Imiquimod: Unexpected Killer

Martin Leverkus
Department of Dermatology, University of Würzburg Medical School, Würzburg, Germany

Melanoma incidence and, mortality rates in fair-skinned populations are increasing, and despite a major effort in clinical research scrutinizing various treatment regimens the prognosis for these patients still remains poor. The treatment options tested have ranged from monochemotherapy and polychemotherapeutic approaches as well as immunomodulatory therapies using defined cytokines and more recently cell-based vaccination strategies with dendritic cells. Although immuno-modulatory approaches are currently regarded as the most promising, to date no improved overall survival has been achieved by any of these measures, especially if examined in multicenter trials (Eigentler *et al*, 2003). Melanoma is the most immunogenic tumor among all neoplasia and therefore the initiation of a melanoma-specific immune response by the patient's immune system may be crucial for a successful tumor attack. Thus, it represented a "hypothesis-driven" approach to investigate the potential of immune response modifiers like imiquimod for the topical treatment of melanoma (Powell *et al*, 2004). Imidazoquinoline compounds have potent antiviral and antitumor properties in animals, have been clinically approved for the treatment of genital warts caused by human papillomavirus, and show promise for the treatment of pre-cancerous cells of the skin (Sauder, 2003). Extensive studies over the past years have indicated that imiquimod affects the innate as well as the adaptive immune system by indirect (e.g., induction of immune modulatory cytokines) or direct (activation of toll-like receptors (TLRs) 7 and 8) action on diverse immune cells including dendritic cells (Hemmi *et al*, 2002). Based on this knowledge, the clinical regression of epithelial or melanocytic malign lesions following treatment with imiquimod was thought to mainly involve the activation of the innate and adaptive cellular immune response of the tumor host (Hurwitz *et al*, 2003). In this issue of the JID, Schön *et al* demonstrate that more pleiotrophic antitumoral responses have to be considered when studying imidazoquinolines. They demonstrate that imiquimod, but not resiquimod (R-848) is able to act not only as synthetic adjuvant but also as direct inducer of apoptosis for melanoma cells *in vitro* and *in vivo*. Remarkably, this proapoptotic effect did not require cells of the adaptive immune system as shown by *in vitro* studies with melanoma cell lines. Similar to recent findings by the same group for keratinocyte-derived skin neoplasias (Schön *et al*, 2003), this proapoptotic signal is selectively activated in melanoma cells, but not in primary human melanocytes. The study was initially prompted by the observation that imiquimod directly interferes with cell viability of melanoma cells *in vitro*. The authors then observed that doses of imiquimod in a range likely obtained *in vivo* directly induce tumor cell death. Further experiments confirmed that cell death was exerted by apoptosis rather than necrosis. Having examined the proapoptotic effect on melanoma cells *in vitro*, the authors went on and showed that imiquimod indeed induces apoptotic cell death *in vivo* when applied to cutaneous metastases. Intriguingly, melanoma cell lines generated from cutaneous metastases that did not respond to the topical application of imiquimod were also resistant to imiquimod *in vitro*, giving further circumstantial evidence for the biological relevance of the pro-apoptotic response to imiquimod.

How might imiquimod activate the apoptotic program in melanoma cells? Several pathways leading to the induction of apoptosis have been described over the last years. Schön *et al* started to examine the role of these different cell death pathways and first showed that imiquimod-induced apoptosis requires the activation of the "work horses" of apoptosis necessary for the unique phenotype of apoptotic cells, namely, the caspase family of proteases (Nicholson, 1999). A widely studied pathway for the induction of apoptosis is the "extrinsic" cell death pathway, when apoptosis is triggered from the outside of the cell by death receptors like TNF-R1, TRAMP, CD95, TRAIL-R1 and -R2, DR6, and EDA-R (Locksley *et al*, 2001). These transmembrane proteins trigger apoptosis by multimerization upon ligand binding via a cytoplasmic "death domain" that recruits the adaptor molecule Fas-associated death domain (FADD) and subsequently the initiator caspases 8 and/or 10 (Leverkus *et al*, 2003). Schön *et al* then demonstrated that imiquimod initiated the apoptotic program of melanoma cells independent of the "extrinsic" pathway of apoptosis in that blocking of individual members of the death receptor family (CD95, TRAIL-R1, -R2, or TNF-R1) was unable to interfere with imiquimod-induced cell death. Alternatively, apoptosis can be triggered via an "intrinsic" cell death pathway converging at the mitochondria. Many chemotherapeutic drugs are known to activate this intrinsic cell death pathway, eventually leading to the release of proapoptotic molecules like AIF, Smac, HtrA2, cytochrome c, and endonuclease G (Debatin *et al*, 2002). These molecules either activate caspase 9 in a multimeric complex called the apoptosome or exert their deleterious function for the cell in a caspase-independent manner (van Loo *et al*, 2002). In contrast to the signals activated by death receptors, the initiation of the intrinsic death cascade is less well understood and may involve p53-dependent and -independent modulation of Bcl-2 proteins. The Bcl-2 family of proteins

Abbreviation: TLR, toll-like receptor

consists of antiapoptotic (e.g., Bcl-2, Bcl-XL, A1, Boo) and proapoptotic subgroups (e.g., Bax, Bak, or "BH3-only" proteins like Bim, Bid, Bmf, Noxa, and Puma). Since their discovery, Bcl-2 and its homologues have been associated with cancer, and are of crucial importance for the regulation of the intrinsic cell death pathway (Coultas and Strasser, 2003). To ask the question if imiquimod activates the intrinsic cell death pathway, Schön *et al* have studied the role of Bcl-2 and Bax during imiquimod-induced apoptosis. When they examined the mitochondria-associated levels of the proapoptotic Bax and the antiapoptotic Bcl-2, they could show that in imiquimod-susceptible melanoma lines increased Bax translocation to mitochondria is detected, whereas imiquimod-resistant lines did not translocate Bax to mitochondria. Finally, functional studies using a murine Bcl-2 viral expression construct confirmed that Bcl-2 overexpression was able to block imiquimod-induced apoptosis, narrowing down the cellular pathways leading to imiquimod-induced cell death. It thus appears that selective interference with components of the proapoptotic cascade upstream of mitochondria is responsible for imiquimod-induced apoptosis of melanoma cells.

Several important questions arise from this study. What is the direct cellular target of imiquimod? Why does imiquimod, but not resiquimod activates a proapoptotic response? Imidazoquinolines activate TLR7 and TLR8, and proinflammatory signals elicited by these receptors are mediated via the MyD88-IRAK-TRAF6 pathway, subsequently leading to the activation of transcription factors like Jun N-terminal kinase (JNK), p38, activating protein-1 (AP-1) and nuclear factor-κB (NF-κB) (Yamamoto *et al*, 2004). In addition, MyD88 was shown to interact with FADD and the stoichiometry of these proteins at the TLR receptor complex may modify the outcome of receptor triggering (Bannerman *et al*, 2002; Ruckdeschel *et al*, 2002). It is tempting to speculate that imiquimod exerts its proapoptotic potential by recruitment of death domain proteins like FADD to TLR7 or TLR8 via MyD88, thereby activating downstream targets in the apoptotic signalling cascade. Alternatively, TLR triggering may differentially activate MyD88-independent apoptic pathways involving TRAM-TRIF-PKR as reported for TLR4 (Hsu *et al*, 2004). Intriguingly, the balance between antiapoptotic (e.g., NF-κB activation) and proapoptotic (e.g., caspase activation) signals may be modulated in a cell type or tumor-specific manner, ultimately shifting the fine-tuned balance between inflammation and cell death. Therefore, it will be interesting to study the quantitative responses to different imidazoquinolines in either resistant or sensitive melanoma cells in more detail. Obviously there are more possibilities, including a role for different affinities of these compounds to distinct TLR's or differences in the internalization following ligation by TLR agonists as recently suggested (Heil *et al*, 2003). In order to test these hypotheses, further studies selectively interfering with receptor-mediated pro- and antiapoptotic signals are required.

Once the exact mechanism of imiquimod-induced apoptosis is clarified, it may lead to the identification of related compounds that preserve the proapoptotic ability but lack the proinflammatory potential of these TLR ligands. Moreover it may enable the identification of more successful treatment regimens that utilize knowledge about tumor-specific properties of apoptosis resistance. Thus more efficient killers from the same family of compounds may allow for the initiation of the apoptotic program without a prominent proinflammatory response, and these agents might be useful for future tumor therapies in melanoma.

DOI: 10.1111/j.0022-202X.2004.22537.x

References

Bannerman DD, Tupper JC, Kelly JD, Winn RK, Harlan JM: The Fas-associated death domain protein suppresses activation of NF-kappa B by LPS and IL-1 beta. J Clin Invest 109:419–425, 2002

Coultas L, Strasser A: The role of the Bcl-2 protein family in cancer. Semin Cancer Biol 13:115–123, 2003

Debatin KM, Poncet D, Kroemer G: Chemotherapy: Targeting the mitochondrial cell death pathway. Oncogene 21:8786–8803, 2002

Eigentler TK, Caroli UM, Radny P, Garbe C: Palliative therapy of disseminated malignant melanoma: A systematic review of 41 randomised clinical trials. Lancet Oncol 4:748–759, 2003

Heil F, Ahmad-Nejad P, Hemmi H, *et al*: The toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily. Eur J Immunol 33:2987–2997, 2003

Hemmi H, Kaisho T, Takeuchi O, *et al*: Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat Immunol 3:196–200, 2002

Hsu LC, Mo PJ, Zhang K, *et al*: The protein kinase PKR is required for macrophage apoptosis after activation of Toll-like receptor 4. Nature 428:341–345, 2004

Hurwitz DJ, Pincus L, Kupper TS: Imiquimod: A topically applied link between innate and acquired immunity. Arch Dermatol 139:1347–1350, 2003

Leverkus M, Sprick MR, Wachter T, *et al*: Proteasome inhibition results in TRAIL sensitization of primary keratinocytes by removing the resistance-mediating block of effector caspase maturation. Mol Cell Biol 23: 777–790, 2003

Locksley RM, Killeen N, Lenardo MJ: The TNF and TNF receptor superfamilies: Integrating mammalian biology. Cell 104:487–501, 2001

Nicholson DW: Caspase structure, proteolytic substrates, and function during apoptotic cell death. Cell Death Differ 6:1028–1042, 1999

Powell AM, Russell-Jones R, Barlow RJ: Topical imiquimod immunotherapy in the management of lentigo maligna. Clin Exp Dermatol 29:15–21, 2004

Ruckdeschel K, Mannel O, Schrottner P: Divergence of apoptosis-inducing and preventing signals in bacteria-faced macrophages through myeloid differentiation factor 88 and IL-1 receptor-associated kinase members. J Immunol 168:4601–4611, 2002

Sauder DN: Imiquimod: Modes of action. Br J Dermatol 149 (Suppl. 66):5–8, 2003

Schön M, Bong AB, Drewniok C, *et al*: Tumor-selective induction of apoptosis and the small-molecule immune response modifier imiquimod. J Natl Cancer Inst 95:1138–1149, 2003

van Loo G, Saelens X, van Gurp M, MacFarlane M, Martin SJ, Vandenabeele P: The role of mitochondrial factors in apoptosis: A Russian roulette with more than one bullet. Cell Death Differ 9:1031–1042, 2002

Yamamoto M, Takeda K, Akira S: TIR domain-containing adaptors define the specificity of TLR signaling. Mol Immunol 40:861–868, 2004