



PERGAMON

International Journal of Immunopharmacology 21 (1999) 1–14

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INTERNATIONAL JOURNAL  
OF  
IMMUNOPHARMACOLOGY

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## Review Article

# Imiquimod applied topically: a novel immune response modifier and new class of drug

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Received 29 May 1998; accepted 31 July 1998

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### Abstract

Imiquimod (S-26308, R-837) (1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4 amine), an immune response modifier, demonstrates potent antiviral and antitumor activity in animal models (see structure in Fig. 1). The drug exhibits no direct antiviral or antiproliferative activity when tested in a number of cell culture systems. Imiquimod's activity was discovered while screening for anti-herpes virus activity. One of the first analogs in the series, S-25059 was tested in the early 1980's and due to slight toxicity, caused slightly reduced herpes cytopathology in Vero cell cultures. Follow-up testing in herpes infected guinea pigs showed complete protection toward lesion development. Activity of these drugs results primarily from interferon alpha (IFN- $\alpha$ ) induction and other cytokine induction. At least part of the cytokine induction is mediated through NF- $\kappa$ B activation. These cytokines stimulate several other aspects of the innate immune response. In addition, imiquimod stimulates acquired immunity, in particular the cellular arm which is important for control of viral infections and various tumors. This effect is mediated by drug induced IFN- $\alpha$  and Interleukin-12 (IL-12) and IFN- $\gamma$  induced by these cytokines. Imiquimod is expected to be effective where exogenous IFN- $\alpha$  has shown utility and where enhancement of cell-mediated immunity is needed. The following is a brief review of the preclinical pharmacology of imiquimod and the clinical results of genital wart trials. The mechanism of action of topically applied imiquimod will likely lead to benefits in several other chronic virus infections and tumors of the skin. Two other reviews on imiquimod that focus mainly on the clinical results have been published (Beutner & Geisse, 1997; Slade, Owens, Tomai & Miller, 1998). © 1999 International Society for Immunopharmacology. Published by Elsevier Science Ltd.

*Key words:* Cell mediated immunity; Condylomata acuminata; Cytokines; Human papillomavirus; Imiquimod; Immune response modifier; Interferon; Mechanism of action; Monocytes; Preclinical; Review; Therapy

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## 1. Effects on innate immunity

When incubated with mouse spleen cells *in vitro*, imiquimod at 0.2  $\mu\text{g}/\text{ml}$  induces the synthesis and release of IFN, IL-6, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and probably other cytokines (Reiter, Testerman, Miller, Weeks & Tomai, 1994). Imiquimod also causes non-specific B-cell proliferation which may be mediated directly by the drug and not through cytokine induction (Tomai, Imbertson, Wagner, Reiter & Miller, 1994).

The mouse macrophage cell line, RAW 264.7, produces TNF in response to 3  $\mu\text{g}/\text{ml}$  of imiquimod. Saturable specific binding of closely related analogs of imiquimod to membrane fractions from these cells suggests the presence of a membrane receptor for these drugs (Miller et al., 1995).

When administered orally or parenterally to mice, imiquimod induces increased serum concentrations of IFN- $\alpha$ , TNF- $\alpha$  and IL-6 between 1 and 4 h after dosing (Reiter et al., 1994). Effective oral doses range from 1–250 mg/kg. Multiple doses of imiquimod on the same day cause augmented IFN- $\alpha$  levels, however high daily doses of imiquimod to mice results in a hyporesponsive state characterized by reduced cytokine induction. Separation of the doses by four or more days causes normal levels of cytokine induction. Oral imiquimod also causes increased levels of serum 2',5' oligoadenylate synthetase (2',5' AS) (Miller et al., 1994) which is an IFN inducible enzyme believed to be partly responsible for IFN's antiviral properties (DeBenedetti, Pytel & Baglioni, 1987). A single drug treatment causes elevated 2',5' AS levels for 3–4 days which supports 2–3 times per week dosing in efficacy studies (Miller et al., 1994). The use of knock-out mice indicate that STAT-1 is needed for priming and maximal production of IFN in mice treated with imiquimod (Bottrel, Levy, Tomai & Reis, 1997).

Topical application of the 1% or 5% cream formulation of imiquimod to the skin of hairless mice induces increased IFN- $\alpha$  messenger RNA (mRNA) levels and increased protein concentrations of IFN and TNF- $\alpha$  in the skin at the treatment site (Tomai et al., 1997; Imbertson et al., 1998). Cytokine increases are seen from 1–4 h after application and are not seen in skin taken from the untreated side of the mice or the site where placebo cream was applied. Topical treatment of hairless mice with imiquimod causes Langerhans cells in the skin to enlarge, appear activated and migrate from the treatment site to the regional lymph node (Suzuki et al., 1998). These activated Langerhans cells may enhance antigen presentation to T cells.

Intravaginal application of imiquimod cream to mice induces increases in vaginal tissue con-

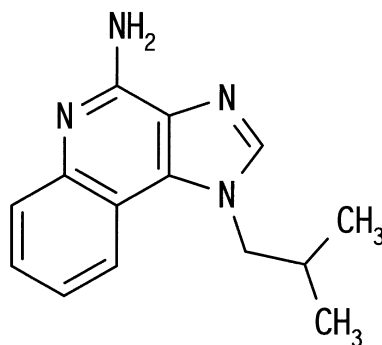


Fig. 1. 5-26308, R 837, Imiquimod.

centrations of IFN, TNF- $\alpha$  and 2',5'-AS. Vaginal washes also contained increased 2',5'-AS concentrations. Serum levels of IFN, TNF- $\alpha$  and 2',5'-AS are increased after intravaginal drug application. The serum cytokine levels and kinetics are similar to an equivalent dose of imiquimod given orally.

In rats, oral administration of 3 mg/kg or more of imiquimod induces increased serum levels of IFN- $\alpha$  and TNF- $\alpha$ . The kinetics of induction are similar to those seen in mice. Hyporesponsiveness is seen in rats after multiple high daily doses. As in mice, topical application of imiquimod cream (1% or 5%) to the skin of hairless rats leads to local induction of TNF- $\alpha$  at the application site (Imbertson et al., 1998). In guinea pigs, 3 mg/kg of imiquimod induces serum levels of IFN- $\alpha$  when the drug is administered orally, intravaginally, topically, or parenterally (Miller, Imbertson, Reiter, Pecore & Gerster, 1986). In monkeys, multiple oral imiquimod doses of 3 mg/kg induce serum levels of IFN- $\alpha$ , interleukin-1 receptor antagonist (IL-1RA) and, in rare instances, low levels of IL-6. Peripheral blood mononuclear cell (PBMC) cultures from monkeys produce increased levels of messenger RNA (mRNA) and cytokine for IFN, IL-1 $\beta$ , IL-6 and IL-8 after treatment with imiquimod in vitro (Wagner et al., 1997). Hyporesponsiveness is not seen in guinea pigs or monkeys when low doses of imiquimod are used. Generally, 2–3 mg/kg is a minimum effective oral dose for IFN- $\alpha$  induction in different species including humans.

In human PBMCs, specifically monocytes, imiquimod at 1–5  $\mu$ g/ml induces the production of several cytokines including several subtypes of IFN- $\alpha$ , TNF- $\alpha$ , IL-1, IL-1RA, IL-6, IL-8, IL-10, IL-12 p40, granulocyte colony stimulating factor (G-CSF), granulocyte/macrophage colony stimulating factor (GM-CSF), and macrophage inflammatory protein 1- $\alpha$  (MIP-1), MIP-1 $\beta$ , and macrophage chemotactic protein (MCP-1) (Weeks & Gibson, 1995; Gibson et al., 1995). Generally, a low drug concentration (about 0.5  $\mu$ g/ml) is found at which IFN- $\alpha$  and IL-1RA are the only cytokines increased. Cytokines are detected as early as 1–4 h after stimulation with drug and this induction requires both mRNA and protein synthesis (Testerman, Gerster, Imbertson et al., 1995; Megyeri et al., 1995). Induction of these cytokines occurs through activation of transcription factors that bind to the promoter regions of IFN- $\alpha$  ( $\alpha$ 4F1 complexes) and a number of the proinflammatory cytokines (Nuclear factor kappa-B (NF- $\kappa$ B)) and activate transcription (Megyeri et al., 1995).

Keratinocytes isolated from human skin and cells from a human epidermal cell line also respond to 1  $\mu$ g/ml imiquimod by producing increases in mRNA for IFN- $\alpha$ , IL-6 and IL-8 but not TNF- $\alpha$  or IL-1 (Kono et al., 1994). Slight increases in IL-8 protein levels are seen; however, imiquimod does not increase protein concentrations of IFN, IL-1 $\alpha$ , IL-6 and TNF- $\alpha$  in these cultures at 6 or 24 h (Miller et al., 1995; Kono et al., 1994).

In addition to IFN and other cytokine induction, imiquimod causes stimulation of several other aspects of the innate immune response. For example, natural killer cell activity is stimulated in mice, probably due to induction of IFN- $\alpha$  and other cytokines induced by imiquimod. Macrophages are activated to secrete cytokines and nitric oxide. Proliferation and differentiation of B-lymphocytes is also caused by imiquimod and appears to be a direct drug effect on these cells.

As a result of these effects on innate immune responses, imiquimod has been shown to be effective in animal models against a number of viral infections and a variety of transplantable tumors. In herpes simplex virus (HSV) infected guinea pigs, a single treatment of 2–3 mg/kg of imiquimod given orally, parenterally, intravaginally, or topically is protective against primary infection when given between 72 h before and 24 h after inoculation (Miller et al., 1985; Harrison,

Jenski, Voychehovski & Bernstein, 1988). In mice, imiquimod causes an increase in survival in Rift Valley Fever virus infection and Banzi virus infection (Kende, Lupton & Canonico, 1988). In Rift Valley Fever virus infected mice, production of IFN- $\alpha$  is critical for the antiviral effect since antibody to murine IFN- $\alpha$  blocks much of the increase in survival induced by imiquimod. The duration of antiviral activity lasts for 3–4 days after each oral imiquimod administration and correlates with elevation of 2',5'-AS activity. Elevated 2',5'-AS has been observed in the serum of mice, rats, guinea pigs, monkeys, and humans (Tomai et al., 1997) from 24–72 h after oral treatment with imiquimod. Induction of 2',5'-AS is indirect through the production of IFN- $\alpha$  since production is abrogated in IFN- $\alpha/\beta$  receptor knock-out mice (Bottrel et al., 1997). Acute antiviral activity is also seen in cytomegalovirus infection models, both in guinea pigs (Chen, Griffith, Lucia & Hsiung, 1988) and in mice. However, topical application of 5% imiquimod cream was ineffective in the human papillomavirus (HPV) type 11 infected external human-severe combined immunodeficiency (SCID) mouse model (Bonnez, DaRin, Borkhuis, Rose & Miller, 1996). **Finally, in a rabbit papillomavirus infection model in rabbits, topically applied imiquimod was ineffective, which is likely due to the drug's inability to induce IFN and possibly other cytokines in this species.**

Antitumor activity of imiquimod is also seen in a number of transplantable mouse tumor models (Sidky et al., 1992). When given acutely, the drug is effective at reducing tumor volumes in mice given cells from a number of lines including MC-26 colon carcinoma, B16-F10 melanoma, Lewis lung carcinoma, FCB bladder carcinoma, RIF-1 sarcoma and MBT-2 bladder cell carcinoma. Imiquimod was also effective at inhibiting growth of the human mammary tumor MCF-7 when transplanted into nude mice which lack T cells. This indicates that acutely, T cells are not required for the antitumor effects of imiquimod. Much of the antitumor effect with imiquimod is again blocked by administration of antibodies to IFN- $\alpha$ ; however, TNF- $\alpha$  also seems to be involved. These results suggest that imiquimod's effects on the innate immune response, in particular its ability to induce IFN- $\alpha$  and other cytokines, are largely responsible for its acute antiviral and antitumor effects.

## 2. Effects on acquired immunity

Although imiquimod does not stimulate T cells to divide or directly induce T cell cytokines such as IL-2, IL-4 or IL-5, imiquimod is capable of indirectly stimulating production of the T helper type 1 (Th1) cytokine, IFN- $\gamma$ , in mouse splenic and bone marrow cultures as well as human PBMC cultures. Production of IFN- $\gamma$  in response to imiquimod is inhibited by antibodies to IL-12 and IFN- $\alpha$ , demonstrating the importance of these monocyte/macrophage cytokines (Tomai et al., 1998). The mechanism of interaction between these cytokines has recently been defined (Rogge et al., 1997; Szabo, Dighe, Gubler & Murphy, 1997). Results show that IFN- $\alpha$  induces the IL-12 receptor  $\beta$ 2 subunit on Th1 cells. These cells can then respond to IL-12 and produce IFN- $\gamma$ . Thus, Th1 cells are the major source of IFN- $\gamma$ ; however, cytotoxic T cells and NK cells are also able to produce IFN- $\gamma$  in response to imiquimod.

The ability of imiquimod to stimulate IFN- $\alpha$ , IL-12 and IFN- $\gamma$ , cytokines known to be involved in driving the cellular arm of the acquired immune response and imiquimod's ability when applied topically to stimulate Langerhans cells are likely important in models where imiquimod has demonstrated long-lasting protection. For example, treatment of guinea pigs after primary HSV-

2 infection with imiquimod reduces recurrences both during the treatment period and even after treatment has stopped (Harrison, Miller & Bernstein, 1994). The prolonged effect after treatment is likely due to increased cellular immunity to HSV antigens and HSV infected cells (Bernstein & Harrison 1989; Harrison, Stanberry & Bernstein, 1991; Bernstein, Miller & Harrison, 1993a; Harrison et al., 1994). In addition, imiquimod can serve as a vaccine adjuvant for a HSV glycoprotein vaccine preparation in guinea pigs when given both prophylactically and therapeutically (Bernstein, Miller & Harrison, 1993b; Bernstein, Harrison, Tepe, Shahwan & Miller, 1995). Imiquimod is more effective than complete Freund's adjuvant in this model. In mice, imiquimod also enhances rejection of tumors caused by cells which express the HPV 16 E7 gene. Imiquimod causes a reduction in the control of EL4 tumors by only 9% and a reduction of the E7 expressing tumors by 51% in sham immunized mice. In the E7 immunized mice, imiquimod has no effect on the control EL4 tumors but reduces the weight of the E7 expressing tumors by 84%, which is associated with stimulation of a delayed type hypersensitivity skin test reaction (Th1) to the E7 protein. Finally, in mice implanted with FCB bladder carcinoma cells, certain regimens of imiquimod actually lead to total eradication of the tumor. These mice are totally resistant for at least eight months to rechallenge with the same FCB tumor cells but remain sensitive to challenge with a different tumor cell (Borden, Sidky & Weeks, 1991). The long-lasting immunity observed is likely via the cell mediated arm.

Imiquimod also has been shown to inhibit production of the Th2 cytokine IL-5 in both mouse and human cell systems. Inhibition of IL-5 production is mediated by IFN- $\alpha$  and IFN- $\gamma$ . As a result of this ability to inhibit IL-5 production, imiquimod has also been found to inhibit both antigen and sephadex induced eosinophilia in several animal models (Hammerbeck et al., 1997). In addition, imiquimod has been found to inhibit virus induced eosinophilia in rats (Stokes et al., 1998). These results suggest the possibility that imiquimod may be useful in atopic diseases as well as other diseases where an increased Th1 response is needed.

Imiquimod treatment of murine B-cells also changes the immunoglobulin (Ig) response to antigens (Tygrett, Li, Tomai & Waldschmidt, 1995). Levels of the Th1 Ig, IgG2a, are increased and levels of the Th2 Igs, IgG1 and IgE, are decreased.

In summary, imiquimod induces cytokines in skin cells and blood cells and stimulates or enhances both the innate response and the cellular immune system. Long lasting immunity is demonstrated in the recurrent HSV guinea pig model and in the FCB mouse tumor model. Increased delayed type hypersensitivity is demonstrated in the HPV mouse model. Table 1 summarizes the lowest effective concentration of imiquimod in the various models. These preclinical pharmacology results indicate the potential of imiquimod for treatment of virus infections or tumors in humans.

### 3. Clinical mechanism of action study

The data generated in animal models suggest that imiquimod's antiviral and antitumor effects are largely mediated through the induction of cytokines that drive the innate and cell-mediated immune response. A study was carried out in humans to further explore the drug's mechanism of action (Tyring et al., 1998). The objective of this study was to evaluate the mechanism of action of imiquimod 5% cream when applied topically to genital warts in human patients by: (1) invest-

Table 1  
Lowest effective concentrations of imiquimod

Species	In vivo/in vitro	Results	Dose (mg/kg) or concentration ( $\mu\text{g/ml}$ ) of imiquimod
Mouse	In vitro (spleen cells)	IFN, IL-6, TNF- $\alpha$	0.2 $\mu\text{g/ml}$
Mouse	In vitro (spleen cells)	IL-5 inhibition	0.5 $\mu\text{g/ml}$
Mouse	In vitro (spleen cells)	IFN $\gamma$ induction	0.1 $\mu\text{g/ml}$
Mouse	In vitro (spleen cells)	B-cell proliferation	0.1 $\mu\text{g/ml}$
Mouse	In vitro (RAW cells)	TNF- $\alpha$ induction	3 $\mu\text{g/ml}$
Mouse	In vivo (oral)	IFN, TNF- $\alpha$ , IL-6 induction in serum	1–3 mg/kg
Mouse	In vivo (oral)	Antiviral (RVF, Banzi, Influenza)	3 mg/kg
Mouse	In vivo (oral)	Antitumor	50 mg/kg
Mouse	In vivo (topical)	IFN, TNF- $\alpha$ induction	(10 $\mu\text{l}$ 1%) 4 mg/kg
Rat	In vivo (oral)	IFN Induction	3 mg/kg
Rat	In vivo (topical)	TNF- $\alpha$ induction	(100 $\mu\text{l}$ 1%) 4 mg/kg
Rat	In vivo (oral)	Hyperreactivity	3 mg/kg
Guinea Pig	In vivo (oral, intravaginal, sub cut)	Antiviral (HSV-1, HSV-2, CMV)	3 mg/kg
Guinea Pig	In vivo (oral, intravaginal, sub cut)	IFN induction	3 mg/kg
Monkey	In vitro (PBMC)	IFN- $\alpha$ induction	0.5 $\mu\text{g/ml}$
Monkey	In vivo (oral)	IFN- $\alpha$ induction	3* mg/kg
Monkey	In vivo (oral)	Antiviral (Yellow Fever Virus)	10 mg/kg
Human	In vitro (PBMC)	IFN- $\alpha$ , IL-1RA induction	0.5 $\mu\text{g/ml}$
Human	In vitro (PBMC)	IL-5 inhibition	0.1 $\mu\text{g/ml}$
Human	keratinocytes	IFN- $\alpha$ , IL-6, IL-8 mRNA induction	1.0 $\mu\text{g/ml}$
Human	In vivo (oral)	IFN, IL-1RA induction in serum	2–3 mg/kg
Human	Intravaginal	IFN, IL-1RA induction in serum	(5 g 3%) 2–3 mg/kg
Human	Topical	Antiviral (warts)	(5% 3/Week) 0.1 mg/kg

\* Multiple dosing required.

igating local and systemic cytokine induction; (2) assessing cellular infiltration into the warts; and (3) evaluating effects of imiquimod on HPV DNA and gene expression. In this Phase I double-blind, randomized, parallel group study, imiquimod 5% cream or placebo was applied to warts three times a week for up to 16 weeks. Serum and biopsies of warts were taken at predose, after six weeks of treatment, and at the end of study. As an inclusion criteria, HPV infection was confirmed in the predose biopsy. Biopsies were analysed by the polymerase chain reaction (PCR) for HPV DNA (copies/cell) and by reverse transcriptase (RT)-PCR for mRNAs to a number of cytokines, cellular markers and viral gene products. Changes from baseline at six weeks and at end of treatment were compared between treatments.

Results showed that all imiquimod treated patients had a  $\geq 75\%$  reduction in wart area. Safety analysis revealed that most (15/16) patients receiving imiquimod cream experienced erythema at the application site that was significantly different from the vehicle treated patients. Imiquimod treatment stimulated significant increases in IFN- $\alpha$  and increases in TNF- $\alpha$  mRNAs, cytokines previously found to be induced by imiquimod in animal studies (Miller et al., 1986; 1994; 1995;

Reiter et al., 1994; Tomai et al., 1997; Wagner et al., 1997) and in human PBMC studies (Weeks & Gibson, 1994; Gibson et al., 1995; Testerman et al., 1995). A number of IFN- $\alpha$  and TNF- $\alpha$  inducible effects were also increased in imiquimod treated patients (Mx-B, 2',5'-AS, IFN- $\beta$ ). IL-12 p40 and IL-8 mRNA were also increased in many patients receiving imiquimod, but these were not statistically significant. Decreases in CD1a mRNA associated with Langerhans cells were seen in imiquimod treated patients, suggesting that these cells were activated or migrated to the draining lymph node. Cytokines associated with a Th1 immune response (IFN- $\gamma$ , IL-2 and IL-12 p40) were increased in many imiquimod treated patients as were CD4 and CD8 mRNAs which indicates activation of a cell mediated immune response. Increased CD4 mRNA correlated with increases in expression of CD29 and CD45Ro mRNA which are expressed on activated cells and memory cells, respectively. Wart regression strongly correlated with a decrease in viral load as measured by a decrease in HPV-DNA and a decrease in expression of both HPV early (E7) and late (L1) mRNAs. Coincident with wart regression and diminished virus was a decrease in mRNA expression for markers associated with hyperproliferation (PCNA, *c-myc*) and an increase in markers associated with differentiation (Fillagrin, involucrin, p53 and Rb). These changes are likely a result of taking wart tissue at baseline and normal appearing skin at the wart site at the end of treatment. In conclusion, wart regression by imiquimod is associated with an induction of local cytokines and cellular infiltrates that are involved with generation of a cell mediated immune response. These results in humans are consistent with the preclinical results generated with imiquimod in animal models.

#### 4. Summary of clinical efficacy trials

Imiquimod cream was applied topically and tested for efficacy in patients with external genital and/or perianal warts (condylomata acuminata). Genital warts, the most common viral sexually transmitted disease, was chosen as the first clinical target because injectable IFN- $\alpha$  had demonstrated some benefit and the current therapies do not meet the patient's or physician's needs. Patient dissatisfaction with current therapeutic options is significant due to pain, tissue destruction, high recurrence rates, expense, and time required for treatment. In addition, current treatments only treat the visible wart symptoms and do not treat the underlying HPV infection. Published results indicate that biopsies of warts from these patients show little immune recognition but biopsies from warts undergoing spontaneous regression show monocytic cellular infiltration and increased Th1 cytokine expression (Tagami, Oku & Iwatsuki, 1985; Coleman et al., 1994). Similar results are seen in patients treated with interferon (Arany & Tyring, 1996). We reasoned that an immune response modifier that stimulates cell mediated immunity should be an improved therapy for genital warts.

A Phase II study in 108 patients with genital warts compared topically applied 5% imiquimod cream to vehicle cream with 23–24 h application, three days/week for eight weeks (Beutner et al., 1998). The imiquimod group had 40% complete wart clearance compared to no complete clearance in the vehicle group. In addition, there was a median 90% reduction in wart area at the end of treatment among the imiquimod group but no change in wart area in the vehicle treated group. Patients who totally cleared their lesions entered a 10 week follow-up period to observe wart

recurrence and 81% of the imiquimod treated group remained wart free. In this trial, acceptable safety and efficacy was demonstrated by 5% imiquimod cream in genital wart patients.

A Phase III multi-centered, randomized, double blind, placebo controlled trial compared the safety and efficacy of imiquimod 5% cream and 1% cream with vehicle (Edwards et al., 1998). Patients applied the cream to their warts overnight for 8 h three times per week until their warts were totally cleared or for a maximum of 16 weeks. The main outcome measurements were the number of patients experiencing the complete elimination of all baseline warts and the recurrence of these warts. In addition, the reduction in baseline wart area, the duration of therapy required to eliminate warts, and the frequency and severity of adverse reactions were monitored. Patients who totally cleared their warts were entered into a 12 week follow up period to monitor recurrence of their warts.

The three times per week trial included 180 men and 131 women 18 years or older having 2–50 external anogenital warts. In the intent to treat analysis, 50% (54/109) of the patients who received 5% imiquimod cream, 21% (21/102) of those who received 1% imiquimod cream, and 11% (11/100) of patients treated with vehicle completely cleared all their baseline warts. In the treatment failures analysis, clearance was observed in 56, 27 and 14%, respectively. The difference between the effectiveness of 5% cream and vehicle was statistically significant ( $P < 0.0001$ ) using either method of analysis. The results using 1% cream were not significantly different from vehicle. The median time to clearance was 10 weeks, 12 weeks, and 12 weeks, respectively. Females had a higher clearance rate (77, 46 and 28%, respectively) than males (40, 10 and 6%, respectively). In addition, females had a shorter median time to clearance (8 weeks) than males (12 weeks) in both imiquimod groups. The better response in females could be due to several factors including shorter duration of warts in females (3.4 months median) vs males (6.7 months median), better compliance in females or better drug absorption in females. Of the patients whose warts completely cleared during therapy, 13% (6/45) of the patients treated with 5% cream had a recurrence of at least one wart. No recurrences (0/18) were seen in the 1% patients who cleared and recurrences were seen in 10% (1/10) of the vehicle patients who totally cleared their baseline warts. Since the initial clearance rate was highest for the 5% group, the sustained wart free period was also greatest for the 5% group.

The treatment was well tolerated. Local erythema was the most common adverse reaction (67, 26 and 24%, respectively) but the majority of patients in each group experienced no or only mild local inflammatory reactions. Less than 1.2% of the patients discontinued due to side effects. There were no differences in the incidence of flu-like symptoms among the treatment groups indicating no systemic effects from cytokine induction by the drug. System effects were not expected since results of a study using radiolabeled imiquimod showed that <1% of the radiolabeled imiquimod applied was absorbed into the systemic circulation (Owens et al., 1997). In addition, a 21 day cumulative irritation study demonstrated that imiquimod cream 5% was less irritating than Vaseline<sup>®</sup> Intensive Care Lotion<sup>®</sup>, the reference cream used in the study (Owens et al., 1997).

A second Phase III trial was carried out in 154 male and 125 female patients with genital warts using daily application of 5%, 1%, or vehicle with 8 h application until wart clearance or 16 weeks maximum (Beutner et al., 1996; Beutner et al., 1998a). In this trial, 71% (49/94) of the 5% patients, 16% (13/90) of the 1% patients and 4% (3/95) of the vehicle patients had complete clearing of their baseline warts,  $P < 0.0001$  when comparing the 5% and vehicle groups. Clearance in the 1% group and the vehicle group were not significantly different. Recurrence rates were 19% (9/48) for



5% imiquimod group, 17% (2/12) for the 1% group, and 0% (0/3) for the vehicle group. The low recurrence rate in the vehicle groups is not surprising since the mechanism of spontaneous clearance has been shown to be due to immune recognition (Tagami et al., 1985; Coleman et al., 1994). Local skin reactions were more common and more severe with daily treatment but there were no systemic adverse reactions. The daily treatment regimen resulted in somewhat increased rate of total wart clearance when compared to three times per week application but also resulted in greater local skin reactions.

A vehicle controlled safety and efficacy trial was also carried out in HIV-positive genital wart patients (Conant et al., 1998). The primary objective of this multi-national, multi-center, double blind, vehicle controlled, parallel group trial was to evaluate the safety of imiquimod 5% cream in HIV-positive patients. A secondary objective was to assess wart clearance and reduction in wart area. A total of 100 patients (97 males and 3 females) were enrolled and treated three times per week for up to 16 weeks or until wart clearance. Imiquimod was applied to 65 patients and vehicle was applied to 35 patients. No local skin reactions were seen in a majority of patients and only mild erythema was seen in most of the others. The intent to treat analysis of all patients showed that 11% of the imiquimod patients achieved complete wart clearance compared to 6% of the vehicle group, which was not significantly different. However, there was a statistically significant difference between treatment groups for patients who achieved >50% reduction in wart area; 38% for imiquimod and 14% for vehicle ( $P = 0.013$ ). This was a clinically meaningful reduction in wart area since wart area increases are frequently seen in these patients. These results suggest that in HIV patients, imiquimod induces the innate response which stops wart growth and causes wart area reduction and may, in part, be IFN- $\alpha$  mediated. However, the reduced total wart clearance in HIV patients compared to immunocompetent genital wart patients suggests a role for T-cell responses in initial wart clearance as well as in long term protection from recurrence. Imiquimod has an acceptable safety profile in HIV-positive and AIDS patients.

## 5. Oral delivery

Some clinical testing of imiquimod was also carried out by the oral route. A single dose study in normal volunteers indicated that measurable serum IFN levels were obtained in four of six subjects after 200 mg and in six of six subjects after 300 mg. Peak levels of IFN were seen at 12 h after dosing and levels returned to baseline by 24 h. Activity of 2,5-AS was elevated for 96 h after the 300 mg dose and this activity correlated with antiviral activity in the subject PBMC's. The drug was well tolerated (Imbertson et al., 1992). Phase I multiple dose studies using imiquimod were completed in cancer patients using different dosing schedules (Witt et al. 1993; Savage, Horton, Moore, Owens, Witt & Gore, 1996). An oral Phase I dose escalating study was also carried out in asymptomatic HIV positive individuals (Goldstein et al., 1998). Both cancer patients and HIV positive individuals responded to imiquimod with serum IFN production. Despite activity when given orally, topical administration of imiquimod is the preferred route of delivery.

## 6. New class of drug

The preclinical and mechanism of action study in patients indicate that topically applied imiquimod results in the induction of several cytokines at the treatment site. Cytokines such as IFN and

others inhibit virus production and inhibit tumor cell growth. The drug also enhances aspects of the cell mediated immune (CMI) response and may result in long term protection from the initial virus or tumor. Application of the drug to warts by wart patients, in the privacy of their own home produced a high genital wart clearance rate with limited side effects. Results were better in women, perhaps due to a shorter duration of the warts, to better compliance with treatment or enhanced drug absorption through poorly keratinized and occluded epithelium. Both three times per week and daily treatment regimens were acceptable for safety and efficacy, however in the final analysis, the three times per week regimen was preferred for most patients. Imiquimod 5% cream (ALDARA™, 3M Pharmaceuticals) received approval by the FDA in February 1997 and is currently available in the U.S.A. for the treatment of external genital and perianal warts. Approvals are expected in additional countries in the future.

The cytokines induced by topically applied imiquimod include IFN- $\alpha$ , TNF- $\alpha$ , and IL-12p40 and indirectly, IFN- $\beta$  and IFN- $\gamma$ . Induction of these cytokines stimulates the Th1 CMI response and the preclinical data suggest the suppression of Th2 immune responses. This mechanism of action should cause imiquimod to be an effective treatment for chronic virus infections of the skin such as Human Papillomavirus in genital warts, and theoretically in common warts, plantar warts, Herpes simplex virus infection and Molluscum contagiosum.

Skin lesions caused by other intracellular pathogens that might also respond include intracellular bacteria such as leprosy and intracellular parasites such as leishmania. Preliminary in vitro studies using imiquimod in mouse bone marrow derived macrophages showed inhibition of *Leishmania donovani* proliferation and the topical application of imiquimod cream to mice infected with *L. major* caused a reduction in the severity of lesions (Buates & Matlashewski, 1997a,b). In addition, more potent analogs of imiquimod are able to inhibit in vitro growth of Mycobacterium avium in human monocytes (Shiratsuchi, Sherman, Miller & Ellner, 1995). Further studies are needed to confirm these activities.

Other possible uses include ultraviolet induced skin lesions such as actinic keratosis and skin tumors such as basal cell carcinoma, squamous cell carcinoma, and perhaps even melanoma. Results of a small pilot trial of imiquimod 5% cream in patients with the skin cancer, Bowen's disease, showed that 14 of 16 patients cleared their lesions (MacKenzie-Wood et al., 1998) Other skin tumors that might respond include Kaposi's sarcoma and Cutaneous T-cell lymphoma.

Since Th2 responses can be inhibited in preclinical animal models by imiquimod, atopic based skin inflammation such as atopic dermatitis might also benefit. Other conditions that may respond to topically applied imiquimod include Alopecia areata, keloids and cutaneous symptoms of the Th2 mediated autoimmune disease, Systemic Lupus Erythematosus (SLE). Another possible use for these drugs is application with a vaccine for adjuvant activity. The imidazoquinolines are expected to enhance a Th1 response to the vaccine which could be beneficial for virus or tumor vaccines. Drug application topically or transdermally could be explored with the injectable vaccine. On the other hand, skin inflammation due to excessive Th1 responses, such as psoriasis and contact dermatitis, might be worsened by topical treatment with imiquimod. Among drugs, imiquimod is unique in being a topically active cytokine inducer and stimulant for the CMI response. Overall, imiquimod applied topically is an Immune Response Modifier which should be a useful addition to the drugs that can be used to treat significant and chronic conditions of the skin. As such, imiquimod applied topically represents a new class of drug.

The Th1 CMI response is very effective in most people in controlling virus infections and tumors.

For example, Chicken pox infection is almost universal and after the outbreak, the Varicella zoster virus responsible is carried in the dorsal root ganglia for the rest of the individual's life with no further lesions. However, lesions can occur following suppression of cellular immunity. Epidemiology studies report the Human Papillomavirus is also a frequently occurring infection with 50–75% of sexually active adults having an antibody response to the virus (Koutsky, 1997). About 15% of these individuals carry the virus and if the cellular immune response is suppressed due to anti-graft rejection drugs following transplantation, anti-cancer chemotherapy, acquiring HIV infection or in some cases of pregnancy, a severe outbreak of warts can occur. These are just two examples that demonstrate the role of the Th1 CMI response in suppressing virus lesions.

One can consider why some patients develop chronic virus lesions like warts but many times more people are infected and develop an appropriate immune response and have minor or even no symptoms (Koutsky, 1997). Infected patients who have symptoms may have mounted a Th2 response to their infection rather than the Th1, CMI response needed to eliminate the infected cells. Their Th2 response to the virus may result from a dominant Th2 response to a bacterial infection that was ongoing at the time they first encountered the virus. Elevated IL-4 levels at that time would suppress the needed Th1 response (Szabo et al., 1997). On the other hand, an ongoing Th1 response to a viral infection with IFN- $\alpha$  and IFN- $\gamma$  induction could prevent a Th2 response and cause an inappropriate and ineffective immune response to a subsequent bacterial infection. This might explain the propensity patients with viral pneumonia or influenza have toward development of bacterial pneumonia which can be severe and even cause death (Couch et al., 1986). Animals also have severe bacterial infections after viral infections. For example, shipping fever in cattle results from a *Pasteurella* bacterial infection developing after a respiratory herpes virus infection (Frank, 1983). If this explanation is true, early treatment with an imidazoquinoline in an acute viral infection may be beneficial in boosting the Th1 antiviral response but prolonged Th1 stimulation may lead to problems with bacterial infections. In addition, use of these drugs after a severe bacterial infection might be beneficial in shifting away from the dominant Th2 response so as to prevent the subsequent establishment of chronic virus infections. Thus, successful manipulation of the immune response by use of the imidazoquinolines or other similar drugs could benefit patients with many different infections or conditions. These drugs may provide an entirely new means of helping patients when compared to existing treatment methods.

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