of the potential benefits of mTOR inhibitors in this patient group is required.

Summary

This chapter outlines possible carcinogenic mechanisms of three immunosuppressive agents, namely cyclosporin, prednisolone, and azathioprine, all in routine use until recently, and their contribution to the development of post-transplant skin cancer. Many transplant units are now using other immunosuppressive regimens, comprising newer agents such as mycophenolate mofetil, tacrolimus, and sirolimus. The longer-term effects of these drugs on skin and other cancers in organ transplant recipients, whose life expectancy post transplant is now considerable, will become clearer in future.

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