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2. Imiquimod: Mode of Action and Therapeutic Potential

OLIVIER CHOSIDOW and REINHARD DUMMER

INTRODUCTION

The immune system's main function is to protect the host against infection and can be divided into two main parts, innate (non-specific) and acquired (specific) immunity. Innate immunity provides the first line of defense against pathogens, and includes mechanisms already present that can be activated immediately, as in the skin and mucous membranes, e.g. the interferon alpha (IFNα) response, the cytokine response, and the neutrophil and macrophage response. In contrast, acquired immunity is specific for each pathogen and consists of humoral and cellular responses. Humoral immunity involves the production of immunoglobins (antigens) by B lymphocytes, which bind specifically to the antigen that induced them. A humoral response is initiated when an antigen activates a specific B-cell with the support of CD4 T cells. The B-cell proliferates and differentiates to form plasma cells, which then produce the antibodies with high affinity against the target antigen. Cell-mediated immunity depends on direct interactions between T-cell lymphocytes and cells primed by professional antigen presenting cells lymphocytes and cells; the cells bearing the antigen the T cells recognize in the HLA class I/II complex. A cellular response is initiated when an antigen on the surface of an abnormal cell is identified by and activates T-helper and cytotoxic T cells.

Immunomodulators orchestrate the immune response, either up-regulating (immunostimulation) or down-regulating (immunosuppression) the immune response. Imiquimod belongs to the family of immunostimulators and is a novel synthetic molecule which enhances both the innate and acquired immune response, in particular the cell-mediated pathways.

MECHANISM OF ACTION OF IMIQUIMOD

Imiquimod, an imidazoquinoline, has shown antiviral and antitumor properties in animal models (1). However, the precise mechanism of action is unknown. The data from pre-clinical studies suggest that imiquimod acts as a potent immune response modifier through its ability to induce the production of cytokines, which in turn stimulate T cells, thereby enhancing innate and acquired cellular immunity (1, 2).

Imiquimod's effects on the innate immune response, in particular its ability to induce IFNα and other cytokines, are largely responsible for its acute antiviral and antitumor effects (1). Induction of the cytokines IFNα, interleukin (IL) 6 and IL12 and tumor necrosis factor (TNFα) by imiquimod has been observed in pre-clinical studies (1, 2). In addition to this, imiquimod also stimulates other aspects of the innate response in animal models. Natural killer cell activity is stimulated, macrophages are activated to secrete both cytokines and nitric oxide, and B lymphocytes are induced to proliferate and differentiate (1). The action of imiquimod to stimulate innate immunity indicates its potential to treat viral infections and tumors.

The cellular arm of the two pathways in the acquired immune response is induced by imiquimod, although this is not a direct effect (Fig. 1). Imiquimod does not directly stimulate T-cell division or directly induce T-cell cytokines (3). Instead imiquimod indirectly stimulates the production of the T-helper type 1 (Th1) cytokine IFNγ. One mechanism by which this may occur is that imiquimod induces IFNγ, which upregulates the expression of the IL12 receptor β2 subunit on Th1 cells, increasing their responsiveness to IL2 and their production of IFNγ (4). Imiquimod also acts to suppress the humoral arm of acquired immunity by inhibiting the production of the Th2 cytokines, IL4 and IL5 (4). IFNα is believed to play a major role in this inhibition by imiquimod (4).

An additional effect of imiquimod on the immune response is its activation of Langerhans' cells, the major antigen-presenting cells within the epidermis. Imiquimod enhances the migration of these cells to the regional lymph node (2) potentially enhancing antigen presentation to T cells.

In the last two years, results have shown that cells of the immune response recognize pathogens by special pattern recognition receptors on the immune cell surface (5). These receptors are called Toll-like receptors (TLRs) and they discriminate between specific components derived from pathogens (5). Recognition of microbial invasion by TLRs triggers activation of a signalling cascade, which leads to the production of inflammatory cytokines. In humans TLRs subtypes 1 to 10 have been identified. In animal models, it has been shown that imiquimod acts through TLR7 and stimulates rapid synthesis and release of cytokines from monocyte, macrophage and dendritic cells (5). Imiquimod is the first small molecule disclosed to act through TLR activation, especially TLR7 (5). Hemmi et al. showed that activation of immune cells by imidazoquinoline compounds such as imiquimod, is elicited through the MyD88-dependent signalling

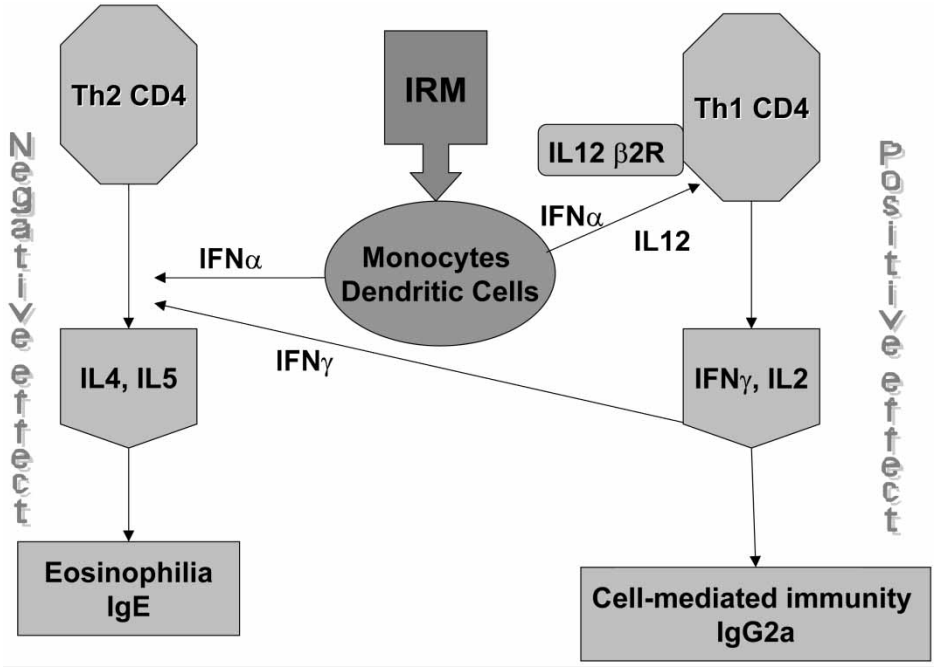

Fig. 1. Immune response modifier (IRM) effects on Th1 & Th2 balance.

cascade (5). In the study, generation of TLR7-deficient mice showed that TLR7 is an essential receptor for stimulation by the imidazoquinolines (5). Clarifying how TLR7 is involved in viral infection or in type I IFN production will shed further light on host-virus interactions (5). The stimulatory effect of imiquimod on the immune response can be used to improve the host's response to a virus, for example human papillomavirus (HPV), which often evades the immune system.

Human papillomavirus

HPV has numerous manifestations, including common, plantar and genital warts, and genital cancers. Different HPV types have different anatomic preferences and the severity of infection varies widely, some with low oncogenic risk and some with high oncogenic risk. HPV infection is common across all races and socio-economic groups and is prevalent throughout the world. In most viral infections the presence of viral proteins within a cell stimulates production of cytotoxic T cells. When a virus-infected cell is damaged, viral proteins leak from the cell, and these are detected by dendritic (antigen-presenting) cells and taken to the draining lymph nodes, where they activate T-helper cells and cytotoxic T cells. These cells then seek out and destroy the virus-infected cells. The target cells for HPV infection are epithelial basal keratinocytes. After viral penetration, the viral capsid proteins are produced. However, unlike many viruses, HPV does not cause cell lysis; infection spreads through the shedding of infected epithelial cells from the surface of the skin. This means that there is no release of viral proteins to the circulating dendritic cells and therefore, limited or no antigen presentation. More specifically, cytotoxic T cells are needed to kill virus-infected cells. HPV has a limited number of proteins, many of them mimicking 'self' proteins, which are not immunogenic.

Despite the absence of antigens, HPV can induce an immune response, and spontaneous regression of anogenital warts, associated with HPV types 6 and 11, is seen in 10% to 30% of patients (6). This regression may be due in part to the production of specific immunity against the HPV capsid, thought to be due to cell-mediated immunity. The lack of a cellular immune response means that although many treatments are available for the skin conditions caused by HPV, few are uniformly successful. The majority of treatments work by destroying affected tissues, by either a cytotoxic or physically ablative mode of action. Therapies for cutaneous warts include surgical excision, ablation by cryotherapy, electrocautery or laser therapy, podophyllotoxin or trichloroacetic acid. Although these treatment methods remove visible genital and nongenital lesions, they are associated with pain and a high recurrence rate (7). Latent HPV can remain in the skin or mucous membranes surrounding the skin of the original wart resulting in recurrence. Analysis of skin biopsies from warts undergoing spontaneous regression suggests that immune enhancement may be an alternative to ablative therapies.

The ideal way to combat HPV infection would be to improve the immune response to the virus so it is specific and directed against early viral proteins. One way of achieving this would be by better presentation of viral antigens to the immune response. Stimulation

of cell-mediated immunity by topical application of imiquimod has been shown to be an effective strategy for the treatment of HPV infection, in particular, genital warts (8–12). In a mechanism of action study of 22 patients, Tyring reported that when genital warts were treated with imiquimod three times a week for up to 16 weeks, HPV DNA started to disappear by the sixth week of treatment (13). This was mirrored by the disappearance of the genital warts. The study also found up-regulation of IFNα, IFNβ, IFNγ and TNFα, both at week 6 and at the end of therapy. These findings are substantiated by the increasing number of case reports of imiquimod's successful treatment of other HPV-associated conditions, such as common warts, as well as other cutaneous viral conditions, for example molluscum contagiosum (14–17). Imiquimod 5% cream (Aldara^{TM}) has been approved for the treatment of external genital and perianal warts. In addition to the treatment of viral skin conditions, imiquimod could also be a successful treatment for conditions where the immune system affects the regression of the disease such as cutaneous oncological conditions, for example basal cell carcinoma (BCC) and actinic keratosis (AK).

Nonmelanoma skin cancers

Non melanoma skin cancer (NMSC) is one of the most common types of cancer in the world. Caucasians are the most affected, particularly in areas exposed to sunlight, such as head, neck, forearm and back of the hand. There is an increased risk with fair skin, blue eyes and a history of repeated sunburns (18). The main forms of NMSC are BCC and squamous cell carcinoma (SCC), which account for 80% and 16% of cases, respectively (18). Actinic keratosis is a pre-cancerous lesion that may develop into SCC. The risk of progression of AK to invasive SCC has been estimated as ranging from 0.25% to 20% per year.

Ultraviolet radiation (UVR) has a profound effect on both the local and systemic immune system and has been proposed as a contributing factor in the development of NMSC. One mechanism that is thought to mediate the immunosuppressive effect of UVR is the alteration of Langerhans' cells, as UVR impairs their ability to present antigens to Th1-lymphocytes (19). UVR also stimulates keratinocytes to produce certain cytokines including TNF and IL-10, which promote the development of suppressor T cells. Despite the effect of UVR to suppress the immune response, both the innate and acquired immune pathways are thought to play some role in skin cancer immunosurveillance (20). For BCC regression, it is thought that the interactions between T lymphocytes and tumor cells that process and present tumor-associated antigens are critical. A recent study has shown that in regressing BCCs compared with non-regressing BCCs, there is a significant increase in the expression of the Th1 cytokine IFNγ and also elevated levels of IL2 and TNFβ (21).

The putative role of Th1 cytokines in the spontaneous regression of BCCs highlights the fact that successful clearance may occur with application of imiquimod by stimulation of these same pathways. An additional factor that encourages the use of imiquimod for the treatment of BCC is that BCCs are known to respond well to IFN treatment (20, 22, 23). Treatment with IFNα, however, requires intralesional injections three times a week for at least three weeks, which can be painful and requires multiple clinic visits. The side effects of IFNα treatment include local and systemic inflammatory responses and sometimes severe flu-like symptoms, which may cause the patient to discontinue therapy.

Imiquimod offers an alternative to intralesional IFN injections, as it is a patient-applied cream, so treatment can take place in the home. In a number of clinical trials, imiquimod has successfully treated BCC (24–30). Imiquimod has also been shown to successfully treat AK, in a number of trials and case studies (31–33). Drs Shumack and Rigel discuss these results in more depth in this supplement. Further phase III clinical studies are being carried out to confirm this evidence.

CONCLUSIONS

In summary, immunotherapy is already a valid treatment option for several types of cutaneous viral infections. Imiquimod is especially interesting because it is a treatment that the patient can administer at home. The clinical efficacy of imiquimod in the treatment of HPV-associated conditions has been shown in numerous clinical trials (8–12) and it is a recommended treatment option for external genital and perianal warts (34, 35). The effectiveness of imiquimod for the treatment of BCC and AK has also been demonstrated in clinical studies (24, 25, 27–33). However, in order to supplement the growing clinical evidence, further multicenter, international, randomized clinical trials with imiquimod in various cutaneous viral and oncological diseases are ongoing.

REFERENCES

1. Miller RL, Gerster JF, Owens ML, Slade HB, Tomai MA. Imiquimod applied topically: a novel immune response modifier and new class of drug. Int J Immunopharmacol 1999; 21: 1–14.
2. Suzuki H, Wang B, Shivji GM, Toto P, Amerio P, Tomai MA, et al. Imiquimod, a topical immune response modifier, induces migration of Langerhans' cells. J Invest Dermatol 2000; 114: 135–141.
3. Sauder DN. New immune therapies for skin disease: imiquimod and related compounds. J Cut Med Surg 2001; 5: 2–6.
4. Wagner TL, Ahonen CL, Couture AM, Gibson SJ, Miller

RL, Smith RM, et al. Modulation of TH1 and TH2 cytoki–ne production with the immune response modifiers, R-848 and imiquimod. Cell Immunol 1999; 191: 10–19.
5. Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K, et al. Small anti-viral compounds activate immune cells via the TLR7 MYD88-dependent signalling pathway. Nat Immunol 2002; 3: 196–200.
6. Coleman N, Birley HDL, Renton AM, Hanna NF, Ryait BK, Byrne M, et al. Immunological events in regressing genital warts. Am J Clin Pathol 1994; 102: 768–774.
7. Beutner KR, Wiley DJ. Recurrent external genital warts: a literature review. Papillomavirus Rep 1997; 8: 69–74.
8. Edwards L, Ferenezy A, Eron L, Baker D, Owens ML, Fox TL, et al. Self-administered topical 5% imiquimod cream for external anogenital warts. Arch Dermatol 1998; 134: 25–30.
9. Buck HW, Fortier M, Knudsen J, Paavonen J. Imiquimod 5% cream in the treatment of anogenital warts in female patients. Int J Gyn & Obs 2002; 77: 231–238.
10. Fife KH, Ferenczy A, Douglas JM, Brown DR, Smith M, Owens ML, the HPV Study group. Treatment of external genital warts in men using 5% imiquimod cream applied three times a week, once daily, twice daily or three times a day. Sex Trans Dis 2001; 28: 226–231.
11. Garland SM, Sellors JW, Wikstrom A, Peterson CS, Aranda C, Aractingi S, et al. Imiquimod 5% cream is a safe and effective self-applied treatment for anogenital warts – results of an open-label, multicentre Phase IIIB trial. Int J STD AIDS 2001; 12: 722–729.
12. Gollnick H, Barasso R, Jappe U, Ward K, Eul A, Carey-Yard M, Milde K. Safety and efficacy of imiquimod 5% cream in the treatment of penile genital warts in uncircumcised men when applied three times weekly or once per day. Int J STD AIDS 2001; 12: 22–28.
13. Tyring S. A randomized, controlled, molecular study of condylomata acuminata clearance during treatment with imiquimod. J Infect Dis 1998; 178: 551–555.
14. Yesudian PD, Parslew RAG. Treatment of recalcitrant plantar warts with imiquimod. J Dermatol Treat 2002; 13: 31–33.
15. Weisshaar E, Gollnick H. Potentiating effect of imiquimod in the treatment of verrucae vulgares in immunocompromised patients. Acta Derm Venereol 2000; 80: 306–307.
16. Schwab RA, Elston DM. Topical imiquimod for recalcitrant facial flat warts. Cutis 2000; 43: 555–556.
17. Buckley R, Smith K. Topical imiquimod therapy for chronic giant molluscum contagiosum in a patient with advanced human immunodeficiency virus 1 disease. Arch Dermatol 1999; 135: 1167–1169.
18. Diepgen TL, Mahler V. The epidemiology of skin cancer. Br J Dermatol 2002; 146: 1–6.
19. Bergstresser PR. Ultraviolet immunosuppression. Dermatol Found 2000; 34: 1–12.
20. Urosevic M, Dummer R. Immunotherapy for nonmelanoma skin cancer: does it have a future? Cancer 2002; 94: 477–4585.
21. Wong DA, Bishop GA, Lowes MA, Cooke B, Barnetson RStC, Halliday GM. Cytokine profiles in spontaneously regressing basal cell carcinoma. Br J Dermatol 2000; 143: 91–98.
22. Cornell RC, Greenway HT, Tucker SB, Edwards L, Ashworth S, Vance JC, et al. Intralesional interferon therapy for basal cell carcinoma. J Am Acad Dermatol 1990; 23: 694–700.
23. Greenway HT, Cornell RC, Tanner DJ, Peets E, Bordin GM, Nagi C. Treatment of basal cell carcinoma with intralesional interferon. J Am Acad Dermatol 1986; 15: 437–443.
24. Marks R, Gebauer K, Shumack S, Amies M, Bryden J, Fox TL, et al. Imiquimod 5% cream in the treatment of superficial basal cell carcinoma: results of a multi-center 6-week dose response trial. J Am Acad Dermatol 2001; 44: 807–13.
25. Beutner KR, Geisse JR, Helman D, Fox TL, Ginkel A, Owens ML. Therapeutic response of basal cell carcinoma to the immune response modifier imiquimod 5% cream. J Am Acad Dermatol 1999; 41: 1002–1007.
26. Geisse JK, Rich P, Pandya A, Gross K, Andres K, Ginkel A, Owens M. Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: a double-blind, randomized, vehicle-controlled study. J Am Acad Dermatol 2002; 47: 390–398.
27. Chen TM, Rosen T, Orengo I. Treatment of a large superficial basal cell carcinoma with 5% imiquimod cream: a case report and review of the literature. Dermatol Surg 2002; 28: 344–346.
28. Drehs MM, Cook-Bolden F, Tanzi EL, Weinberg JM. Successful treatment of multiple superficial basal cell carcinomas with topical imiquimod: case report and review of the literature. Dermatol Surg 2002; 28: 427–429.
29. Shumack S, Robinson J, Kossard S, Golitz L, Greenway H, Schroeter A, et al. Efficacy of topical 5% imiquimod cream for the treatment of nodular basal cell carcinoma. Arch Dermatol 2002; 138: 1165–1171.
30. Sterry W, Ruzicka T, Herrera E, Takwale A, Bichel J, Andres K, Ding L. Imiquimod 5% cream for the treatment of superficial and nodular basal cell carcinoma: randomised studies comparing low-frequency dosing with and without occlusion. Br J Dermatol 2002; 147: 1227–1236.
31. Stockfleth E, Meyer T, Benninghoff B, Christophers E. Successful treatment of actinic keratosis with imiquimod cream 5%: a report of six cases. Br J Dermatol 2001; 144: 1050–1053.
32. Salasche SJ, Levine N, Morrison L. Cycle therapy of actinic keratoses of the face and scalp with 5% topical imiquimod cream: an open label trial. J Am Acad Dermatol 2002; 47: 571–577.
33. Stockfleth E, Ulrich C, Salasche SJ, Christophers E. A randomized, double-blind, vehicle-controlled study to assess imiquimod 5% cream for the treatment of actinic keratosis. Arch Dermatol 2002; 138: 1498–1502.
34. von Krogh G, Lacey CJ, Gross G, Barrasso R, Schneider A. European course on HPV-associated pathology: guidelines for the diagnosis and management of anogenital warts. Sex Trans Infec 2000; 76: 162–168.
35. Centers for Disease Control & Prevention (CDC). 2002 Sexually Transmitted Diseases Treatment Guidelines Morbidity & Mortality weekly report (MMWR) 2002; 51(RR6): 53–56.