The Psoriatic Transcriptome Closely Resembles That Induced by Interleukin-1 in Cultured Keratinocytes

Dominance of Innate Immune Responses in Psoriasis

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Psoriasis has been considered an autoimmune, T cell-mediated disorder in which adaptive immune responses predominate over those of non-antigen-specific innate immunity. To test this hypothesis, we profiled the transcriptome of psoriatic tissue and compared the data with that from cultured human keratinocytes exposed to the proinflammatory cytokine interleukin (IL)-1 α and the Th1 cytokine interferon- γ . When compared with patient-matched, non-lesional skin biopsies, psoriatic samples exhibited regulation of 90 transcripts including several members of the epidermal differentiation complex, molecules with antimicrobial activity, and hyperproliferation-associated keratins. Stimulation of keratinocytes with interferon- γ resulted in regulation of 252 transcripts, with particularly strong expression of the CXCR3-binding ligands CXCL9, -10, and -11 and class II major histocompatibility complex genes, primarily those of the HLA-DR and -DP families. In contrast, the transcriptome resulting from exposure of keratinocytes to IL-1 α elicited differences in just 19 transcripts, particularly genes within the epidermal differentiation complex and antimicrobial molecules, including *PI3* and *DEFB4*. Major differences between the two keratinocyte transcriptomes were exhibited with only five induced IL-1 α transcripts also regulated in the interferon- γ set. Unexpectedly, there was a high correlation between psoriatic lesional tissue and the IL-1 α transcriptome. These findings suggest that the inflammatory milieu in the epidermal microenvironment in psoriasis is more likely dependent on evolutionarily ancient cytokines such as IL-1, rather

than those of the adaptive immune response. (*Am J Pathol* 2007, 171:32–42; DOI: 10.2353/ajpath.2007.061067)

Psoriasis affects approximately 2% of Western populations and is characterized by epidermal inflammation and hyperplasia, chronic neutrophilic and T-lymphocytic infiltrates, and abnormal keratinocyte differentiation.¹ Previous studies have suggested a predominance of type 1 (Th1 and Tc1 T cell subset)-associated cytokines, such as interferon (IFN)- γ and interleukin (IL)-2, within psoriatic lesions.^{2,3} Xenotransplantation experiments involving grafting of nonlesional skin from psoriatic patients to immunodeficient mice and the subsequent introduction of autologous, stimulated immunocytes to induce plaque formation⁴ have demonstrated the obligatory role of these cells in the pathology. Further, many effective antipsoriatic therapies, including cyclosporin A, alefacept, and efalizumab, directly target T cells as their major mode of action.⁵

As the predominant cell type within the epidermis, keratinocytes have been demonstrated to be pivotal in the initiation, maintenance, and regulation of immune responses in the skin.⁶ They can synthesize a diverse array of immunomodulatory molecules, including a number of primary cytokines [eg, IL-1 and tumor necrosis factor (TNF)- α] and chemokines such as IL-8 required to induce vascular endothelial molecules and recruit T cells to the epidermis. In addition, stimulation of keratinocytes with T-cell-derived molecules such as IFN- γ up-regulates a number of major histocompatibility complex and co-

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stimulatory molecules inducing amplification of cytokine cascades essential in the potentiation of cutaneous inflammation.⁷

A key difference between the immunological responses induced in T cells and keratinocytes is that of antigen specificity. Keratinocytes react to infection or other trauma with an evolutionarily ancient, non-antigen-specific set of responses (innate immunity), whereas T cells use somatically recombined receptor genes to mediate an antigen-specific response (adaptive immunity). Effective host defense activities within the skin require interactions between the innate and adaptive immune systems. For example, innate proinflammatory mediators such as IL-1 α released from keratinocytes following trauma are a prerequisite for the extravasation of memory T cells to the epidermis and subsequent antigen-specific responses.⁸

Such interactions suggest that keratinocyte-mediated (innate) responses may have a fundamental role to play in psoriatic pathology.^{9,10} A number of transgenic mice exhibiting psoriasiform phenotypes, in which keratinocyte molecules were either overexpressed^{11,12} or deleted,^{13,14} support this hypothesis, and recent data indicating an essential role for resident plasmacytoid predendritic cells in psoriatic pathology¹⁵ further underpin the concept that innate immune mechanisms are central to this disease.

Novel insights into the molecular mechanisms driving psoriatic plaques have been provided by a number of recent studies using high-density cDNA microarrays to perform global comparisons of gene expression in lesional skin with biopsies from clinically normal skin.^{16–19} Thus, the psoriatic transcriptome may be broadly defined as comprising molecules found on the epidermal differentiation complex on chromosome 1q (eg, S100A7, SPRR1B), molecules with antimicrobial activity (eg, DEFB4, LCN2), hyperproliferation-associated molecules (eg, KRT6A, KRT16), and molecules involved in the regulation of proteolysis (eg, SERPINB3, PI3). In addition, numerous cytokines or cytokine-induced molecules associated with both keratinocytes (eg, IL1B, CXCL8/IL-8) and T cells (eg, IFNG, STAT1) were commonly induced in these studies.

To explore whether psoriasis is associated with the innate or adaptive immune response at the molecular level, we used microarray techniques to analyze psoriatic skin and compared this with the transcriptomes generated from primary human keratinocytes exposed to either IL-1 α or IFN- γ , as simple models of innate and adaptive immunity, respectively.

Materials and Methods

Patients

Six unrelated patients with chronic plaque psoriasis were recruited from a local dermatology outpatient clinic. None had received any systemic or topical therapy (except emollients) for at least 6 weeks before biopsy. Following local ethical committee approval and informed consent, a pair of shave biopsies was excised from each patient using a keratome, one intralesionally and the other from

clinically normal skin, at least 20 mm from any plaque. All biopsies were snap-frozen in liquid nitrogen to maximize *ex vivo* transcriptome accuracy before RNA extraction. In two additional patients, 2-mm punch biopsies were removed from the center of the keratomes subsequent to excision, fixed, and processed according to standard techniques for histological examination.

Keratinocyte Culture and Cytokine Treatment

Following local ethical committee approval and informed consent, primary human keratinocyte cultures were established as described previously²⁰ from breast reduction tissue derived from six unrelated female donors. Second-passage cells were grown to approximately 90% confluence in 25-cm² flasks and quiesced in supplement-free keratinocyte growth medium (EpiLife, Cascade Biologics, Mansfield, UK) for 24 hours before the addition of either recombinant human IL-1 α (100 ng/ml final concentration), recombinant human IFN- γ (20 ng/ml final concentration; specific activity = 1×10^7 IU/mg) (R&D Systems, Abingdon, UK), or vehicle alone (equivalent dilution of 1% bovine serum albumin in phosphate-buffered saline). Cultures were incubated for a further 24 hours at 37°C, 5% CO₂, before RNA extraction.

RNA Extraction and Preparation for Hybridization

Total RNA was isolated from all samples using TRIzol reagent (Invitrogen, Paisley, UK) according to the manufacturer's instructions. Skin biopsies were processed in 2 ml of TRIzol using a Polytron PT 1200CL homogenizer (Kinematica, Lucerne, Switzerland) for 30 seconds to commence RNA extraction. First-strand cDNA was derived from 10 μ g of total RNA using a T7-(dT)₂₄ primer and Superscript II reverse transcriptase (Invitrogen) (42°C, 1 hour). Second-strand cDNA synthesis was performed using *Escherichia coli* DNA ligase, *E. coli* DNA polymerase I, and RNase H (Invitrogen) (16°C, 2 hours). Biotin-labeled antisense cRNA was synthesized from purified double-stranded cDNA using the BioArray HighYield RNA transcript labeling kit (Enzo Diagnostics, Farmingdale, NY) according to the manufacturer's instructions (37°C, 5 hours).

Array Hybridization and Scanning

Two Affymetrix microarrays were used in the present studies. For the psoriatic biopsies, the HuGeneFL array, representing approximately 5600 full-length human genes, was used, whereas the HG-U133A array, containing approximately 18,000 characterized genes, was used in the analysis of the cytokine-exposed keratinocytes. For the HuGeneFL arrays, fragmentation was performed according to the Affymetrix protocol (20 μ g of biotinylated cRNA in 40 mmol/L Tris-acetate, pH 8.1, 100 mmol/L potassium acetate, and 150 mmol/L magnesium acetate at 94°C for 35 minutes). Ten micrograms of fragmented cRNA were hybridized to HuGeneFL arrays according to standard

Figure 1. Keratome biopsies from psoriatic skin provide a rich source of epidermal tissue. Representative histological sections from 2-mm punch biopsies removed from paired nonlesional (**a**) and lesional (**b**) keratome biopsies from a psoriatic patient. Scale bar = 100 μ m.

Affymetrix protocols in 100 mmol/L MES buffer, 1 mol/L $[\text{Na}^+]$, 20 mmol/L ethylenediamine tetraacetic acid, 0.01% Tween 20, 0.1 mg/ml herring sperm DNA, 0.5 mg/ml acetylated bovine serum albumin, and control oligos B2, BioB, BioC, BioD, and cre at 45°C for 16 hours. The fragmentation of antisense cRNA and hybridization to HG-U133A arrays was performed at the Clinical Sciences Centre/Imperial College Microarray Centre using in-house protocols based on those provided by Affymetrix. Further details are available online (<http://microarray.csc.mrc.ac.uk>, accessed March 2006). For both sets of microarrays, washing and subsequent staining with streptavidin phycoerythrin (two rounds at 10 μ g/ml) were performed according to standard Affymetrix protocols before scanning (Affymetrix GeneChip scanner 3000).

Analysis of GeneChip Data

Data from Affymetrix Microarray Suite 5.0 scanning software (CEL files) were imported into GeneSpring (version 7.2, Agilent Technologies, Palo Alto, CA) for analysis. For each experimental set of chips, array normalization was performed using the Robust Multichip Average preprocessing module of GeneSpring 7.2, followed by normalization of each probe set relative to the mean intensity value calculated on the control array. For each experi-

ment, probe sets were initially filtered to exclude all those called as “absent” on all arrays, then for those exhibiting a twofold minimum difference in normalized expression levels. The resulting probe lists were exported to a custom-written database that was used to filter the probe sets further to include only those regulated in at least four of the six replicate experiments for all three comparisons, ie, lesional versus nonlesional skin, IL-1 α versus no treatment, and IFN- γ versus no treatment, to minimize false-positive data and provide final transcript lists. Biological Process description data from the Gene Ontology Consortium, supplied for each probe set by Affymetrix (Q4 2005 update), were used for transcript annotation.

Results

Transcriptome of Psoriatic Skin

To better understand molecular dysregulation within psoriatic tissue, we were first interested in defining the transcriptional changes occurring in lesional skin compared with adjacent clinically normal tissue. Two shave biopsies were taken from each of six patients with chronic plaque psoriasis. The use of keratome sections reduced the dermal content of these biopsies relative to standard

punch or ellipse methodologies (Figure 1). RNA was extracted from all biopsies and subsequently processed for microarray analysis using the Affymetrix HuGeneFL array, comprising approximately 5600 well-characterized human transcripts. Analysis of these data revealed 95 probe sets (representing 90 separate transcripts) regulated by a factor of two or more in at least four of the six patients examined (Table 1 and Supplemental Table S1 at <http://ajp.amjpathol.org>). Seventy-five transcripts were induced and 15 were repressed. The serine protease inhibitor (SERPIN) B4 was the most strongly up-regulated transcript, with a mean induction of 64-fold. In accordance with previous studies,^{18,19,21} we found marked enrichment for epidermal differentiation complex genes on chromosome 1q21, including S100A7/psoriasin, S100A9/calgranulin B, S100A12/calgranulin C, and the small proline-rich proteins (SPRR) 1A, 1B, 2A, 2B, 2D, and 2E. In addition to SERPINB4, a number of other protease inhibitors were induced (PI3, SERPINB3, and cystatin A), along with the antimicrobial genes β 4 defensin and lipocalin 2. Marked enhancements in the transcription of the hyperproliferative keratins K6, K16, and K17 were also observed as demonstrated previously.²² Functional classification of transcripts revealed clusters of genes involved in immune responses, cellular and electron transport, and epidermal differentiation all markedly up-regulated. Surprisingly, no T cell-specific transcripts were consistently up-regulated. No functional groupings could be discerned among the 15 down-regulated transcripts, with the marked reduction of hemoglobin subunit mRNA likely indicative of greater reticulocytic contamination of the nonlesional biopsies.

Transcriptome of IL-1 α - and IFN- γ -Treated Keratinocytes

We were interested in dissecting out the influence of individual cytokines on the chronic inflammatory micro-environment observed in psoriatic skin through *in vitro* stimulation of keratinocytes with either IL-1 α or IFN- γ . Following exposure of second-passage normal human epidermal keratinocytes to either 100 ng/ml IL-1 α or 20 ng/ml IFN- γ cytokine for 24 hours, cRNA was generated for microarray analysis using the Affymetrix HG-U133A microarray. Keratinocytes expressed approximately 10,000 of the 22,283 probe sets present on the arrays. Many genes are represented by more than one probe set on the U133A array, hence 266 separate transcripts were regulated consistently in these experiments (247 by IFN- γ alone, 14 by IL-1 α alone, and five by both cytokines), according to the filtering criteria used.

The small set of 19 transcripts induced by IL-1 α exposure in keratinocytes contained a number of genes found in the epidermal differentiation complex, namely S100A7, S100A9, S100A12, and SPRR2B (Table 2). In addition, a group of proteases (MMP1 and MMP10) and protease inhibitors (SERPINB4 and PI3) were identified, along with the antimicrobial transcripts β 4 defensin and lipocalin 2 and a number of genes involved in regulation of the key proinflammatory nuclear factor κ B (NF- κ B) signaling

pathway (IL-32, IL-1F9, I κ B, and TNF- α -induced protein 3). Bioactivity of the IL-1 α used in the present study was confirmed by induction of IL-8 release from keratinocytes, observed from 1 ng/ml IL-1 α (data not shown).

By contrast, 252 transcripts (156 increased, 96 decreased) were regulated by IFN- γ exposure (Table 3 and Supplemental Table S2 at <http://ajp.amjpathol.org>). Particularly strong induction (mean >100-fold) of CXCL9, -10, and -11, all ligands of the Th1 cell chemokine receptor CXCR3, and a large number of genes located in the major histocompatibility complex on chromosome 6p, particularly those of the class II family including HLA-DR and -DP, were observed. In addition to the immune response genes, a number of proteolysis-associated transcripts were induced, whereas groups of genes involved in cell cycle regulation and DNA replication and repair were markedly down-regulated following IFN- γ exposure.

In all experiments, much less transcriptional activity was induced in keratinocytes following exposure to IL-1 α compared with IFN- γ , and the subsets of transcripts over-expressed showed surprisingly low levels of overlap (Figure 2). Overall, just five of the 252 transcripts from the IFN- γ set were also present in the IL-1 α group, ie, IL32, MMP1, mitochondrial superoxide dismutase 2, PDZK1-interacting protein 1, and SERPINB4, which was up-regulated by IL-1 α but repressed following IFN- γ treatment.

Comparison of Three Transcriptomes

Boolean analysis of the three sets of transcripts derived from the present study revealed a marked proportional overlap between the psoriasis and IL-1 α exposure groups (nine common transcripts out of 19; 47%) compared with the much larger IFN- γ and psoriasis transcript subsets (10 common transcripts out of 252; 4%). Only two transcripts, PDZK1-interacting protein 1 and SERPIN B4, were common to all three groups (Figure 3a). Analysis of the 90 regulated psoriatic transcripts in the cytokine-exposed keratinocytes revealed particularly strong and specific correlation with the IL-1 α transcriptome in the most markedly up-regulated genes (Figure 3b).

Discussion

The primary pathogenic defect in psoriasis is unclear, although evidence from numerous mouse models and experimental therapies suggests that contributions from both residing keratinocytes and infiltrating immunocytes may be required to induce disease. Both cell types can release proinflammatory cytokines within psoriatic lesions, although the relative contribution from each remains poorly understood. The present study sought to use microarray technology to dissect out molecular signals associated with two key cutaneous proinflammatory cytokines from the transcriptome of established psoriatic skin, IL-1 α (released predominantly by keratinocytes) and IFN- γ (an immunocyte-specific cytokine). These cytokines were selected on the basis of their cellular specificity, cutaneous expression levels, and known associations with psoriatic lesions. In addition to finding a

Table 1. Differentially Expressed Transcripts between Lesional and Nonlesional Psoriatic Skin

Probe ID	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Fold Change	Gene	Definition	Chromosome
U19557_s_at	86.21	51.23	94.40	61.51	62.20	30.06	64.27	<i>SERPINB4</i>	Serpin peptidase inhibitor, clade B (ovalbumin), member 4	18q21
L10343_at	36.44	27.65	31.64	27.38	28.71	26.07	29.65	<i>PI3</i>	Peptidase inhibitor 3, skin-derived (SKALP)	20q12-q13
M26311_s_at	28.49	40.85	25.30	38.12	17.37	23.51	28.94	<i>S100A9</i>	S100 calcium binding protein A9 (calgranulin B)	1q21
M86757_s_at	19.10	29.37	14.65	40.07	7.84	33.03	24.01	<i>S100A7</i>	S100 calcium binding protein A7 (psoriasin 1)	1q21
Z71389_at	36.69	18.27	33.58	15.66	25.77	8.49	23.08	<i>DEFB4</i>	Defensin, β 4	8p23-p22
V01516_f_at	7.62	26.36	39.05	22.30	26.04	9.20	21.76	<i>KRT6A</i>	Keratin 6A	12q12-q13
L42583_f_at	8.14	24.00	32.52	25.65	24.68	9.44	20.74	<i>KRT6A</i>	Keratin 6A	12q12-q13
L42601_f_at	7.69	24.18	34.30	21.50	24.53	11.31	20.58	<i>KRT6A</i>	Keratin 6A	12q12-q13
S66896_at	34.16	21.44	14.43	16.62	16.19	11.92	19.13	<i>SERPINB3</i>	Serpin peptidase inhibitor, clade B (ovalbumin), member 3	18q21
L05188_f_at	16.68	14.42	13.28	33.66	8.04	12.70	16.46	<i>SPRR2B</i>	Small proline-rich protein 2B	1q21-q22
Z19574_rna1_at	4.20	18.58	16.29	24.23	18.64	7.00	14.82	<i>KRT17</i>	Keratin 17	17q12-q21
S72493_s_at	10.82	20.46	17.56	16.95	10.38	6.78	13.82	<i>KRT16</i>	Keratin 16 (focal nonepidermolytic palmoplantar keratoderma)	17q12-q21
X53065_f_at	12.59	8.89	9.55	26.14	7.18	10.94	12.55	<i>SPRR2A</i>	Small proline-rich protein 2A	1q21-q22
M21302_at	15.41	12.11	15.98	14.00	9.76	6.12	12.23	<i>SPRR2D</i>	Small proline-rich protein 2D	1q21-q22
M86849_at	4.91	6.13	14.80	7.60	17.32	5.89	9.44	<i>GJB2</i>	Gap junction protein, β 2, 26 kd (connexin 26)	13q11-q12
L42611_f_at	12.69	13.21	12.98	5.38	7.24	2.88	9.06	<i>KRT6E</i>	Keratin 6E	12q13
L00205_at	8.80	19.90	8.19	4.32	8.83	2.36	8.73	<i>KRT6B</i>	Keratin 6B	12q12-q13
D88422_at	26.61	3.38	2.81	2.80	2.68	3.61	6.98	<i>CSTA</i>	Cystatin A (stefin A)	3q21
M19888_at	3.51	4.19	13.67	12.26	5.65	2.44	6.95	<i>SPRR1B</i>	Small proline-rich protein 1B (cornifin)	1q21-q22
J05068_at	14.07	4.50	6.26	4.50	6.91	5.47	6.95	<i>TCN1</i>	Transcobalamin I (vitamin B12 binding protein, R binder family)	11q11-q12
L05187_at	5.36	9.31	9.43	7.35	6.72	3.50	6.95	<i>SPRR1A</i>	Small proline-rich protein 1A	1q21-q22
L33930_s_at	11.74	2.36	6.40	5.13	6.65	3.63	5.99	<i>CD24</i>	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	6q21
S75256_s_at	7.89	7.11	9.67	3.46	5.41	2.25	5.96	<i>LCN2</i>	Lipocalin 2 (oncogene 24p3)	9q34
M20030_f_at	4.61	2.45	3.98	14.80	2.91	5.77	5.75	<i>SPRR2E</i>	Small proline-rich protein 2E	1q21-q22
M94856_at	7.73	4.20	5.31	5.19	5.04	5.65	5.52	<i>FABP5</i>	Fatty acid binding protein 5 (psoriasis-associated)	8q21
HG880-HT880_at	1.03	0.71	0.45	0.39	0.35	0.35	0.55	<i>MUC6</i>	Mucin 6, gastric	11p15
M57710_at	0.59	0.29	0.42	0.50	0.43	1.03	0.54	<i>LGALS3</i>	Lectin, galactoside-binding, soluble, 3 (galectin 3)	14q21-q22
HG3115-HT3291_at	1.43	0.79	0.33	0.19	0.18	0.15	0.51	<i>MBP</i>	Myelin basic protein	18q23
S73591_at	0.36	0.34	0.40	0.46	0.55	0.88	0.50	<i>TXNIP</i>	Thioredoxin interacting protein	1q21
U62800_at	0.21	0.29	0.44	0.59	0.49	0.96	0.50	<i>CST6</i>	Cystatin E/M	11q13
HG3925-HT4195_at	0.79	0.36	0.42	0.72	0.38	0.20	0.48	<i>SFTPA2</i>	Surfactant, pulmonary-associated protein A2	10q22-q23
X13839_at	0.19	0.22	0.39	0.86	0.60	0.49	0.46	<i>ACTA2</i>	Actin, α 2, smooth muscle, aorta	10q23
M94077_at	0.38	0.24	0.09	0.58	0.37	1.08	0.46	<i>LOR</i>	Loricrin	1q21
X58072_at	0.43	0.12	0.58	0.39	0.38	0.82	0.45	<i>GATA3</i>	GATA binding protein 3	10p15
X76717_at	0.40	0.21	0.66	0.39	0.47	0.54	0.44	<i>MT1X</i>	Metallothionein 1X	16q13
HG2149-HT2219_at	1.11	1.04	0.14	0.13	0.12	0.11	0.44	<i>MUC5B</i>	Mucin 5, subtype B, tracheobronchial	11p15
L11672_r_at	0.47	0.17	0.46	0.53	0.41	0.51	0.42	<i>ZNF91</i>	Zinc finger protein 91 (HPF7, HTF10)	19p13-p12
M25079_s_at	0.22	0.14	0.40	1.05	0.09	0.58	0.41	<i>HBB</i>	Hemoglobin, β (aka β -globin)	11p15
S43646_at	0.28	0.20	0.20	0.58	0.23	0.85	0.39	<i>KRT2A</i>	Keratin 2A (epidermal ichthyosis bullosa of Siemens)	12q11-q13
Z84721_cds2_at	0.10	0.04	0.31	0.96	0.09	0.68	0.36	<i>HBA2</i>	Hemoglobin, α 2	16p13
HG1428-HT1428_s_at	0.06	0.03	0.19	1.22	0.07	0.50	0.35	<i>HBB</i>	Hemoglobin, β	11p15

Data represent the fold increases in expression between the two tissue types in six unrelated psoriatic patients (Pt 1–6), ranked by mean fold change. Probe IDs refer to Affymetrix HuGeneFL microarrays. Values in dark shading represent increases, light shading denotes decreases, and no shading indicates a fold change below the twofold threshold used. Note that only the top 25 of 79 induced probe sets are displayed (see Supplemental Table S1 at <http://ajp.amjpathol.org> for full data set).

Table 2. Differentially Expressed Transcripts between IL-1 α -Exposed and Vehicle-Treated Keratinocytes

Probe ID	D1	D2	D3	D4	D5	D6	Fold change	Gene	Definition	Chromosome
205916_at	122.40	30.20	15.86	10.42	10.17	8.17	32.87	<i>S100A7</i>	S100 calcium binding protein A7 (psoriasin 1)	1q21
211906_s_at	91.46	9.21	3.12	1.77	1.54	6.03	18.85	<i>SERPINB4</i>	Serpin peptidase inhibitor, clade B (ovalbumin), member 4	18q21
219554_at	55.00	9.92	7.09	2.99	3.61	3.51	13.69	<i>RHCG</i>	Rhesus blood group, C glycoprotein	15q25
207356_at	45.49	11.93	5.62	1.55	1.55	4.73	11.81	<i>DEFB4</i>	Defensin, β 4	8p23-p22
220322_at	20.94	7.83	4.47	3.40	5.35	4.96	7.83	<i>IL1F9</i>	IL-1 family, member 9	2q12-q21
212531_at	16.07	6.54	2.80	2.56	2.25	2.78	5.50	<i>LCN2</i>	Lipocalin 2 (oncogene 24p3)	9q34
203535_at	7.78	8.52	3.38	3.40	3.55	4.56	5.19	<i>S100A9</i>	S100 calcium binding protein A9 (calgranulin B)	1q21
41469_at	13.67	7.38	2.81	2.41	2.30	2.58	5.19	<i>PI3</i>	Peptidase inhibitor 3, skin-derived (SKALP)	20q12-q13
203691_at	12.83	7.21	3.30	2.43	2.32	2.85	5.16	<i>PI3</i>	Peptidase inhibitor 3, skin-derived (SKALP)	20q12-q13
210413_x_at	14.64	5.07	2.08	1.31	1.34	2.70	4.52	<i>SERPINB4</i>	Serpin peptidase inhibitor, clade B (ovalbumin), member 4	18q21
219630_at	5.02	6.46	4.12	2.72	3.24	4.98	4.42	<i>PDZK1IP1</i>	PDZK1 interacting protein 1	1p33
215223_s_at	10.21	4.36	2.88	1.50	2.62	3.19	4.13	<i>SOD2</i>	Superoxide dismutase 2, mitochondrial	6q25
204475_at	4.79	3.86	1.88	2.94	4.94	3.57	3.66	<i>MMP1</i>	Matrix metalloproteinase 1 (interstitial collagenase)	11q22
208539_x_at	4.40	6.43	2.73	2.39	1.76	4.04	3.62	<i>SPRR2B</i>	Small proline-rich protein 2B	1q21-q22
205863_at	9.39	3.39	2.27	1.10	1.34	3.53	3.50	<i>S100A12</i>	S100 calcium binding protein A12 (calgranulin C)	1q21
205680_at	1.74	5.38	3.47	2.63	2.10	5.25	3.43	<i>MMP10</i>	Matrix metalloproteinase 10 (stromelysin 2)	11q22
218810_at	4.11	2.61	2.06	1.96	2.13	2.11	2.50	<i>ZC3H12A</i>	Zinc finger CCCH-type containing 12A	1p34
203180_at	3.52	3.28	1.99	2.11	1.72	2.25	2.48	<i>ALDH1A3</i>	Aldehyde dehydrogenase 1 family, member A3	15q26
202643_s_at	4.99	2.34	1.47	2.23	2.06	1.74	2.47	<i>TNFAIP3</i>	TNF- α -induced protein 3	6q23
203828_s_at	2.30	1.67	2.16	2.71	2.50	3.02	2.39	<i>IL32</i>	IL-32	16p13
202644_s_at	4.49	2.10	1.47	2.04	2.10	1.73	2.32	<i>TNFAIP3</i>	TNF- α -induced protein 3	6q23
201502_s_at	3.43	2.49	2.00	1.76	2.42	1.49	2.27	<i>NFKBIA</i>	NF- κ light polypeptide gene enhancer in B-cell inhibitor α	14q13

Data represent the fold increases in expression between the two treatment types in second-passage cultured keratinocytes derived from six unrelated donors (D1–6), ranked by mean fold change. Probe IDs refer to Affymetrix HG-U133A microarrays. Shading is as in Table 1.

marked difference in keratinocyte response to the two cytokine stimuli, a significant correlation was noted between transcripts up-regulated in psoriatic lesional skin and those induced by IL-1 α *in vitro*.

Several major studies have previously defined the transcriptome of psoriatic lesional skin.^{16–19} Because psoriasis affects the epidermis, we were interested in focusing on this compartment of the skin using epidermis-rich keratome shave biopsies, unlike the previous studies that used full-thickness biopsies, which comprise a significant number of dermal cells. There was a high degree of concordance between the previously published psoriatic transcriptomes and the present study with strong expression of transcripts in four main groups: 1) genes located on the epidermal differentiation complex on chromosome 1q21 (also known as the psoriasis susceptibility locus, PSORS4²³), 2) proteases and protease inhibitors, 3) molecules with antimicrobial activity, and 4) hyperproliferation-associated keratins.

As a prototypic proinflammatory cytokine, we chose IL-1 α as a suitable candidate to investigate innate immune responses in keratinocytes and found marked similarities between the transcriptome generated and that observed in lesional psoriatic plaques. This supports the significant literature associating members of the IL-1 family with psoria-

sis.^{24–27} Although IL-1 α is known to be down-regulated in established psoriatic plaques,²⁸ this most likely reflects consumption during plaque initiation²⁷ and measurable levels remain intralesionally. The relatively small number of transcripts induced by IL-1 α , compared with IFN- γ , may reflect the recently described homeostatic control of IL-1 in murine epidermis, in which a small amount of IL-1 α is constitutively released by keratinocytes, and excess IL-1 results in a type I IL-1 receptor-dependent up-regulation of IL-1ra release.²⁹ Consistent with this, a previous study observed an unexpectedly small number of transcripts regulated in ovarian epithelial cells following exposure to IL-1 α .³⁰

In addition to those molecules common to the psoriatic lesional transcriptome, a number of genes with broadly opposing roles were induced by IL-1 α . Two recently characterized cytokines, IL-1F9 and IL-32, both of which activate the NF- κ B proinflammatory pathway,^{31,32} were induced, the former having been shown previously to be up-regulated in psoriatic plaques.²⁶ Conversely, TNF- α induced protein 3³³ and I κ B both inhibit NF- κ B signaling. Likewise, the induction of both proteases (MMP-1 and -10) and protease inhibitors (SERPINB4 and PI3) are further suggestive of complex counter-regulatory mechanisms.

Table 3. Differentially Expressed Transcripts between IFN- γ -Exposed and Vehicle-Treated Keratinocytes

Probe ID	D1	D2	D3	D4	D5	D6	Fold change	Gene	Definition	Chromosome
203915_at	141.80	209.00	86.13	96.86	79.26	87.41	116.74	<i>CXCL9</i>	Chemokine (C-X-C motif) ligand 9	4q21
204533_at	92.33	80.28	117.50	132.90	72.28	184.20	113.25	<i>CXCL10</i>	Chemokine (C-X-C motif) ligand 10	4q21
211122_s_at	112.90	138.70	79.78	111.00	132.80	88.00	110.53	<i>CXCL11</i>	Chemokine (C-X-C motif) ligand 11	4q21
210163_at	168.70	154.20	25.57	96.43	92.06	77.27	102.37	<i>CXCL11</i>	Chemokine (C-X-C motif) ligand 11	4q21
208894_at	58.10	27.55	57.63	94.60	36.54	48.18	53.77	<i>HLA-DRA</i>	Major histocompatibility complex, class II, DR α	6p21
209312_x_at	19.91	11.36	20.24	80.62	43.34	84.52	43.33	<i>HLA-DRB1</i>	Major histocompatibility complex, class II, DR β 1	6p21
211990_at	39.09	23.07	61.62	48.33	34.28	35.74	40.36	<i>HLA-DPA1</i>	Major histocompatibility complex, class II, DP α 1	6p21
209619_at	48.73	28.77	26.54	34.73	22.48	36.79	33.01	<i>CD74</i>	CD74 antigen (invariant polypeptide of MHC, class II antigen-associated)	5q31-q33
210982_s_at	46.99	21.13	38.09	34.30	25.70	31.27	32.91	<i>HLA-DRA</i>	Major histocompatibility complex, class II, DR α	6p21
200629_at	29.24	35.95	31.21	20.51	25.74	48.18	31.81	<i>WARS</i>	Tryptophanyl-tRNA synthetase	14q32
217478_s_at	21.96	16.48	40.57	45.60	17.03	41.52	30.53	<i>HLA-DMA</i>	Major histocompatibility complex, class II, DM α	6p21
205890_s_at	21.88	93.25	11.41	35.65	7.99	7.40	29.60	<i>UBD</i>	Ubiquitin D	6p21
213797_at	34.94	39.61	15.99	18.93	24.11	22.99	26.10	<i>RSAD2</i>	Radical S-adenosyl methionine domain containing 2	2p25
200986_at	18.96	20.25	28.74	19.51	27.42	30.13	24.17	<i>SERPING1</i>	Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1 (angioedema, hereditary)	11q12-q13
204070_at	13.33	11.10	33.82	37.63	12.02	33.65	23.59	<i>RARRES3</i>	Retinoic acid receptor responder (tazarotene-induced) 3	11q23
202269_x_at	21.81	39.47	13.06	12.33	26.23	28.20	23.52	<i>GBP1</i>	Guanylate binding protein 1, IFN-inducible, 67 kd	1p22
211991_s_at	23.66	14.29	26.18	20.78	17.44	25.98	21.39	<i>HLA-DPA1</i>	Major histocompatibility complex, class II, DP α 1	6p21
200628_s_at	14.77	19.37	17.58	19.32	21.05	28.57	20.11	<i>WARS</i>	Tryptophanyl-tRNA synthetase	14q32
202270_at	18.71	36.80	10.14	9.95	31.23	12.98	19.97	<i>GBP1</i>	Guanylate binding protein 1, IFN-inducible, 67 kd	1p22
215193_x_at	12.81	15.09	23.30	25.01	16.62	26.01	19.81	<i>HLA-DRB1</i>	Major histocompatibility complex, class II, DR β 1	6p21
202531_at	15.85	23.64	20.34	21.12	10.58	25.69	19.54	<i>IRF1</i>	IFN regulatory factor 1	5q31
823_at	31.68	30.39	13.17	14.52	11.85	9.17	18.46	<i>CX3CL1</i>	Chemokine (C-X3-C motif) ligand 1	16q13
203828_s_at	8.91	18.27	6.47	14.38	36.01	16.97	16.83	<i>IL32</i>	IL-32	16p13
204698_at	11.38	7.96	16.64	15.10	17.10	28.69	16.15	<i>ISG20</i>	IFN-stimulated exonuclease gene 20 kd	15q26
204670_x_at	10.37	6.72	20.95	12.42	14.26	23.20	14.65	<i>HLA-DRB1</i>	Major histocompatibility complex, class II, DR β 1	6p21
204023_at	0.38	0.28	0.38	0.28	0.30	0.53	0.36	<i>RFC4</i>	Replication factor C (activator 1) 4, 37 kd	3q27
201417_at	0.51	0.39	0.25	0.25	0.30	0.40	0.35	<i>SOX4</i>	SRY (sex determining region Y)-box 4	6p22
201362_at	0.40	0.19	0.48	0.32	0.25	0.38	0.34	<i>IVNS1ABP</i>	Influenza virus NS1A binding protein	1q25-q31
205891_at	0.38	0.16	0.31	0.24	0.35	0.56	0.33	<i>ADORA2B</i>	Adenosine A2b receptor	17p12-p11.2
218039_at	0.36	0.23	0.52	0.20	0.42	0.28	0.33	<i>NUSAP1</i>	Nucleolar and spindle-associated protein 1	15q15
201890_at	0.38	0.22	0.38	0.15	0.41	0.45	0.33	<i>RRM2</i>	Ribonucleotide reductase M2 polypeptide	2p25-p24
208691_at	0.21	0.28	0.26	0.18	0.73	0.32	0.33	<i>TFRC</i>	Transferrin receptor (p90, CD71)	3q29
201291_s_at	0.42	0.24	0.58	0.27	0.30	0.15	0.33	<i>TOP2A</i>	Topoisomerase (DNA) II α 170 kd	17q21-q22
202870_s_at	0.37	0.21	0.51	0.021	0.31	0.33	0.32	<i>CDC20</i>	CDC20 cell division cycle 20 homolog (<i>Saccharomyces cerevisiae</i>)	1p34

(table continues)

Table 3. *Continued*

Probe ID	D1	D2	D3	D4	D5	D6	Fold change	Gene	Definition	Chromosome
207324_s_at	0.70	0.32	0.34	0.29	0.15	0.12	0.32	<i>DSC1</i>	Desmocollin 1	18q12.1
201185_at	0.37	0.21	0.42	0.28	0.30	0.34	0.32	<i>HTRA1</i>	HtrA serine peptidase 1	10q26
202589_at	0.36	0.14	0.53	0.41	0.21	0.26	0.32	<i>TYMS</i>	Thymidylate synthetase	18p11
204151_x_at	0.23	0.31	0.27	0.30	0.44	0.30	0.31	<i>AKR1C1</i>	Aldo-keto reductase family 1, member C1	10p15-p14
204026_s_at	0.32	0.23	0.35	0.30	0.26	0.25	0.28	<i>ZWINT</i>	ZW10 interactor	10q21-q22
211653_x_at	0.17	0.28	0.21	0.30	0.40	0.32	0.28	<i>AKR1C2</i>	Aldo-keto reductase family 1, member C2	10p15-p14
209699_x_at	0.19	0.22	0.28	0.37	0.34	0.26	0.28	<i>AKR1C2</i>	Aldo-keto reductase family 1, member C2	10p15-p14
211756_at	0.64	0.15	0.12	0.04	0.36	0.27	0.26	<i>PTH1H</i>	Parathyroid hormone-like hormone	12p12-p11
202503_s_at	0.34	0.24	0.31	0.24	0.26	0.12	0.25	<i>KIAA0101</i>	KIAA0101	15q22
214710_s_at	0.44	0.14	0.28	0.23	0.24	0.16	0.25	<i>CCNB1</i>	Cyclin B1	5q12
210559_s_at	0.31	0.12	0.28	0.26	0.20	0.12	0.22	<i>CDC2</i>	Cell division cycle 2, G ₁ to S and G ₂ to M	10q21
218002_s_at	0.37	0.15	0.30	0.14	0.10	0.24	0.22	<i>CXCL14</i>	Chemokine (C-X-C motif) ligand 14	5q31
203083_at	0.49	0.13	0.27	0.14	0.09	0.14	0.21	<i>THBS2</i>	Thrombospondin 2	6q27
209773_s_at	0.22	0.12	0.29	0.31	0.17	0.11	0.20	<i>RRM2</i>	Ribonucleotide reductase M2 polypeptide	2p25-p24
218009_s_at	0.21	0.14	0.25	0.20	0.20	0.13	0.19	<i>PRC1</i>	Protein regulator of cytokinesis 1	15q26
221805_at	0.54	0.04	0.12	0.05	0.08	0.06	0.15	<i>NEFL</i>	Neurofilament, light polypeptide 68 kd	8p21

Conditions are as in Table 2. Note that only the top 25 of 220 induced probe sets and the top 25 of 119 repressed probe sets are displayed (see Supplemental Table S2 at <http://ajp.amjpathol.org> for full data set). Shading is as in Table 1.

In contrast with the psoriatic and IL-1 α transcriptomes, we found a distinct set of transcripts up-regulated by IFN- γ in keratinocytes. This was surprising, given the

strong evidence for the role of T-helper cells in psoriasis.^{34,35} There was considerably more transcriptional activity in these cells compared with IL-1 α treatment, and

Figure 2. Major differences between transcriptomes induced by IL-1 α and IFN- γ in human keratinocytes. **a:** Cluster analysis showing all probe sets exhibiting a twofold or greater change between cytokine and vehicle treatment in a representative donor ($n = 452$). Probe set intensity values normalized to control (1.0) and colored according to fold induction or repression following treatment with respective cytokine. **b:** Venn diagram of same data, indicating relative lack of overlap between the two data sets.

Figure 3. Transcriptome overlap between IL-1 α - or IFN- γ -stimulated keratinocytes and psoriatic lesional skin. **a:** Boolean analysis of the three data sets generated. Numbers refer to transcripts rather than probe sets. **b:** Table indicating relative expression of the 10 most strongly induced transcripts in the psoriatic transcriptome in human keratinocytes following exposure to either IL-1 α or IFN- γ .

the induced transcripts were dominated by the ligands of the CXCR3 chemokine receptor, CXCL9, -10, and -11 and class II major histocompatibility complex transcripts, involved in antigen presentation to CD4⁺ T-helper cells. Subsequent real-time polymerase chain reaction studies verified the marked induction of CXCL9 by IFN- γ in keratinocytes. Although CXCR3 is expressed primarily on Th1 memory T cells, Flier et al³⁶ showed that psoriatic skin expresses very low levels of these three ligands compared with other inflammatory dermatoses.

A further illustration of the differences between the two cytokine-induced transcriptomes is the observation that SERPINB4, the most up-regulated transcript in the psoriatic data set, was markedly down-regulated in all keratinocyte cultures treated with IFN- γ , in contrast to the consistent induction observed in IL-1 α -treated samples. Further, the coordinated repression of transcripts involved in cell cycle regulation and DNA repair and replication with IFN- γ treatment, important in antiviral activity, was not observed in the psoriatic data set, where both of the regulated cell cycle-associated genes (*ACPP* and *MPHOSPH6*) were induced. A previous microarray analysis of the keratinocyte transcriptome following 24 hours of IFN- γ exposure reported a similar set of induced transcripts, although large increases were also seen in the macrophage-recruiting chemokines CCL2 and CCL8, which were not replicated in the present data set, and a higher number of repressed, rather than induced, transcripts was observed at this time point in the former study.³⁷ However, a key difference between the two analyses is the use of multiple donors in the present work, rather than a single batch of cells used previously. Given the high degree of intra- and interindividual variability commonly observed with microarray studies, the importance of biological replication to transcriptome accuracy cannot be underestimated.

In addition to IFN- γ , Banno et al³⁸ examined the transcriptome of keratinocytes exposed to the other principal primary cytokine, TNF- α , and reported clear differences from those found with IL-1 α in the present study. For example, no up-regulation of any of the S100 proteins, PI3, SERPINB4, or β 4 defensin was reported, although significant induction of CXCL10 and -11 was observed.

Whereas this study used numerous differences in protocol from that used in the present one, previous microarray data using a chondrosarcoma cell line confirmed significant differences in transcriptome induction following exposure to IL-1 α or TNF- α .³⁹ Studies by Wei et al⁴⁰ examining the effects of IL-1 β and IFN- γ on organ-cultured skin biopsies reported a greater degree of overlap between these two cytokines than that observed here. However, only seven proteins were analyzed by semiquantitative immunohistochemistry in the former study, with lower thresholds required for significance.

The keratinocyte IL-1 α transcriptome described here represents the closest approximation to that seen in psoriasis of all microarray studies described to date. It correlates better than the transcriptional profiles seen in other chronic inflammatory dermatoses such as atopic dermatitis, where disease-specific overexpression of the Th2-attracting β -chemokines CCL13, -18, and -27 and no up-regulation of highly induced psoriatic/IL-1 genes *DEFB4*, *PI3*, or *SPRR2B* have been reported.¹⁷ Likewise, the IL-1 keratinocyte transcriptome is distinct from that induced following ultraviolet B irradiation⁴¹ and the wound-healing response seen in neonatal mice⁴² where, in neither case, were any of these three key genes up-regulated, despite a number of psoriatic transcripts being expressed as part of the "late effector" cluster in the latter study.

The transcripts shared between lesional psoriatic tissue and IL-1 α -stimulated keratinocytes may suggest a specific role for IL-1 in psoriatic pathology. We have previously demonstrated that transgenic mice overexpressing IL-1 α in basal keratinocytes developed focal inflammatory skin lesions,⁴³ whereas others have reported psoriasiform lesions on BALB/c IL-1 receptor antagonist knockout mice.⁴⁴ The strong induction by IL-1 α of antimicrobial molecules is of particular interest. S100A7 has been shown recently to exhibit potent antimicrobial properties, particularly against *E. coli*.⁴⁵ Our data are in agreement with studies in which lesions from psoriasis and atopic dermatitis were compared, and a marked deficit in antimicrobial molecules was noted in the skin of patients with atopic dermatitis,^{46,47} further suggestive of an important role for innate immunity, specifically in psoriasis. Recent interest in the role of the Th17 effector lineage in psoriasis has yielded data indicating that Th17-associated cytokines up-regulate antimicrobial molecules, including β 4 defensin and S100A9, in keratinocytes.⁴⁸

Although the transcriptome data presented strongly suggest a bias toward innate immune responses in psoriatic lesions, it is important to note that they do not exclude the possibility that IFN- γ responses are actively suppressed within inflamed epidermis. This is supported by the relative infrequency of epidermal keratinocyte HLA-DR expression within psoriatic plaques, despite the widely reported sensitivity of cultured human keratinocytes to induction of this molecule by IFN- γ . Such an effect could be mediated by transcriptional interference involving different members of the STAT family of proteins induced, following receptor engagement by the wide variety of cytokines present in psoriatic epidermis. Alter-

natively, there is evidence from a murine autoimmunity model that Th17 responses may be antagonized by IFN- γ ,⁴⁹ hence the lack of IFN- γ signature transcripts observed in psoriatic tissue.

In this study, we have demonstrated that keratinocytes respond to cytokines known to be strongly associated with either innate or adaptive immune responses in the cutaneous microenvironment through discreet pathways at the transcriptional level and that exposure of human keratinocytes to IL-1 α results in the most psoriatic transcriptome described to date. Consequently, we propose that the inflammatory milieu in established psoriatic lesions is much more suggestive of dominant innate, rather than adaptive, immune responses. Although further work is required to validate this hypothesis, these data provide a novel perspective for investigating the immunopathology of psoriasis.

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