Immune modulation and apoptosis induction: Two sides of the antitumoral activity of imiquimod

M. P. Schöhn and M. Schöhn
Rudolf-Virchow-Center, DFG Research Center for Experimental Biomedicine and Department of Dermatology and Venereology, University of Würzburg, Germany

Imiquimod, the first member of the imidazoquinoline family of immune response modifiers, has proven good clinical efficacy against basal cell carcinomas and actinic keratoses in several independent studies. In addition, there is recent evidence that imiquimod is also efficacious against other tumors such as cutaneous metastases of malignant melanoma or vascular tumors. Imiquimod exerts its antitumoral effect, at least in part, through binding to TLR-7 and TLR-8 on dendritic cells followed by secretion of a multitude of proinflammatory cytokines. The net result of this proinflammatory activity is a profound tumor-directed cellular immune response. However, recent experimental and clinical data indicate that imiquimod also possesses considerable direct pro-apoptotic activity against tumor cells both in vitro and in vivo. This novel mode of action appears to be independent of membrane bound death receptors, but involves caspase activation. Induction of apoptosis by imiquimod is, at least in part, presumably mediated through Bcl-2-dependent release of mitochondrial cytochrome c and subsequent activation of caspase-9. The structural analogue, resiquimod, exhibited very limited, if any, such pro-apoptotic activity, possibly due to its lacking ability to enter the cell. Bypassing molecular mechanisms of apoptosis deficiency by a topical compound may be of great utility for treating certain cutaneous tumors.

Keywords: apoptosis; bcl-2; imiquimod; immune response modifier; skin cancer.

Introduction

Various molecular alterations of cells contribute to the pathogenesis of malignant tumors. In case of epithelial skin cancers, namely basal cell carcinomas (BCC) and squamous cell carcinomas (SCC), UV irradiation, various chemicals, defective genes, point mutations (such as those identified in the patched (PTCH) gene or the p53 tumor suppressor gene), or polymorphisms of carcinogen-metabolizing enzymes have been recognized as etiological factors.1–7 Genetic alterations in these tumors may result in apoptosis deficiency allowing the tumors to grow uncontrolled.7–11 In addition, experimental and clinical evidence is accumulating indicating that impaired T lymphocyte-associated immune surveillance may also significantly contribute to the pathogenesis of both types of skin cancer.12 This evidence includes the rapid development of SCC and/or BCC in some patients with drug-induced immunosuppression,13 CD4 lymphocytopenia,14 AIDS,15 or hairy cell leukemia.16 In addition, both BCC and SCC respond to therapies modulating cellular immune mechanisms.17–22 Based on the recognition of immunological dysfunctions as contributing factors in the pathogenesis of skin tumors, searches for compounds have been conducted in order to overcome the immune evasion of such tumors. Imiquimod (1-(2-methylpropyl)-1H-imidazo[4,5-c]quinolin-4-amine) is an imidazoquinoline family member of $M_r = 240.3$. This small-molecule compound is highly efficacious in the treatment of BCC23–27 and intraepidermal keratinocyte neoplasias, the so-called actinic keratoses.27–28 In addition, imiquimod applied systemically in animal experiments has proven efficacy in a variety of transplantable tumors including colon carcinomas, melanomas, lung sarcomas, mammary carcinomas, and bladder carcinomas.29

It has been thought that imiquimod exerts its antitumoral activity primarily through induction of a profound cellular tumor-directed immune response.30 Recent experimental and clinical data, however, have demonstrated that the mode of action of this interesting compound clearly extends far beyond its known function as an immune response modifier inasmuch as imiquimod also confers direct pro-apoptotic activity against malignant and benign tumors of different origin.31–34 We will focus here
on the molecular mode of action of imiquimod which underlies its antitumoral activity.

Immunomodulatory effects of imiquimod in skin cancer treatment

The immunological aspects of imiquimod are summarized in Figure 1A. Dendritic cells (DC) are considered the primary responsive cell population in the skin, a notion that is supported by observations that DC respond to lower concentrations of imiquimod in vitro as compared to several other cell types.53–59 Recent studies demonstrated that members of the imidazoquinoline family are recognized independently by Toll Like Receptor-(TLR)-7 and TLR-8, thereby activating the signal transduction cascade downstream of these receptors.40,41 The activity of imiquimod is then mediated, at least in part, through intracellular activation of the transcription factor NF-κB, which upon activation migrates to the nucleus and upregulates transcription of various cytokines including TNFα, IL-2, IL-6, IL-8, IL-12, G-CSF, GM-CSF, IFNγ, and IFNα, as well as chemokines such as MIP-1α, MIP-1β, and MCP-1.35–38,42,43 The net result of these cytokine effects is a profound stimulation of a tumor-directed cellular immune response. As exemplified in Figure 1B, such a tumor-directed immune response is also seen in human skin tumors following exposure to topical imiquimod.55 In addition, imiquimod activates dendritic cells (resident Langerhans cells in the skin) possibly resulting in prolonged protective Th1-skewed immunity against viral infections and malignant tumors.44–47

Induction of tumor cell apoptosis by imiquimod in vitro and in vivo

It was thought that neither imiquimod nor resiquimod, a related compound of M, 314.4 inducing even more pronounced cytokine secretion, macrophage activation and enhancement of cellular immunity as compared to imiquimod,42,45,46–52 exhibit direct antineoplastic activities. It was, therefore, a surprise when it was found that tumor cells cultured in the absence of immune cells consistently exhibited reduced cell numbers when they were exposed to imiquimod (25–50 µg/ml; i.e., concentrations approximately 3 logs lower than the marketed formulation) as compared to otherwise identical cultures without imiquimod.31 In contrast, resiquimod showed very little, if any, such direct activity in the absence of immune cells. Further investigations revealed that this effect was due to marked induction of apoptosis by imiquimod, but not resiquimod (Figure 2A). The pro-apoptotic activity was dose-dependent and affected transformed keratinocytes (i.e., tumor cells) stronger than normal keratinocytes. When the generation of histone-bound DNA fragments was assessed as a parameter of apoptosis in cultured cells, it was found that in most cases imiquimod induced apoptosis in the cell cultures by 200–700% after 24 h of incubation, in some experiments to an even higher degree. Pro-apoptotic activity of imiquimod was also observed when superficial BCC of three patients were treated topically with the marketed formulation of imiquimod (Aldara 5% cream) for 4 days, although it could not be ruled out that indirect mechanisms of apoptosis induction contributed under in vivo conditions where many different cell types including dendritic cells and other immune cells were present. In any case, given that imiquimod causes apoptosis in concentrations approximately 1000-fold below the marketed formulation, it is reasonable to assume that the apoptosis observed in vitro is at least in part a direct effect. In subsequent studies, we and others have confirmed the pro-apoptotic activity of imiquimod in vivo in other tumors such as some cutaneous metastases of human malignant melanoma32 (Figure 2B) or a murine hemangiendothelioma model.32

Imiquimod-induced apoptosis is death receptor-independent, but involves caspase activation

Two major routes have been identified through which cytostatic drugs induce apoptosis. The first involves activation of membrane-bound death receptors, such as the CD95 (Fas/APO-1), TNF or TRAIL receptor systems, and the other is dependent on direct mitochondrial cytochrome c release, both resulting in subsequent cell death.11,53–55 Thus, at least three major hypotheses can be delineated (which are not mutually exclusive) concerning the molecular mechanisms involved in imiquimod-induced apoptosis of tumor cells: First, imiquimod could act directly on membrane-bound death receptors, thus initiating the apoptotic signal transduction cascade triggered by CD95 or other death receptors. Second, as described for ceramides,56 imiquimod could affect downstream molecular interactions triggering the caspase cascade and subsequent cell death, thus bypassing the membrane-bound receptors. Third, imiquimod could influence complementary (intrinsic) pathways of apoptosis in the tumor cells such as the Bcl-2-dependent mitochondrial cytochrome c release leading to activation of caspase-9 and subsequent cell death.

Several surface-bound death receptor systems and their ligands have been described thus far. The best-known example is the TNF receptor family member CD95 (Fas/APO-1) which plays important roles for immune evasion and apoptosis deficiency of many malignant tumors (reviewed in).57,58 Another, more recently identified receptor system of the TNF receptor family is the TRAIL (TNF-related apoptosis inducing ligand) system.59 While