

Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: A gene microarray analysis

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Background: Atopic dermatitis (AD) and psoriasis are the two most common chronic inflammatory skin diseases. Both of these diseases have distinct clinical findings and specific inflammatory cell infiltrates. Previous reports have focused individually on one or two genes or gene products in the lesions of both skin diseases. However, they have not captured the complex gene expression that must occur to induce specific cellular infiltrates in the skin lesions of these two diseases. DNA microarray studies allow the simultaneous comparison of thousands of messenger RNAs that may identify the disease-specific pattern of tissue inflammatory responses.

Objective: To compare the complex gene expression pattern of AD versus psoriasis skin lesions.

Methods: RNA was extracted from skin biopsy specimens of 6 patients with AD and 7 patients with psoriasis and analyzed with the use of Hu-U95Av.GeneChip microarrays. To confirm GeneChip results, real-time PCR of selected genes were performed.

Results: In AD skin, a total of 18 genes including the CC chemokines, CCL-13/MCP-4, CCL-18/PARC, and CCL-27/CTACK showed a statistically significant, >2-fold increase of gene expression compared with psoriasis. In psoriasis skin, a total of 62 genes including CCL-4/MIP-1 β , CCL-20/MIP-3 α , CXCL-2/GRO- β , CXCL-8/IL-8, and CXCR2/IL-8R showed a >2-fold increase of gene expression compared with AD skin. Real-time PCR confirmed several of these GeneChip results. **Conclusions:** These results show a very distinctive gene expression pattern in AD as compared with psoriasis that may explain several features of AD and psoriasis including the specific inflammatory cell infiltrates observed in these disorders, that is, T_H2 cells, eosinophils, and mast cells in AD and T_H1 cells and neutrophils in psoriasis. Such observations may contribute to a characteristic "signature" for these two skin diseases. (*J Allergy Clin Immunol* 2003;112:1195-1202.)

Key words: Atopic dermatitis, chemokines, psoriasis, genes

Atopic dermatitis (AD) and psoriasis are the two most common chronic inflammatory skin diseases.^{1,2} Although it is well established that both skin diseases are genetically inherited and mediated by immunologic processes, the exact mechanisms leading to skin inflammation are poorly understood. AD skin lesions are characterized by intensely pruritic erythematous papules associated with epidermal intercellular edema as well as increased numbers of IgE-bearing Langerhans cells, inflammatory dendritic epidermal cells, macrophages, eosinophils, and CD4-activated T_H2 cells.^{1,3} In contrast, the clinical picture of psoriasis is associated with erythematous scaly papules and plaque formation.⁴ The immune response in psoriasis is markedly polarized to a T_H1 cell response and associated with local infiltration of T cells, monocytes, and neutrophils.⁵ In addition, psoriasis is often associated with epidermal hyperproliferation.

Previous reports have described isolated observations on changes in the proinflammatory and chemokine expression in skin lesions of both skin diseases.⁶⁻¹¹ However, they have not captured the complex gene expression that must occur to induce these different clinical skin findings. DNA microarray studies allow the simultaneous comparison of thousands of messenger RNAs that may identify the disease-specific pattern of tissue inflammatory responses. Our current study used microarray analysis to compare the patterns of gene expression in AD versus psoriasis in an effort to better characterize new aspects of their pathology and identify potentially new gene targets that may be useful in the diagnosis and treatment of these two common skin diseases.

METHODS

Subjects

This study was approved by the Institutional Review Board at National Jewish Medical and Research Center. Skin biopsy specimens were obtained from the skin lesions of 15 different patients with moderate to severe AD (mean age, 33.0 years; skin involvement, 20% to 60%; skin biopsy specimens were used from 6 different patients [1 with acute AD and 5 with chronic AD] for GeneChip study, 10 different patients for real-time PCR; some patients' skin biopsy specimens were used in both assays), 14 psoriasis patients (mean age, 39.7 years; skin involvement, 15% to 40%; skin biopsy specimens of chronic skin lesions were used from 7 different patients for GeneChip study, 11 different patient samples were used for real-time PCR, thus some patients' skin biopsy specimens were

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Abbreviations used

AD:	Atopic dermatitis
CCL:	CC chemokine ligand
CCR:	CC chemokine receptor
CTACK:	Cutaneous T-cell activating chemokine
CXCL:	CXC chemokine ligand
CXCR:	CXC chemokine receptor
GAPDH:	Glyceraldehyde-3-phosphate dehydrogenase
MCP-4:	Monocyte chemoattractant protein-4
MIP-1 β :	Macrophage inflammatory protein 1-beta
MIP-3 α :	Macrophage inflammatory protein 3-alpha
PARC:	Pulmonary and activation-regulated chemokine

used in both assays), and 6 different healthy individuals (mean age, 44.2 years; examined by real-time PCR) were included in this study. None of the patients had previously received any systemic immunosuppressive drugs such as corticosteroids or cyclosporine. All patients were off topical corticosteroids or calcineurin inhibitor for more than 1 week before their skin biopsy. Informed consent was obtained from all subjects before the study. The skin samples were immediately submerged in 1 mL of RNA Later (RNeasy protect mini kit, Qiagen, Valencia, Calif) and frozen at -80°C for future RNA isolation.

RNA extraction from skin biopsy specimens

Skin biopsy specimens, preserved in RNA Later, were put into 600 μL of Buffer RLT (Qiagen RNeasy mini-kit, Qiagen, Valencia, Calif) and homogenized with the use of a Pyrex tissue grinder. Total RNA was then extracted according to the manufacturer's protocol in the presence of DNase I (Qiagen).

DNA microarray analysis

Total RNA (5 μg) isolated from skin samples were individually converted to double-stranded cDNA (ds-cDNA) through the use of the Superscript Choice system (Life Technologies, Rockville, Md). An oligo-dT primer containing a T7 RNA polymerase promoter (Promega Corp, Madison, Wis) was used. After second-strand synthesis, the reaction mixture was extracted with phenol-chloroform-isoamyl alcohol and the ds-cDNA was recovered by ethanol precipitation. In vitro transcription and biotin labeling were then performed with an RNA transcript labeling kit (Enzo Biochem, Inc, New York, NY). A ds-cDNA template was transcribed in the presence of a mixture of unlabeled ATP, CTP, GTP, UTP, biotin-labeled CTP, and UTP (Enzo Biochem, Inc). Synthesized cRNA was fragmented in a fragmentation buffer at 94°C for 35 minutes. Samples were then resuspended in a hybridization solution containing 100 mmol/L 2-[N-Morpholino]ethanesulfonic acid (MES), 1 mol/L NaCl, and 20 mmol/L EDTA in the presence of 0.01% Tween 20. Each of the 6 AD samples and 7 psoriasis samples were loaded individually into respective (total, 13) human U-95Av2 GeneChip probe arrays (Affymetrix Inc, Santa Clara, Calif) and hybridized at 45°C for 16 hours. After washing and staining with streptavidin-phycoerythrin, the probe arrays were scanned with a Gene-array scanner (Hewlett Packard, Palo Alto, Calif).

The data were analyzed with MicroArray Suite Version 5.0 software (Affymetrix Inc, Santa Clara, Calif). Detailed protocols for data analysis of Affymetrix microarrays and extensive documentation of the sensitivity and quantitative aspects of the method have been described (References 12 through 14 and Affymetrix Microarray Suite, version 5.0, User's guide, 2001).¹²⁻¹⁴ Affymetrix software Microarray suite 5.0 was used for analysis of raw microarray data. Data are presented as mean $\pm$ SEM. Means of several groups were analyzed by using an unpaired 2-tailed *t* test and a matrix analysis of all possible combinations of AD samples to each of the psoriasis

samples. In this matrix analysis, a very stringent requirement was that more than 70% of the 42 possible comparisons (6 AD samples against 7 psoriasis samples) must show concordance in the "increase" or "decrease" call generated by the Affymetrix Microarray Suite 5.0 (MAS 5.0) algorithm. Genes called "increase" or "decrease" in more than 70% of comparisons and at least a 2-fold change were picked and grouped for statistical tests. Before analysis by any of the statistical tests, variability filters were applied to the data set to minimize multiple testing errors. The first filter used the Affymetrix mRNA detection "call" (MAS version 5.0) to exclude all genes with an "absolute call" or "absent" in all experiments. The second filter identified genes with intensity value smaller than 100 in all experiments. Intensity value of 100 was close to the background value for the array system in our settings. These preprocessing steps screened out genes with low variance and low levels of mRNA expression. The remainder of the genes was then fed to an unpaired 2-tailed *t* test, producing a final list of genes whose expression levels are significantly different between two conditions. A total of 12,000 genes were screened in AD versus psoriasis skin biopsy specimens. The unpaired 2-tailed *t* test analysis was considered significant when there was an associated probability level of $P < .05$.

Quantitative real-time PCR

Primers and probes for human CCL-4/MIP-1 β , CCL-18/PARC, CCL-27/CTACK, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Perkin-Elmer Applied Biosystems (Norwalk, Conn). Total RNA was isolated as described above. One microgram of RNA was reverse-transcribed in a 20- μL reaction containing Random Primers (Invitrogen, Carlsbad, Calif) and Superscript II enzyme (Invitrogen). Each PCR reaction system (50 μL) contained 1 μL of cDNA, 1 $\times$ gold buffer II, 4 mmol/L MgCl_2 , 500 $\mu\text{mol/L}$ dNTP, primers (200 nmol/L), 100 nmol/L probe (labeled with 6-carboxyfluorescein), and 1.25 U AmpliTaq Gold DNA polymerase (Perkin-Elmer Applied Biosystems, Norwalk, Conn). Real-time PCR was performed and analyzed by the dual-labeled fluorogenic probe method, using an ABI Prism 7700 sequence detector (Perkin-Elmer/Applied Biosystems). After amplification, the relative gene expression was determined for each sample. For each target gene, a threshold was set above the mean baseline value for fluorescence determined on the basis of the first 15 cycles. Amplification reactions in which the intensity of fluorescence exceeded the threshold were defined as positive reactions. The threshold cycle (*Ct*) is the PCR cycle at which an increase in reporter signal above the baseline signal can first be detected. A standard curve was then generated with the use of the fluorescence data from the 10-fold serial dilutions of total RNA from a sample that highly expressed that particular gene. This curve was used to calculate the relative amounts of target gene expression in test samples. Quantities of target gene expression in test samples were normalized to the corresponding GAPDH according to the manufacturer's instructions (Applied Biosystems; Foster City, Calif).

Statistical methods

Data are presented as mean $\pm$ SEM. Data were analyzed by unpaired 2-tailed *t* test (Tables I and II) or 1-way analysis of variance followed by the Scheffe *F* test for multiple comparisons (Fig 1). An associated probability (*P* value) of $< .05$ was considered significant.

RESULTS

In each GeneChip oligonucleotide microarray study, we included an analysis of GAPDH and β -actin as internal controls for our analysis of AD and psoriasis skin biopsy specimens. We verified the integrity of our RNA by checking hybridization intensity of 5', midportion,

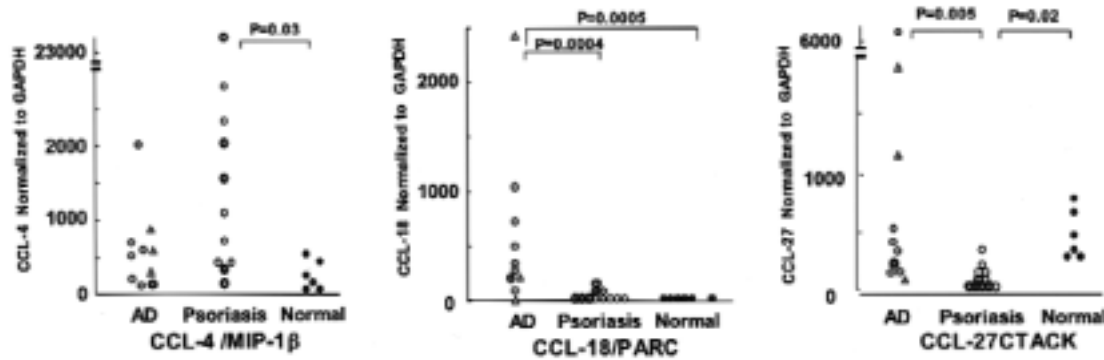


FIG 1. Real-time PCR of CCL-4/MIP-1 β , CCL-18/PARC, and CCL-27/CTACK in skin samples of AD, psoriasis, and healthy control subjects. In these experiments, selected chemokine genes were compared by real-time PCR in RNA samples isolated from skin biopsy specimens of AD (green), psoriasis patients (yellow), and normal donors (red). Acute lesions of AD are expressed as triangles and chronic samples are expressed as circles. Thick blue circled symbols are samples used both in GeneChip microarray and real-time PCR.

TABLE I. Genes with statistically significant, more than two-fold expression in atopic dermatitis as compared with psoriasis

Gene name	Probe set	GeneBank accession	Relative intensity (mean $\pm$ SEM)		P value	Ratio of AD: psoriasis gene expression
			AD	Psoriasis		
Nel-like 2	32598_at	D83018	2825 $\pm$ 491	374 $\pm$ 81	.004	7.56
CCL-18/PARC	32128_at	Y13710	3078 $\pm$ 874	422 $\pm$ 108	.028	7.30
CCL-27/CTACK	31644_at	AJ243542	5972 $\pm$ 1283	972 $\pm$ 326	.010	6.14
CCL-13/MCP-4	37454_at	AJ001634	1100 $\pm$ 327	203 $\pm$ 36	.041	5.41
Osteoblast specific factor 2	1451_s_at	D13666	2278 $\pm$ 436	463 $\pm$ 53	.009	4.92
Carbonic anhydrase II	40095_at	J03037	1074 $\pm$ 171	223 $\pm$ 31	.004	4.82
PPP1R5	39366_at	N36638	1095 $\pm$ 205	243 $\pm$ 30	.008	4.50
Tenascin precursor	32818_at	X78565	1951 $\pm$ 381	572 $\pm$ 95	.014	3.41
Cysteine-rich protein 1	33232_at	AI017574	9515 $\pm$ 1545	2939 $\pm$ 389	.007	3.24
PPP1R5	39364_s_at	Y18207	480 $\pm$ 87	167 $\pm$ 23	.014	2.88
Plasminogen activator inhibitor, type I	38125_at	M14083	305 $\pm$ 52	111 $\pm$ 18	.012	2.76
CD1a	34926_at	M28825	1246 $\pm$ 264	475 $\pm$ 58	.032	2.62
CD26 dipeptidylpeptidase IV	34823_at	X60708	207 $\pm$ 19	86 $\pm$ 19	.001	2.41
Lymphocyte-specific protein 1	36493_at	M33552	1210 $\pm$ 174	549 $\pm$ 68	.011	2.20
Cysteine-rich protein 2	35828_at	D42123	5314 $\pm$ 923	2450 $\pm$ 249	.026	2.17
AQP3 gene for aquaporine 3 (water channel)	39249_at	AB001325	16,912 $\pm$ 1418	8053 $\pm$ 1008	.001	2.10
Collagen VI α -1 C-terminal globular domain	38722_at	X15880	4872 $\pm$ 905	2369 $\pm$ 200	.039	2.06
N-myc downstream regulated gene 1	36933_at	D87953	5605 $\pm$ 770	2749 $\pm$ 213	.013	2.04

and 3' regions of GAPDH and β -actin gene. In all samples used in this study, hybridization intensity of these 3 portions were all the same, indicating that the integrity of the RNA used was intact (data not shown). Next, we focused on genes that demonstrated the greatest differences in hybridization signal intensity between AD and psoriasis skin lesions.

As shown in Table I, the expression of 18 genes were found to have a statistically significant, >2-fold increase in skin biopsy specimens from 6 different AD skin lesions as compared with skin lesions from 7 different patients with psoriasis. Notably, the gene encoding Nel-like 2 protein, a neural tissue-specific epidermal growth factor (EGF)-like repeat domain-containing protein,¹⁵ was most highly expressed (7.6-fold increase) in AD as

compared with psoriasis skin samples. The next greatest difference was observed for the CC chemokines¹⁶ CCL-18/PARC, CCL-27/CTACK, and CCL-13/MCP-4, which were increased in AD as compared with psoriasis skin. Other genes of known function that were significantly increased in AD as compared with psoriasis skin lesions were Tenascin C, an extracellular matrix molecule, which contributes to repair and remodeling events in allergic inflammation¹⁷; plasminogen activator inhibitor 1, which plays an essential role in tissue remodeling and has been previously reported to be increased in bronchi of asthma patients¹⁸; and the CD1a dendritic cell marker.

Table II shows 62 genes that had a statistically significant >2-fold expression in psoriasis skin lesions as compared with AD skin lesions. These genes included small

TABLE II. Gene with more than two-fold expression and with statistical significant difference in psoriasis as compared with atopic dermatitis

Gene name	Probe set	GeneBank accession	Relative intensity (mean $\pm$ SEM)		P value	Ratio of psoriasis: AD gene expression
			AD	Psoriasis		
A.						
Small proline-rich protein 2C, keratinocyte differentiation markers	36242_at	M21539	446 $\pm$ 104	4903 $\pm$ 1168	.009	11.0
Transcobalamin I neutrophil granule protein	35919_at	J05068	210 $\pm$ 32	2206 $\pm$ 385	.002	10.5
CXCL-8/IL-8	35372_r_at	M17017	63 $\pm$ 12	600 $\pm$ 210	.044	9.5
Lipocalin 2, α -2-microglobulin-related subunit of MMP-9	32821_at	AI762213	589 $\pm$ 158	4739 $\pm$ 1046	.007	8.0
ATPase, H+/K+ transporting, nongastric, α - polypeptide, found in skin and kidney	32482_at	L42563	268 $\pm$ 60	1983 $\pm$ 523	.017	7.4
Kallikrein 6	37554_at	U62801	1182 $\pm$ 234	8621 $\pm$ 2778	.037	7.3
Airway trypsin-like protease	31345_at	AB002134	81 $\pm$ 25	459 $\pm$ 78	.002	5.7
Paraoxonase 3	41424_at	L48516	149 $\pm$ 41	835 $\pm$ 222	.021	5.6
Kynureninase	40671_g_at	AI148772	158 $\pm$ 28	804 $\pm$ 143	.004	5.1
iNOS	1948_f_at	U31511	65 $\pm$ 9	299 $\pm$ 71	.016	4.7
Kallikrein 13	36406_at	AA401397	677 $\pm$ 117	3118 $\pm$ 854	.029	4.6
Serum amyloid A1	36674_at	J04130	378 $\pm$ 146	1516 $\pm$ 296	.008	4.0
CCL-4/MIP-1 β	36103_at	D90144	47 $\pm$ 20	187 $\pm$ 51	.035	4.0
Deoxyribonuclease I-like 2	31704_at	U62647	458 $\pm$ 158	1716 $\pm$ 501	.047	3.7
Aldehyde dehydrogenase 5 family, member A1	41790_at	AL031230	128 $\pm$ 27	455 $\pm$ 59	.001	3.6
ATPase, Na+/K+ transporting, beta 1 polypeptide	37669_s_at	U16799	1936 $\pm$ 337	6852 $\pm$ 575	.001	3.5
HBD2	32464_at	AF071216	13,312 $\pm$ 4264	45,863 $\pm$ 4177	.0002	3.4
Protease inhibitor 3, skin-derived (SKALP)	41469_at	L10343	10,176 $\pm$ 2677	33,397 $\pm$ 4219	.001	3.3
Arginase	1962_at	M14502	1576.9 $\pm$ 466	5065 $\pm$ 787	.004	3.2
S100 calcium binding protein A12	38879_at	D83664	168 $\pm$ 33	532 $\pm$ 84	.004	3.2

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proline-rich protein 2C, which is a component of the cornified cell envelope and has a protective skin barrier function^{19,20} and showed the largest (11-fold) increase in psoriasis skin lesions as compared with AD. Lipocalin 2, a marker for dysregulated keratinocyte differentiation in human skin^{21,22} and airway trypsin-like protease, a molecule thought to play a role in host defense,²³ was also increased in psoriasis as compared with AD skin. Protease inhibitor 3 (Elafin) is an elastase-specific inhibitor with antimicrobial activity^{24,25}; it was increased in psoriasis as compared with AD as well as a number of other molecules listed in Table II.

The clearest differences between AD versus psoriasis skin lesions, with obvious links to differences in their pathophysiology, involved chemokine gene expression. As shown in Table I, chemokines that can attract inflammatory cells found in AD skin histology are increased in AD skin. Also, chemokines that can attract inflammatory cells participating in psoriasis skin histology are increased in psoriasis skin (Table II). Statistically significant increases with >2-fold increases in gene expression in psoriasis as compared with AD skin lesions included CCL-4/MIP-1 β , CCL-20/MIP-3 α , and CXCL-2/GRO- β . The increased expression of CXCL-8/IL-8, CXCR2/IL-8R, iNOS, and HBD-2 has previously been reported in Reference 26 as part of a gene microarray analysis of defective innate immune responses in AD as compared with psoriasis and is included in Table II to provide a

complete picture of significant differences in gene expression patterns between these two skin diseases.²⁶

The gene expression changes of CCL-4/MIP-1 β , hCCL-18/PARC, and CCL-27/CTACK in the skin lesions of patients with AD versus patients with psoriasis have not been previously reported. To confirm the validity of our GeneChip data for these novel genes identified in the current study, we performed real-time PCR for these genes in the skin samples of AD, psoriasis, and normal control subjects (Fig 1). Consistent with the GeneChip data, we found by real-time PCR statistically significant increases in gene expression of CCL-18/PARC in AD as compared with psoriasis skin lesions and normal skin. CCL-27/CTACK showed a unique pattern of expression, as it was significantly decreased in psoriasis skin samples compared with AD ($P = .005$) and normal skin samples ($P = .02$). In contrast, expression levels of CCL-4/MIP-1 β in psoriasis skin samples but not in AD skin were significantly higher than that of normal control subjects ($P = .006$).

DISCUSSION

The current study shows for the first time the distinctive pattern of gene expression that characterizes AD versus psoriasis skin lesions and provides a potential "signature" for identification of these two skin diseases. Although it is well established that AD is associated with

TABLE II. Continued

Gene name	Probe set	GeneBank accession	Relative intensity (mean $\pm$ SEM)		P value	Ratio of psoriasis: AD gene expression
			AD	Psoriasis		
B.						
Arylsulphatase	35056_at	X97868	218 $\pm$ 60	688 $\pm$ 99	.002	3.2
17- β -Hydroxysteroid dehydrogenase	38178_at	L40802	80 $\pm$ 27	251 $\pm$ 42	.006	3.1
Promyelocytic leukemia zinc finger protein	39681_at	AF060568	53 $\pm$ 17	156 $\pm$ 35	.028	3.0
CXCL-2/GRO β	37187_at	M36820	50 $\pm$ 11	143 $\pm$ 38	.050	2.9
L-kynurenine hydrolase	40672_at	U57721	343 $\pm$ 49	978 $\pm$ 90	.001	2.8
Tissue-type plasminogen activator	33452_at	M15518	231 $\pm$ 55	636 $\pm$ 129	.020	2.8
G0S2 protein	38326_at	M69199	405 $\pm$ 99	1109 $\pm$ 281	.049	2.7
Flavin-containing monooxygenase	31806_at	M64082	55 $\pm$ 19	149 $\pm$ 29	.021	2.7
CCL-20/MIP-3 α	40385_at	U64197	54 $\pm$ 11	143 $\pm$ 31	.030	2.7
GM2 ganglioside activator protein	35820_at	X62078	2484 $\pm$ 117	6534 $\pm$ 860	.003	2.6
Pre-B-cell colony-enhancing factor	33849_at	U02020	194 $\pm$ 19	507 $\pm$ 74	.005	2.6
GM2 ganglioside activator protein	33646_g_at	X61094	333 $\pm$ 27	860 $\pm$ 99	.001	2.6
Arcinoembryonic antigen-related cell adhesion molecule	1582_at	M29540	88 $\pm$ 9	226 $\pm$ 35	.007	2.6
3-Hydroxy-3-methylglutaryl-coenzyme A synthase 1	34517_at	X66435	85 $\pm$ 18	219 $\pm$ 41	.018	2.6
Thyroid receptor interactor	269_at	L40387	305 $\pm$ 51	742 $\pm$ 160	.035	2.4
Aldehyde dehydrogenase 6	36686_at	U07919	165 $\pm$ 30	401 $\pm$ 91	.043	2.4
Peptide transporter	32906_at	U21936	96 $\pm$ 9	226 $\pm$ 31	.005	2.4
Paraoxonase 2	40504_at	AF001601	350 $\pm$ 15	816 $\pm$ 86	.001	2.3
Carcinoembryonic antigen BGPa	988_at	X16354	66 $\pm$ 6	152 $\pm$ 31	.032	2.3
5-Aminolevulinate synthase	37674_at	Y00451	572 $\pm$ 67	1295 $\pm$ 208	.012	2.3
C.						
DKFZp586M0723	39616_at	AL050227	370 $\pm$ 78	819 $\pm$ 43	.001	2.2
Keratinocyte transglutaminase gene	35947_at	M98447	1650 $\pm$ 304	3577 $\pm$ 470	.006	2.2
Pirin	35724_at	Y07867	118 $\pm$ 12	255 $\pm$ 19	.001	2.2
LETKI precursor	40315_at	AJ228139	2666 $\pm$ 266	5748 $\pm$ 593	.001	2.2
Squamous cell carcinoma antigen	1549_s_at	U19557	11,206 $\pm$ 2102	24,090 $\pm$ 2494	.002	2.1
SWI/SNF complex 60 KDa subunit	32565_at	U66619	229 $\pm$ 23	491 $\pm$ 85	.021	2.1
G-protein-coupled receptor	649_s_at	L06797	219 $\pm$ 19	467 $\pm$ 81	.022	2.1
Protein-tyrosine kinase EPHB2v (EPHB2)	41678_at	AF025304	177 $\pm$ 21	375 $\pm$ 29	.001	2.1
Malic enzyme 1	31824_at	AL049699	199 $\pm$ 25	419 $\pm$ 73	.023	2.1
Small proline-rich protein	36734_at	M21302	16,149 $\pm$ 4040	33,693 $\pm$ 3226	.007	2.1
Peroxisome proliferator activated receptor- γ	37104_at	L40904	68 $\pm$ 11	142 $\pm$ 17	.004	2.1
Metastasis-associated gene	1348_s_at	S79219	152 $\pm$ 14	315 $\pm$ 23	.001	2.1
Squamous cell carcinoma antigen-serine protease inhibitor	1343_s_at	S66896	3384 $\pm$ 293	6972 $\pm$ 675	.001	2.1
Glutamine synthase	40522_at	X59834	1283 $\pm$ 137	2643 $\pm$ 312	.004	2.1
Small GTP-binding protein	809_at	U57094	169 $\pm$ 18	347 $\pm$ 40	.004	2.1
B-cell activation gene	36575_at	S59049	75 $\pm$ 18	153 $\pm$ 23	.023	2.1
CXCR4	649_s_a	L06797	219 $\pm$ 19	467 $\pm$ 81	.022	2.1
CXCR-2/IL-8R	664_at	L19593	62 $\pm$ 9	122 $\pm$ 12	.002	2.0
12R-lipoxygenase	33029_at	AF038461	3198 $\pm$ 462	6529 $\pm$ 841	.007	2.0
Monoamine oxidase A	41772_at	M68840	235 $\pm$ 32	477 $\pm$ 73	.017	2.0
Normal epithelial cell-specific 1 (NES1)	36838_at	AF055481	1662 $\pm$ 224	3363 $\pm$ 443	.008	2.0
Caspase-like apoptosis regulatory protein 2	1867_at	AF005775	213 $\pm$ 14	429 $\pm$ 66	.017	2.0

Dermatologic and ocular diseases

the infiltration of T_H2 cells and eosinophils whereas psoriasis is associated with the infiltration of T_H1 cells and neutrophils, the events leading to these two distinct histologic pictures are not completely understood. The clearest picture in our GeneChip microarray studies emerged from an examination of the cluster of chemokine genes activated in these two skin diseases.

In AD skin lesions, we found a remarkable increase in the expression of a group of genes encoding CC chemokines, which are known to attract T_H2 cells and eosinophils, whereas psoriasis was associated with

increased expression of CXC chemokines, which are known to attract T_H1 cells and neutrophils.¹⁶ There have been several reports about chemokines and AD or psoriasis skin lesions. These include increased expression of CCL-11/eotaxin, CCL-13/MCP-4, and CCL-17/TARC in AD.^{6,8,10} Adding to these previous reports, we made the novel observation of increased gene expression of CCL-18/PARC, C, and CCL-27/CTACK in AD skin samples as well as increased gene expression of CCL-4/MIP-1 β CXCL-8/IL-8, CCL-20/MIP-3 α , and CXCL-2/GRO β in psoriasis skin samples in the current study.

The CCchemokines observed to be increased in AD are known to have distinct but overlapping properties that augment the T_H2 response and infiltration of eosinophils, basophils, and mast cells. CCL-11/eotaxin and CCL-13/MCP-4 attract CCR-3-bearing eosinophils, basophils, and mast cells.^{6,27} CCL-18/PARC and CCL-27/CTACK have not been established to have action for T_H1 or T_H2 cell chemoattraction. But both were highly upregulated in AD skin lesions compared with psoriasis skin. Also in real-time PCR, CCL-18/PARC expression level showed higher expression in AD skin compared with psoriasis and normal control skin. The receptor of CCL-18/PARC is not known yet, but this chemokine attracts both CD4- and CD8-positive naive T cells.^{28,29}

CCL-27/CTACK has a specific receptor, CCR-10, which attracts cutaneous lymphocyte antigen (CLA)-positive skin homing T cells.^{30,31} Expression of CCL-27/CTACK has been reported to be constitutive and found in normal skin, AD, and psoriasis skin.³² However, in our current study, not only GeneChip data but also real-time PCR of CCL-27/CTACK showed significantly lower expression in psoriasis skin compared with AD and normal control skin samples. This is consistent with previous observations that AD is associated with significant elevations of serum CCL27.³³ Since normal skin samples showed significant expression of CCL-27/CTACK, some factors in psoriasis skin may suppress induction of this molecule. Further investigation is needed to investigate the role of differential CCL-27/CTACK modulation in AD versus psoriasis. A recent study reporting that inhibition of CLA/E-selectin interactions prevents CCR4-mediated recruitment of human T_H2 memory cells to human skin in vivo, however, suggests a potentially important interaction between CCL-27/CTACK and CLA in AD.³⁴

In the current study, we also found certain chemokines to be increased in psoriasis as compared with AD skin (Table II). These included CCL-4/MIP-1 β , which attract CCR-1-bearing or CCR-5-bearing T_H1 cells, immature DC, monocytes, and NK cells.^{35,36} We also found increased gene expression of CCL-20/MIP-3 α , which attracts CCR-6-bearing T_H1 cells,³⁷ as well as CXCL-8/IL-8 and CXCL-2/GRO β , which attracts neutrophils.¹³ Thus, increased expression of these chemokines in psoriasis skin may account for the infiltration of T_H1 cells and neutrophils in this skin condition.

The mechanisms for upregulation of these distinct groups of chemokines in AD versus psoriasis skin requires further investigation. However, several studies suggest that CCchemokines are increased by IL-4 and IL-13.³⁸⁻⁴¹ In AD skin, it has been previously reported that T_H2 cytokines, for example, IL-4, IL-5, IL-10, and IL-13, are increased.⁴²⁻⁴⁴ Thus, these cytokines may contribute to increased gene expression of these chemokines. In contrast, CXCL-2/GRO β and CCL-20/MIP-3 α are known to be induced by T_H1 cytokines.⁴¹ Importantly, psoriasis skin is associated with a marked upregulation of T_H1 cytokines.⁴ Thus, the dichotomy of cytokine and

chemokine profiles found in AD versus psoriasis might be key factors in controlling chronic skin inflammation.

The potential importance of the differences in gene expression described in our current study is supported by the observation that expression of most of the more than 12,000 genes examined were similar in AD and psoriasis (data not shown). In addition to the differences in expression of inflammatory and chemokine genes, other genes of potential pathophysiologic importance included increased expression of the gene encoding Nel-like 2 protein, which showed the greatest increase in AD skin samples compared with psoriasis skin samples ($P = .004$). This gene is thought to act as a growth factor of sensory nerves in the skin and could result in increased sensitivity to itch in AD skin,¹⁵ although further studies are needed to address this point. The finding of increased gene expression of Tenascin C, an extracellular matrix molecule and plasminogen activator inhibitor 1 in AD skin, may reflect repair and remodeling events in response to allergic inflammation, as both have been previously reported to be increased in bronchi of asthmatic patients.^{17,18}

On the other hand, a number of gene expression changes in psoriasis reflected dysregulation in keratinocyte differentiation. Small proline-rich protein 2C, which is necessary for the formation of the cornified cell envelope replacing the plasma membrane by cross-linkage of these proteins,^{19,20} showed an 11-fold-increased expression in psoriasis as compared with AD. Lipocalin 2, another marker for dysregulated keratinocyte differentiation in human skin,^{21,22} was increased in psoriasis skin compared with AD skin. Airway trypsin-like protease, which is thought to play a role in host defense,²³ was also increased in psoriasis skin. Protease inhibitor 3 (Elafin), a secretory protein cross-linked into the cornified cell envelopes from inside of psoriatic keratinocytes,^{24,25} was found to be increased in psoriasis as compared with AD.

In conclusion, the current study has confirmed and extended previous findings accounting for the distinct inflammatory events in AD versus psoriasis. The identification of these genes may lead to new lines of investigation into the pathophysiology not only of skin inflammation but mechanisms of itching and skin remodeling in AD as well as keratinocyte differentiation in psoriasis. In addition to the differences in gene expression pattern described here, we have also reported elsewhere that AD as compared with psoriasis skin lesions express significantly lower levels of antimicrobial peptides (human β -defensins and LL-37) and other innate immune effector molecules.^{26,45} The latter observation may explain the increased susceptibility of patients with AD toward recurrent skin infections. Of more immediate importance is that AD remains a clinical diagnosis with many clinical features that overlap with other skin diseases. It is hoped that by identifying distinct patterns of gene expression in AD, a more objective laboratory-based diagnosis of AD and new therapeutic targets for this common allergic skin disease will become available.

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