Psoriasis Triggered by Toll-like Receptor 7 Agonist Imiquimod in the Presence of Dermal Plasmacytoid Dendritic Cell Precursors

Michel Gilliet, MD; Curdin Conrad, MD; Michael Geiges, MD; Antonio Cozzio, MD, PhD; Wolfgang Thärlimann, MD; Günter Burg, MD; Frank O. Nestle, MD; Reinhard Dummer, MD

Background: It has been proposed that the innate immune system plays a central role in driving the autoimmune T-cell cascade leading to psoriasis; however, there is no direct evidence for this.

Observations: We observed aggravation and spreading of a psoriatic plaque when treated topically with the toll-like receptor (TLR) 7 agonist imiquimod. The exacerbation of psoriasis was accompanied by a massive induction of lesional type I interferon activity, detected by MxA expression after imiquimod therapy. Since imiquimod induces large amounts of type I interferon production from TLR7-expressing plasmacytoid dendritic cell precursors (PDCs), the natural interferon-producing cells of the peripheral blood, we asked whether PDCs are present in psoriatic skin. We identified high numbers of PDCs in psoriatic skin lesions (up to 16% of the total dermal infiltrate) based on their coexpression of BDCA2 and CD123. By contrast, PDCs were present at very low levels in atopic dermatitis and not detected in normal human skin.

Conclusions: This study shows that psoriasis can be driven by the innate immune system through TLR ligation. Furthermore, our finding that large numbers of PDCs infiltrate psoriatic skin suggests a role of lesional PDCs as type I interferon-producing targets for the TLR7 agonist imiquimod.

Arch Dermatol. 2004;140:1490-1495

Toll-like receptors (TLRs) are phylogenetically well-conserved type I transmembrane proteins representing major pattern recognition receptors of the innate immune system for various pathogen-associated molecular patterns (PAMPs). Toll-like receptor signaling leads to activation of nuclear factor κB through the adaptor protein MyD88-dependent pathway and induces a battery of immune adjuvant effects, which are principally mediated by proinflammatory cytokines. Over the last 5 years, 11 TLR family members, along with their agonists, have been identified. Bacterial lipoproteins, lipoteichoic acid, and peptidoglycans have been shown to be agonists for TLR2/TLR1 and TLR2/TLR6. Toll-like receptor 3 binds double-stranded RNA synthesized by viruses, TLR4 recognizes bacterial lipopolysaccharide, TLR5 accepts bacterial flagellin, and TLR9 is activated by unmethylated cytosine guanine oligodeoxynucleotide motifs common to both bacterial and viral DNA (reviewed in Akira et al). A recent addition to this list has been TLR11, which mediates the response to bacteria causing infections of the bladder and kidney.3 Toll-like receptor 7 and TLR8 are required for recognition of the viral single-stranded RNA.4,5 In addition, TLR7 and TLR8 are triggered by a family of low-molecular-weight synthetic compounds called imidazolinoquinolines.6 One of these is called imiquimod, a selective TLR7 agonist, which is currently licensed for the topical treatment of human anogenital warts in a 5% cream formulation (Aldara; 3M Pharmaceuticals, St Paul, Minn).7 Promising clinical results have also been obtained in open-label studies for the treatment of common warts, mollusca contagiosa,8 and skin tumors including Bowenoid keratosis,9 actinic keratosis,10 lentigo maligna,11 and basal cell carcinoma.12 Although imiquimod can directly induce apoptosis of epithelial tumor cells in vitro13 and in vivo,14 the innate antiviral and antitumoral effects are the consequence of the induction of type I interferon (IFN-α/β).15,16 Type I IFNs also shape the adaptive arm of the immune response by driving γδ T cell responses, either through direct action on T cells or through the promotion of dendritic cell maturation.17 In human peripheral blood, the principal prostate...
ducer of type I IFNs in response to imiquimod is represented by plasmacytoid dendritic cell precursors (PDCs), a novel subset of lymphoid-related cells selectively expressing high levels of TLR7 and TLR9. Plasmacytoid dendritic cell precursors are key players in innate antiviral immunity owing to their unique ability to produce large amounts of type I IFN. In addition, on viral stimulation, PDCs differentiate into dendritic cells with the ability to stimulate T-cell–mediated adaptive immunity. The unique link between innate and adaptive immunity has recently generated great interest in the use of imiquimod and other synthetic TLR agonists as adjuvants in cancer immunotherapy strategies. However, the observation that, in murine models, TLR activation may also trigger T-cell–mediated autoimmune disease recommends caution. We report herein a case of psoriasis exacerbated by topical imiquimod therapy, identify large numbers of PDCs in the dermal infiltrate of psoriasis, and suggest that, as IFN-producing targets of TLR-ligands such as imiquimod, PDCs may represent a crucial link between the innate and adaptive immune responses ultimately leading to psoriasis.

**REPORT OF A CASE**

A 58-year-old white man presented with a 3-month history of an erythematous lesion on his back. Physical examination revealed a solitary, well-demarcated erythematous plaque measuring 3.5 × 3.0 cm with an irregular border and surface scaling. The finding of parakeratosis with some degree of disorganization of the epidermal architecture on histological examination led initially to the diagnosis of bowenoid keratosis. Because surgical excision was not suitable as a first-line treatment owing to the size of the lesion, daily application of 5% imiquimod cream (Aldara) was initiated. After 1 week of treatment, the lesion became erosive. Thereafter, the frequency of therapy was reduced to 2 to 3 times weekly. Following this, the lesion became scaly and increased in size. At week 5 of treatment, the erythematous plaque measuring 6.0 × 5.5 cm (Figure 1B), after 10 weeks the enlarged lesion measuring 7.0 × 7.0 cm was surrounded by small erythematous satellite lesions (Figure 1C) and distant, dropletlike lesions appeared disseminated throughout the trunk and the lower extremities reminiscent of a psoriasis guttata. Histological examination at week 10 showed a fully fledged psoriatic phenotype with parakeratosis, acanthosis, focal loss of the granular layer, and papillomatosis. Furthermore, the diagnosis of psoriasis was confirmed by prompt regression of the lesion by treatment with topical calcipotriol cream (Daivonex; Leo Pharma Nordic, Malmö, Sweden) twice a day in combination with 311-nm UV-B therapy 3 times a week. Histological reevaluation of the initial lesion by 2 independent investigators did
not confirm the diagnosis of Bowenoid keratosis, but was rather compatible with the diagnosis of psoriasis (Figure 2A). Hence, imiquimod treatment of a primary psoriasis lesion induced (1) a fully fledged histological psoriasis phenotype with a significant increase in acanthosis and papillomatosis (Figure 2C), (2) a greater than 4-fold enlargement of the surface of the psoriatic lesion, and (3) the spreading of psoriatic lesions to the immediate surroundings as well as to distant sites, with the clinical aspect of a psoriasis guttata.

SKIN BIOPSY SAMPLES

Frozen skin specimens were available from 8 patients with chronic plaque-type psoriasis and 5 patients with atopic dermatitis and were primarily obtained from routine diagnostic procedures after obtaining informed consent from the patient. The healthy skin specimens and lymph node samples were obtained, respectively, from the Departments of Plastic Surgery and Pathology at the University Hospital of Zurich, Zurich, Switzerland. In all patients, the diagnosis of psoriasis has been confirmed by histologic examination. Patients with atopic dermatitis selected for this study obtained informed consent from the patient. The healthy skin specimens and lymph node samples were primarily obtained from routine diagnostic procedures after obtaining informed consent from the patient.

IMMUNOHISTOLOGICAL ANALYSIS

Cryostat sections, prepared from frozen tissue specimen, were fixed in acetone and subsequently stained using a standard alkaline phosphatase anti–alkaline phosphatase technique. Briefly, after blocking of nonspecific binding sites with normal rabbit serum, tissue sections were incubated with an excess of primary antibodies, followed by 3 cycles of sequential incubations with rabbit anti–mouse IgG xenon antibodies conjugated to alkaline-phosphatase and anti–alkaline phosphatase complexes. The immunoreaction was then visualized with a developing solution, containing new fuchsin (DAKO Corp, Glostrup, Denmark) and counterstained with 1% hematoxylin.

Paraffin-embedded sections were deparaffinized and stained by standard alkaline phosphatase anti–alkaline phosphatase technique after antigen retrieval by microwave pretreatment. The following human-specific primary antibodies were used: anti-BDCA2 mAb (Miltenyi Biotec, Bergisch Gladbach, Germany), mouse anti-CD123 mAb (BD Pharmingen, San Jose, Calif), and anti-CD11c (Miltenyi Biotec), anti-MxA mAb (a gift of J. Pavlovic, PhD, Department of Virology, Zurich University Hospital).

IMMUNOFLUORESCENCE

Frozen tissue sections were fixed with acetone and incubated with unlabeled mouse anti-BDCA2 mAb (Miltenyi Biotec) followed by fluorescein isothiocyanate–labeled anti–mouse IgG mAb (DAKO Corp), anti-CD123 PE (BD Pharmingen), and anti-CD3 antigen-presenting cells (BD Pharmingen) for 1 hour each. Immunofluorescence was analyzed by confocal laser scanning microscopy (Leica DM IRBE, Heidelberg, Germany).

RESULTS

Imiquimod is known to exert its immunomodulatory properties principally through the induction of type I IFN. To detect specific changes induced by imiquimod, we investigated lesional type I IFN activity by immunohistochemical examination for MxA, an adenosine triphosphatase selectively induced in response to IFN-α/β and thus representing a surrogate marker for lesional type I IFN activity. While MxA expression was absent before imiquimod treatment (Figure 3A), a massive dermal and epidermal expression of MxA was detected throughout the treated lesion at week 10 (Figure 3B). Given that peripheral blood PDCs express high levels of TLR7 and represent the principal source of type I IFN in response to imiquimod, we sought to investigate whether PDCs are present in psoriatic skin lesions by immunohistochemical examination for BDCA2, a specific marker for blood PDCs. In human lymph node sections, the specificity of BDCA2 for tissue PDC was shown by the typical location of BDCA2-stained cells around high endothelial venules in the T-cell areas and not B-cell areas (Figure 4A). Staining of chronic plaque psoriasis samples revealed high numbers of BDCA2-positive cells among the dermal cellular infiltrate (range, 2.3%-16.9% of the cellular infiltrate; mean 8.0%; n=8) (Figure 4B and Figure 5A). An unequivocal PDC phenotype was demonstrated by the co-staining of BDCA2-positive cells with CD123 ( interleukin 3 receptor α-chain), a marker that is highly expressed on blood PDCs, but not with the T-cell marker CD3 in 3-color confocal microscopy (Figure 4C). Among the samples tested, we did not detect any significant epidermotropism of PDCs. By contrast, PDCs were undetectable in normal human skin (n=5 and were present at significantly lower levels in atopic dermatitis (range, 0.6%-1.8%; mean 0.9%; n=5) (Figure 5A), an inflammatory skin disease with a comparable amount of infiltrate to psoriasis (Figure 5B). Interestingly, psoriatic skin, normal skin, and atopic dermatitis all had comparable percentages of CD11c+ dermal myeloid dendritic cells (Figure 5C), suggesting that the presence of PDCs is characteristic of psoriatic skin and may result from a specific recruitment. Since anti-
BDCA2 antibody is not suitable for staining of paraffin-embedded tissue sections, staining of imiquimod-triggered psoriasis was performed by using anti-CD123 mAb. CD123 was expressed in both PDCs and endothelial cells in control lymph node sections (Figure 6A) and psoriatic skin samples. However, we were able to identify large numbers PDCs in imiquimod-treated psoriatic skin based on their strong CD123 expression and plasmacytoid morphology, which allowed clear distinction from the elongated CD123dim endothelial cells (Figure 6B).

Psoriasis is a chronic-relapsing T-cell–mediated autoimmune disease, in which type 1 cytokine secretion by T cells induces keratinocyte hyperproliferation in geneti-
cally predisposed individuals. In recent years, the observation that psoriatic plaques are resistant to microbial infection despite a compromised skin barrier has led to growing interest in functions of the innate immune system in psoriatic skin. A role for innate immune responses as a trigger of the pathogenic T-cell cascade leading to psoriasis has been suggested; however, there is no direct evidence. Murine models have demonstrated that TLR activation may provide signals to elicit T-cell-mediated autoimmunity. We report herein a case of exacerbation of a chronic psoriasis plaque by repetitive topical administration of the TLR7 agonist imiquimod. The widespread dissemination of the psoriatic lesion allowed us to exclude a Koebner phenomenon, which is typically confined to the location of mechanical stress. A TLR7-mediated effect was suggested by the massive induction of lesional type I IFN after imiquimod therapy. Given that IFN-α may trigger an underlying psoriasis, it would be of great interest to investigate whether the strong lesional type I IFN activity induced by imiquimod is crucial in the triggering of psoriasis. To date the cellular targets of imiquimod in human skin remain unknown. Keratinocytes, as nonprofessional antigen-presenting cells, possess a remarkable production repertoire of proinflammatory cytokines and constitutively express TLR1, 2, 3, 5 and 9, but not TLR7. Among professional antigen-presenting cells, TLR7 is principally expressed by PDCs, which in addition to their unique ability to produce large amounts of type I IFN in response to viral infection, also represent the principal producer of type I IFN in response to imiquimod. We therefore hypothesized that PDCs might represent the type I IFN–producing targets of imiquimod in psoriatic lesions. Plasmacytoid dendritic cell precursors classically described in blood, secondary lymphoid organs, bone marrow, and thymus have recently been described in inflammatory skin lesions. We were indeed able to identify large numbers of PDCs in the dural infiltrate of psoriasis, suggesting that they may represent primary targets for imiquimod. Whether TLR7 expression is exclusive to PDCs remains to be determined. Although TLR7 expression is undetectable or at very low levels on myeloid-related conventional dendritic cells (MDCs), some authors have shown that this dendritic cell subset is able to produce interleukin 12 in response to imiquimod. The recent finding that TLR7 expression on MDCs can be increased by IFN-α has suggested that, in vivo, there might be a coordinated response to imiquimod between PDCs and MDCs. Plasmacytoid dendritic cell precursors may represent the primary target of imiquimod and induce thereafter, through their secretion of IFN-α, a broader immune response by up-regulating TLR7. It is not known whether other cell types in the human skin represent primary targets of imiquimod through the expression of TLR7. In situ analysis of TLR7 expression in human skin will require the availability of a TLR7-specific antibody suitable for immunohistochemical examination.

Our present report provides direct evidence for the ability of the innate immune system to drive psoriasis through the TLR7 agonist imiquimod. To our knowledge, this represents the first description of an autoimmune-related disorder triggered by defined TLR activation in humans. The induction of a strong lesional type I IFN activity and our finding of large numbers of PDCs infiltrating psoriatic skin lesions suggest that PDCs might represent targets for the TLR7 agonist imiquimod in psoriasis. Whether PDCs represent key cellular mediators of innate immune responses driving the pathogenic events leading to psoriasis needs to be determined in future studies. The experimental proof of this model may provide the basis for new therapeutic approaches targeting upstream events in the pathogenesis of psoriasis.

Accepted for Publication: July 23, 2004.

Correspondence: Michel Gilliet, MD, Department of Dermatology, Zurich University Hospital, Gloriastrasse 31, 8091 Zurich, Switzerland (m.gilliet@der.usz.ch).

Funding/Support: This work was supported by a grant from the Swiss National Science Foundation, Berne, Switzerland (Dr Gilliet), the Bonizzi-Theler-Stiftung, Zurich, Switzerland (Dr Conrad), and a research fund by 3M Pharmaceuticals, St Paul, Minn (Dr Dummer).

Acknowledgment: We thank Keith Hoek for proofreading the manuscript. We appreciate the excellent technical assistance by Christa Duddli. We also thank Thomas Baechi (Laboratory of Electron Microscopy, Zurich) for helping in performing confocal microscopy.
REFERENCES


