Increased Expression of Interleukin 23 p19 and p40 in Lesional Skin of Patients with Psoriasis Vulgaris

Edmund Lee,1 William L. Trepicchio,2 Judith L. Oestreicher,3 Debra Pittman,4 Frank Wang,1 Francesca Chamian,1 Madhav Dhodapkar,2 and James G. Krueger1

1Laboratory for Investigative Dermatology, and 2Laboratory for Tumor Immunology and Immunotherapy, The Rockefeller University, New York, NY 10021
3Millennium Pharmaceuticals, Clinical Research-Pharmacogenomics, Cambridge, MA 02139
4Wyeth, Andover, MA 01810

Abstract
Psoriasis is a type I–deviated disease characterized by the presence of interferon (IFN)-γ and multiple IFN-related inflammatory genes in lesions. Because interleukin (IL)-23 is now recognized to play a role in the recruitment of inflammatory cells in a T helper cell (Th)1-mediated disease, we examined psoriasis skin lesions for production of this newly described cytokine. IL-23 is composed of two subunits: a unique p19 subunit and a p40 subunit shared with IL-12. We found a reliable increase in p19 mRNA by quantitative reverse transcription polymerase chain reaction in lesional skin compared with nonlesional skin (22.3-fold increase; P = 0.001). The p40 subunit, shared by IL-12 and IL-23, increased by 11.6-fold compared with nonlesional skin (P = 0.003), but the IL-12 p35 subunit was not increased in lesional skin. IL-23 was expressed mainly by dermal cells and increased p40 immunoreactivity was visualized in large dermal cells in the lesions. Cell isolation experiments from psoriatic tissue showed strong expression of p19 mRNA in cells expressing monocyte (CD14+CD11c+CD83+) and mature dendritic cell (DC) markers (CD14+CD11c+CD83+), whereas in culture, the mRNAs for p40 and p19 were strongly up-regulated in stimulated monocytes and monocyte-derived DCs, persisting in the latter for much longer periods than IL-12. Our data suggest that IL-23 is playing a more dominant role than IL-12 in psoriasis, a Th1 type of human inflammatory disease.

Key words: cytokines • gene expression • dendritic cell • IL-23 • Th1 cells

Introduction
IL-23 was found during a genome scan for the IL-6/IL-12 cytokine family (1). IL-23 is a heterodimer, sharing a p40 subunit with IL-12 (1) but having a distinct p19 subunit. IL-23 binds to IL-12Rβ1 but not IL-12Rβ2. The receptor for this cytokine is heterodimeric and uses a novel second subunit, IL-23R, which is a member of the hematopoietin receptor family (2). IL-23 plays a role in type 1–polarized T cell immune responses. Although IL-12 strongly activates naive T cells, the initial description of IL-23 reported preferential actions on memory T cells to increase IFN-γ production and proliferation (1). Mice with p40-null mutations display reduced IFN-γ production and less resistance to a range of infectious pathogens compared with p35-null mice (3), suggesting an important role for IL-23 in controlling bacterial infections. More recent data indicate that IL-23 is a key cytokine controlling inflammation in peripheral tissues. In transgenic mice, overexpression of p19 produces inflammation in multiple organs and epithelial tissues, including the skin (4). In a recent study, the development of experimental autoimmune encephalitis occurred in mice lacking IL-12, but not in mice with targeted disruption of only IL-23 (5), and IL-23 was shown to be involved in the recruitment of monocytes into the inflamed nervous system.

Psoriasis vulgaris is a T cell–driven disease, with type I (IFN-γ–producing) T cells predominating in lesional skin and excess numbers of type 1 T cells in the peripheral circulation (6). Psoriasis affects ~2.6% of the US population (7) and therefore might be the most common T cell–mediated inflammatory disease in humans. There is a complex cytokine network in psoriatic lesions that consists of elevated levels...
of IFN-γ, TNF-α, several interleukins (IL-1, IL-2, IL-6, IL-8, IL-12 [p40 subunit], IL-17, and IL-19), and multiple chemokines including MIG/CXCL9, IP-10/CXCL10, I-TAC/CXCL11, and MIP3α/CCL20 (8). A central role for IFN-γ as an inflammatory regulator is suggested from array-based gene expression studies that identify increased expression of STAT1 and more than 20 genes controlled by this transcription factor (9–11). IL-12, a 70-kD heterodimer formed from p40 and p35 subunits, also has the ability to stimulate IFN-γ production from T cells. Previously, IL-12 was thought to contribute to the type 1 T cell bias in psoriatic lesions because the p40 subunit is strongly up-regulated (12, 13) and because IL-12 receptor subunits (IL-12Rβ1 and IL-12Rβ2) are also up-regulated (13). However, we were puzzled by the failure to detect up-regulation of mRNA for the IL-12 p35 subunit in psoriatic lesions (13). Because the p40 subunit of IL-12 is shared with IL-23 (as is the β1 receptor subunit), we might have incorrectly assigned p40 overexpression to IL-12 rather than IL-23.

Accordingly, we studied expression of p19, the defining subunit for IL-23, in skin lesions of psoriatic vulgaris. We report consistent large increases in mRNAs encoding IL-23 subunits p19 and p40, but not p35 in psoriatic lesions. We visualized p40 protein in dendritic profiles within the dermis and showed that p19 was enriched in monocytes and DCs stimulated in the dermis and in culture. Therefore, IL-23 could be a key inflammatory cytokine in type 1–deviated human autoimmune and inflammatory diseases.

**Materials and Methods**

**Subjects.** After obtaining informed consent, 22 adults with chronic plaque-type psoriasis had both lesional and nonlesional skin biopsies. The patients had active, untreated skin lesions. Biopsies were stored in liquid nitrogen until analysis.

**RNA Isolation.** The biopsies were processed using the QIAGEN RNasy kit. Using a rotor-stator tissue homogenizer (saw-tooth rotor; PowerGen 700), frozen specimens were homogenized in buffer (RLT; QIAGEN) for 30–60 s and spun in a microcentrifuge for 3 min at room temperature to pellet debris. The supernatant was loaded onto a QIAGEN minicolumn and centrifuged from the column using 30 μl RNase-free water (Sigma-Aldrich). RNA was quantitated by UV spectrophotometry.

**TaqMan RT-PCR Quantitation of mRNA.** The primers and probes for the TaqMan RT–PCR assays for IL-12 p40, p19, and p35 were generated using the Primer Express algorithm version 1.0 from published sequences (National Center for Biotechnology Information). All primers and probes were synthesized by Applied Biosystems–PerkinElmer. RT–PCR reactions were performed according to the manufacturer’s directions (EZ PCR Core Reagents; TaqMan and Applied Biosystems). We used an Applied Biosystems PRISM 7700 thermal cycler for 2 min at 50°C, 30 min at 60°C, 5 min at 95°C, and 40 cycles of 15 s at 95°C followed by 60 s at 60°C. The human acidic ribosomal protein gene (hARP), a housekeeping gene, was used to normalize each sample and each gene. The data were analyzed with Sequence Detection Systems version 1.7 software. Human IL-12 p40 (sequence data available from GenBank/EMBL/DDBJ under accession no. AY008847) forward primer: ACGGACAAGACCTAGCCAC; reverse primer: GCCGCGCCAGCCTA; fluorescent probe: TACCTGCCCAGCAA; Human IL-12 p35 (sequence data available from GenBank/EMBL/DDBJ under accession no. AF044773) forward primer: CCACTCCAGACGGAGAAGT; reverse primer: GACGGCCTCAGAGG; fluorescent probe: TCCCCACTTCGACCCTCAAA. Human IL-12 p19 (sequence data available from GenBank/EMBL/DDBJ under accession no. NM_015684) forward primer: GAGCCCTTCTGCTCTGAT; reverse primer: AGTTGAGCTAGGAGCCAGTAG; fluorescent probe: CCTGTCGGCCGACCTCTGCC. Human hARP (sequence data available from GenBank/EMBL/DDBJ under accession no. NM_000352) forward primer: ACATCTACGGAATGCTCAATGCA; reverse primer: GGAATTGTAATAGCGATCC. Human IL-12 p19 (sequence data available from GenBank/EMBL/DDBJ under accession no. NM_015684) forward primer: CGCTGCTGAACATGCTCA; reverse primer: TGCACAACTCGCTGATG; fluorescent probe: TCCCCCTCTCCTTGGGGCTGG. All probes were conjugated with 6-FAM (dye) at the 5′-end and TAMRA (quench) at the 3′-end (Applied Biosystems).

**Statistical Analysis.** Paired Student’s t test was used to compare lesional and nonlesional gene expression levels. Bonferroni correction was performed on multivariate calculations of p-values for p19, p35, and p40 gene expression.

**DC Generation and Maturation.** Mononuclear cells were isolated by density gradient centrifugation using Ficoll (Amersham Biosciences). Monocytes (purity >95%) were obtained and cultured to mature DCs as previously described (14).

**Immunohistochemistry.** Monoclonal mouse anti–human anti–IL-12 p40 (dye) at the 5′-end of the appropriate antibody-coated magnetic bead. Cells attached to the beads were separated from nonattached cells by magnet and placed in RLT buffer with β-mercap-
to ethanol for p19 and p40 mRNA analysis, whereas unbound flow-through cells were verified by flow cytometry for depletion of the targeted cell type.

Online Supplemental Material. The supplemental material describes gene transduction studies to verify the primer-probe set used for p19 amplification and detection by RT-PCR (TaqMan). Table S1 and Supplemental Results are available at http://www.jem.org/cgi/content/full/jem.20030451/DC1.

Results

Gene Expression Studies. The expression of p40, (IL-23) p19, and (IL-12) p35 was determined in lesional and nonlesional skin of 22 psoriasis patients (Fig. 1). The values obtained for each gene were normalized to the housekeeping gene hARP. To confirm that our primer/probe set correctly identified expression of p19 mRNA, we used recombinant adenovirus-transduced cell lines (see Table S1, which is available at http://www.jem.org/cgi/content/full/jem.20030451/DC1). Expression of p19 mRNA was increased in all 22 lesional skin biopsies (22-fold mean increase; P < 9 × 10⁻⁷) as was p40 mRNA (12-fold mean increase; P < 4.8 × 10⁻⁷). The expression of p35 mRNA was more variable, but the mean values for paired lesional and nonlesional skin samples did not differ significantly (P = 0.153).

Immunohistochemical Studies of IL-12p40 Expression. Because an antibody to the p19 subunit is presently not available, we used a p40-reactive antibody to localize potential cells that might synthesize the IL-23 heterodimer in psoriasis lesions. Intensely positive staining cells were noted in the papillary dermis of lesional skin and were dendritic in appearance (Fig. 2 D). Little background staining was seen with an isotype control antibody in uninvolved or lesional skin (Fig. 2, A and B). The anti-p40 monoclonal antibody produced a variable degree of staining in basal keratinocytes of uninvolved skin, but very few p40-reactive cells were seen in the dermis of uninvolved skin (Fig. 2 C). Variable degrees of epidermal staining with the p40 antibody were noted, but staining of dermal cells was consistent across many different cases of psoriasis vulgaris. Overall, the staining pattern for p40 most resembled the staining of DCs in lesions, as detected previously with antibodies to CD83 or CD11c (15).

Localization of p19/p40 mRNA Expression Mainly to Dermal Cells. Comparison of gene expression in dispase-separated epidermis and dermis from lesions showed that most of the mRNA for p19 and p40 was contained in dermal cells (Fig. 3 A). We then used immunomagnetic beads to enrich specific populations of leukocytes isolated from the dermis of psoriasis lesions (Fig. 3 B). A low level of p19 mRNA was detected in cells isolated with anti-CD3 beads, whereas higher levels were measured in cells selected with anti-CD83, anti-CD11c, and anti-CD68 beads (Fig. 3 B). To characterize the cells isolated by antibody-coated magnetic beads, we assayed the unbound (flow-through) fraction for depletion of HLA-DR⁺ and CD83⁺ or CD3⁺ cells (Fig. 3 D). Using two color flow cytometry and gating parameters appropriate for T cells and DCs (Fig. 3 C, R1 and R2, respectively), we found that CD11c depletion increased the relative concentration of CD83⁺ cells (68%), but decreased the frequency of CD14⁺ cells, as expected if many of the CD11c⁺ cells were monocytes (Fig. 3 D). Analogously, CD83 depletion decreased the relative concentration of CD83⁺ cells in the flow through (Fig. 3 D, 14%), but did not deplete CD14⁺ cells (not depicted), as expected if these cells were mature DCs. CD3 depletion resulted in only a minor increase in CD83⁺ cells in the flow through (Fig. 3 D, 49%). CD3⁺ cells (gate R1) were depleted only with CD3 antibody beads (Fig. 3 D, bottom).
By immunohistochemical and FACS® staining of dermal cells from other biopsies, large increases in presumptive monocytes (CD11c+/CD14+CD83−) and mature DCs (CD11c+/CD14−CD83+) were detected in active psoriasis lesions compared with unaffected skin of the same patients.

Strong and Sustained Expression of p19 and p40 in Mature DCs. To further examine the control of IL-12 and IL-23 expression in monocytes and DCs, we used a standard procedure to generate DCs from peripheral blood monocytes using 4 d of culture in GM-CSF and IL-4 to produce immature DCs and a cytokine cocktail to mature the DCs (14). Fig. 4 displays quantitative expression of p19, p40, and p35 mRNAs in monocytes and in monocyte-derived DCs at various times after the addition of our maturation stimulus, and essentially parallels results seen with CD40L-stimulated DCs (1). Monocytes (−144 h), as well as monocytes
accumulated in GM-CSF and IL-4 to stimulate the differentiation of immature DCs (0 h), expressed relatively low levels of p19 mRNA and virtually undetectable levels of p40 mRNA. However, p19 mRNA was increased up to 10-fold in freshly isolated monocytes by the addition of TNF-α, IL-1β, IL-6, and PGE2 for the stated amount of time. The data bars (open and solid) represent relative gene expression of p19 (A), p40 (B), and p35 (C) from two separate experiments. Note differing abscissa scales.

Figure 4. Expression of p19, p40, and p35 mRNA in monocyte-derived DCs. Monocytes were placed in culture at -144 h, differentiated to immature DCs, and then stimulated beginning at 0 h with TNF-α, IL-1β, IL-6, and PGE2 for the stated amount of time. The data bars (open and solid) represent relative gene expression of p19 (A), p40 (B), and p35 (C) from two separate experiments. Note differing abscissa scales.

Discussion

Accumulating evidence supports the role of type 1 T cells and IFN-γ in the pathogenesis of psoriasis (8). Individual components of the type 1 pathway, both cellular and cytokine, are therapeutic targets in psoriasis. The development of Th1-type helper cells can be driven by IL-12 (1). We and others have previously reported that IL-12 p40 mRNA is elevated in psoriasis (12, 13), but we were perplexed by the failure to find elevated IL-12 p35 mRNA in diseased skin. We would now like to suggest that IL-23, not IL-12, is a key cytokine in psoriatic skin lesions based on the finding that p19 and p40, but not p35 mRNA, are overexpressed.

We believe that this is the first report that IL-23 p19 is up-regulated in a human disease. Recent reports have demonstrated a role for this novel cytokine in enhanced cutaneous immunity in a transgenic mouse model (16) and in inflammatory disorders such as experimental autoimmune encephalitis in a mouse model (5). With our observation that IL-23 is up-regulated in psoriatic skin, it became necessary to identify the cell of origin. The current lack of an antibody to p19 currently hampers this work. However, the reactivity of large cells in psoriasis lesions with antibodies specific for p40 suggests that IL-23 (given the failure to find increased IL-12 p35 mRNA in lesional skin) is likely to be actively synthesized in psoriasis lesions (12) and then act locally on cells that have up-regulated IL-12 receptors (13).

In this study, we also found that IL-23 p19 and p40 mRNA were strongly up-regulated in monocyte-derived human DCs after encountering a maturation stimulus, and that p19 mRNA was strongly expressed in cells bearing both immature and mature DC surface markers in psoriasis lesions (CD11c+ CD14+ CD83+ and CD83+). The sustained expression of IL-23 subunits in cultured fully mature DCs contrasted sharply with the transient expression of p35 mRNA in maturing DCs. Of the cells known to express IL-23 p19, only macrophages and DCs concurrently express p40 and are known to express functional IL-23 protein. Monocytic and DC lines have previously been identified as IL-23 producers, whereas mature human DCs have been shown to secrete IL-23 protein (1, 17). Thus, it seems that the mRNA and protein subunits of IL-23 are coordinately up-regulated in producing cells. One of the cellular features of psoriatic skin lesions is a marked increase in mature CD83+ DCs, whereas uninvolved skin contains mostly immature Langerhans cells and dermal DCs (15, 18, 19). Based on p40 immunostaining, infiltrating mononuclear leukocytes are the likely source of IL-23 in lesions. Given that p35 mRNA is expressed only transiently in activated DCs and p35 mRNA is not elevated in lesional skin, IL-12 is likely to be expressed at lower levels than IL-23 in psoriatic lesions. Adoptive transfer experiments have clearly established leukocytes as the chief source of IL-23 in inflammatory lesions (4).

It is possible that both IL-12 and IL-23 have a role in psoriatic skin disease. However, the work in murine experimental autoimmune encephalitis indicates that IL-23 is critical for a monocyte-rich inflammatory response. Expression of IL-23 may also drive increased production of IL-17 in affected skin (20). IL-17 has been reported to increase the expression of iNOS (21), a gene that is consistently overexpressed in psoriatic skin lesions (13). In model systems, IL-23 acts on DCs to allow for the induction of immunity to otherwise tolerogenic peptides (22), so poten-
tially its expression could contribute to abnormal antigenic recognition of skin-related antigens in psoriasis. Interestingly, a recent report identified skin inflammation in a skin-targeted p40 transgenic overexpressor as due to IL-23 production, but not IL-12 production, and there was a marked increase in the abundance of mature DCs in these lesions associated with increased IL-23 levels (16). In summary, IL-23 antagonists might be the most appropriate treatment for inflammatory skin diseases like psoriasis.

The authors wish to thank Joseph Krasovsky for technical assistance.

J. Krueger and E. Lee were supported by National Institutes of Health (NIH) grants AI-49572, AI-49832, 1-K23-AR49815-01, and M01-RR00102. M. Dhodapkar was supported by NIH grant CA 84512 and a Clinical Investigator Award from Damon Runyon Cancer Research Fund.

Submitted: 20 March 2003
Accepted: 5 November 2003

References


