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Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array

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Zhou, Xianghong, James G. Krueger, Ming-Chih J. Kao, Ed Lee, Fenghe Du, Alan Menter, Wing Hung Wong, Anne M. Bowcock. Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. *Physiol Genomics* 13: 69–78, 2003; 10.1152/physiolgenomics.00157.2002.—A global picture of gene expression in the common immune-mediated skin disease, psoriasis, was obtained by interrogating the full set of Affymetrix GeneChips with psoriatic and control skin samples. We identified 1,338 genes with potential roles in psoriasis pathogenesis/maintenance and revealed many perturbed biological processes. A novel method for identifying transcription factor binding sites was also developed and applied to this dataset. Many of the identified sites are known to be involved in immune response and proliferation. An in-depth study of immune system genes revealed the presence of many regulating cytokines and chemokines within involved skin, and markers of dendritic cell (DC) activation in uninvolved skin. The combination of many CCR7+ T cells, DCs, and regulating chemokines in psoriatic lesions, together with the detection of DC activation markers in nonlesional skin, strongly suggests that the spatial organization of T cells and DCs could sustain chronic T-cell activation and persistence within focal skin regions.

immune signaling; promoter analysis; chemokines; gene expression

PSORIASIS AFFECTS ~2% of the human population of European descent. It is a T-cell, immune-mediated dermatosis characterized by hyperproliferative keratinocytes producing psoriatic lesions with clinical features of erythema, induration, and scaling. Psoriatic arthritis is present in over 10% of psoriasis patients. Psoriatic lesions commonly involve the scalp, elbows, knees, and other sites of repetitive trauma. Throat streptococcal infections are the most common trigger of the onset or exacerbation of psoriasis (6, 26). In addition, it can be triggered by a number of different agents including drugs, as well as bacterial and fungal infections of skin (22, 25, 32).

Both genetic and environmental triggers have been proposed to be responsible for the development of psoriasis. A number of genetic loci have been implicated in its etiology. A major susceptibility locus is the HLA class I region on chromosome 6p21.3. Other loci located on chromosomes 1p35-p34, 1q21, 2p, 3q21, 4qter, 7, 8q, 14q31-q32, 15q, 16q, 17q24-q25, 19p13.3, and 20p have also been proposed on the basis of genome-wide linkage scans (7, 11).

A study of changes in gene expression in psoriasis complements genetic findings and may provide mechanisms for the downstream consequences of genetic alterations and environmental triggers. We recently described comprehensive studies of gene expression changes using the ~7,000-element oligonucleotide array HU6800 (34) and the ~12,600-element array U95A (10). In the current study we extended our analysis to the entire set of ~63,100 Affymetrix gene probes on the U95A, B, C, D, and E arrays. To examine the complex pathophysiology that underlies psoriasis, we exhaustively surveyed the biological processes that are statistically significantly perturbed and performed an in-depth study of genes of the immune system. This led to the identification of chemokines that have not previously been implicated in this disease and of a mechanism for sustaining T-cell activation and chronic inflammation in psoriatic skin lesions through dendritic cell (DC) effects and direct or indirect effects on secondary lymphoid tissue. Finally, we integrated expression and sequence data to identify putative transcription factors for coexpressed genes involved in psoriasis.

METHODS

Samples, Arrays, and Selection of Differentially Expressed Genes

Most of the samples used in this study and target preparation and hybridization are described elsewhere (10). Additional samples used in this study were involved and uninvolved skin from *patient PS1* (I1/U1), involved skin from *patient PS22* (I22), and uninvolved skin from *patients PS04* and *PS23* (U4, U23). Normal skin from two additional indi-

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viduals was also included: *patients N2* (female) and *N14* (male). The mRNAs of these samples were interrogated with the Affymetrix U95A–E arrays. GeneChip 3.2 software (Affymetrix) was used to scan the images. The expression levels of 63,100 probe sets were computed and normalized with the model-based approach implemented in the dChip software (29).

Transcripts were defined to be differentially expressed in two given groups based on the following criteria: *1*) >2-fold change in the means of the expression levels in two groups; *2*) *t*-test *P* value <0.05; *3*) the maximum of groupwise mean expression levels greater than 200, i.e., the genes are significantly expressed in at least one group; and *4*) the sum of the presence-call fractions of all three groups >0.8. To further validate differential expression of the identified genes, we performed permutation *t*-tests (available in the statistical package R at **http://www.r-project.org**). In addition, we performed K-means clustering to identify differentially expressed transcripts to discover those that could not be picked up in the above pairwise comparison analysis due to small fold changes (<2.0). To select transcripts with significant expression variability for K-means clustering, we used the following criteria: *1*) the ratio of standard deviation over the average of expression levels over all samples >0.1, *2*) genes are significantly expressed (≥1,000) in at least 20% of the samples, and *3*) the difference between the maximum expression level and the minimum expression level was ≥500. We were left with 19,269 transcripts, which were then placed into 100 clusters with Eisen's K-means clustering software (14).

TaqMan RT-PCR

Primers and probes specific to IL1HY1 and IL1H1 were designed with PrimerExpress (Perkin-Elmer). Ten nanograms of RNA of each gene was subjected to a RT-PCR reaction using TaqMan chemistry and a model 7700 sequence detector (Perkin-Elmer). Gene expression was quantified by computing the threshold cycle (Ct) at which amplification became linear, as determined by a program of the 7700 sequence detector. Expression of both genes was normalized to expression of human acidic ribosomal protein (HARP) mRNA, a housekeeping gene, that was coamplified from an aliquot of each sample.

Promoter Analysis

To discover transcription factor binding sites (TFBS) among genes under common transcriptional control, we first generated gene sets that were sufficiently homogenous in their expression patterns. We started with the gene clusters resulting either from the K-means clustering or from the pairwise comparison among sample groups. We refined those clusters by a constrained recursive K-means clustering to identify tight expression clusters. Here, a cluster is defined as a set of genes satisfying the following: *1*) the number of genes is less than 15 and more than 3 and *2*) the homogeneity of expression, defined as the average distance between all gene pairs based on normalized expression vectors, should be less than 3. At each step of the constrained recursive K-means clustering, we separated genes into k clusters. Because an ideal cluster for our purpose should contain 5–10 genes, we set k = no. genes/7. Each of the clusters that satisfied the given homogeneity threshold, and that contained more than γ genes and less than λ genes (thresholds here predefined as $\gamma = 3$ and $\lambda = 15$), was selected for the investigation of enrichment of particular TFBSs. For the remaining genes, we further applied the same algorithm recursively. Because of the heuristic nature of the algorithm, we applied this recursive scheme multiple times to select all tight expression clusters.

For each tight expression cluster, we scanned 1,100 bp of the 5′ flanking sequence of each gene (1,000 bp upstream and 100 bp downstream of transcription start site) using MatInspector (**http://www.genomatix.de**) to identify TFBS motifs, defined as either individual TFBSs or paired TFBSs with fixed order and with a distance of 20–80 bp. We use the hypergeometric distribution to assess the statistical significance of the enrichment of all identified motifs against their respective occurrences over the upstream sequences of all genes on the U95A–E arrays. We derived one-tail *P* values for overrepresentation and select only those motifs with $P < 0.02$. Since TFBS motifs are likely to be conserved between the human and the mouse genomes, the presences of the identified motifs in the upstream regions of their corresponding mouse orthologs were determined if available. We identified mouse orthologs using The Institute for Genomic Research (TIGR) Orthologous Gene Alignments (**http://www.tigr.org**). The upstream sequences for mouse genes were obtained from the Celera mouse genome (**http://www.celera.com**).

FACs Analysis

Leukocytes were obtained from explant cultures of split-thickness biopsies of lesional psoriasis skin in patients not undergoing active treatment according to methods described by Ferenczi et al. (15). Four-color flow cytometry analysis of T lymphocytes was performed by gating on CD4+, CD8+, or CD3+ lymphocytes (PERCP-conjugated antibodies, BD Pharmingen). Additional antibodies were FITC-conjugated CD62L, FITC-CLA (HECA452), APC-CD45RA (BD Pharmingen), or PE-CCR7 (R & D Systems).

RESULTS AND DISCUSSION

Summary of Differentially Expressed Genes

RNA from the psoriatic involved/uninvolved skin of 16 patients and 8 controls was used to interrogate the 63,100-element Affymetrix array U95A–E. We used two methods to select transcripts that were differentially expressed among the psoriasis-involved, psoriasis-uninvolved, and normal skin groups. Our first method for identifying differentially expressed genes was based on a combination of fold changes (>2.0) and *t*-tests ($P < 0.05$ in both the Student's *t*-test and a permutation test) and assessed whether the means of the gene expression in any two groups were significantly different. We identified 612 genes that were differentially expressed between involved vs. uninvolved samples, 710 between involved vs. normal samples, and 205 between uninvolved vs. normal samples. To select differentially expressed transcripts that could not be picked up in the above pairwise comparison analysis due to small fold changes (<2.0), we also performed K-means clustering. Out of 100 clusters, we identified 5 in which the transcripts showed higher (4 clusters) or lower (1 cluster) mean expression levels in involved skin than in both uninvolved and normal skin. These 5 clusters include 438 genes, of which 179 were not identified previously with the stringent pairwise mean expression comparison.

In total, we identified 1,338 genes that were differentially expressed among psoriasis-involved, psoriasis-uninvolved, and normal tissues, for which the overlapping and non-overlapping expression changes among the 3 sample groups are illustrated in Fig. 1. All identified genes and their related information can be found on our web site (**http://hg.wustl.edu**). Among them, 600 transcripts were differentially expressed in involved vs. uninvolved/normal skin. These changes are likely to be secondary to the initiating events. All 49 genes differentially expressed in involved/uninvolved samples vs. the controls were upregulated in disease samples, and more than half of them have not been described before. The following seven genes showed fold changes of 5 or more when involved samples were compared with normal samples: FLJ21763, TRIM22, IFI27, S100A7, BAL, FLJ23153, and STAT1. All seven genes showed progressive upregulation from normal to uninvolved skin (≥2.0-fold) and from uninvolved to involved skin (≥2.0-fold).

A breakdown of the three sample group comparisons according to annotated gene function from the Gene-Ontology (GO) database is shown in Fig. 2 and illustrates the wide range of biological processes in psoriasis. When involved skin is compared with that from normal individuals, strongly perturbed biological processes ($P < 0.05$) include the immune and inflammatory responses, response to wounding, response to pest/pathogen, cell proliferation, the JAK-STAT signaling cascade, and cell growth and/or maintenance. Several metabolic processes linked to immune or inflammatory responses, such as nitric oxide biosynthesis, arginine metabolism, and leukotriene metabolism, were also activated. A significant portion of the identified genes in the super-category "developmental processes" belongs to epidermal differentiation or neurogenesis. Epidermal differentiation is the only process consistently altered in comparisons of involved vs. normal as well as uninvolved vs. normal skin. Others that were significantly perturbed in only uninvolved vs. normal skin comparisons included melanin biosynthesis, exocytosis, and cell organization and biogenesis. These differences observed in the uninvolved skin of psoriasis patients may have been triggered by circulating cytokines or may reflect underlying genetic alterations in patients. This will only be resolved once genetic risk factors for psoriasis and their corresponding biochemical pathways are identified.

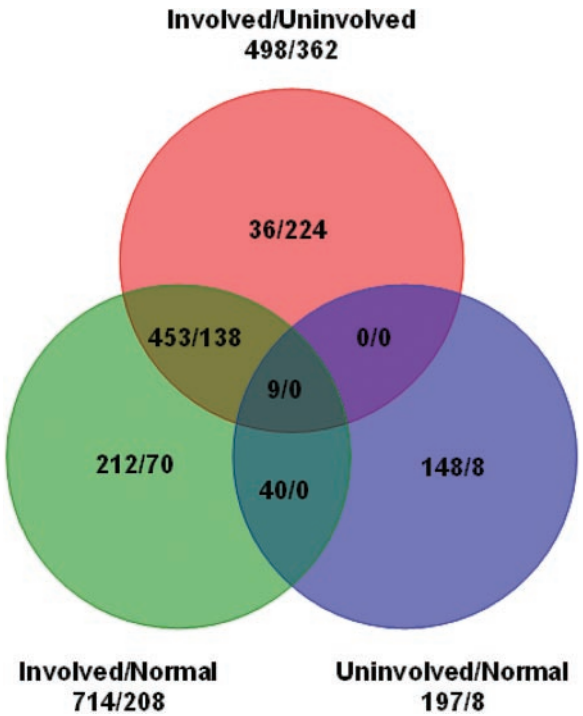
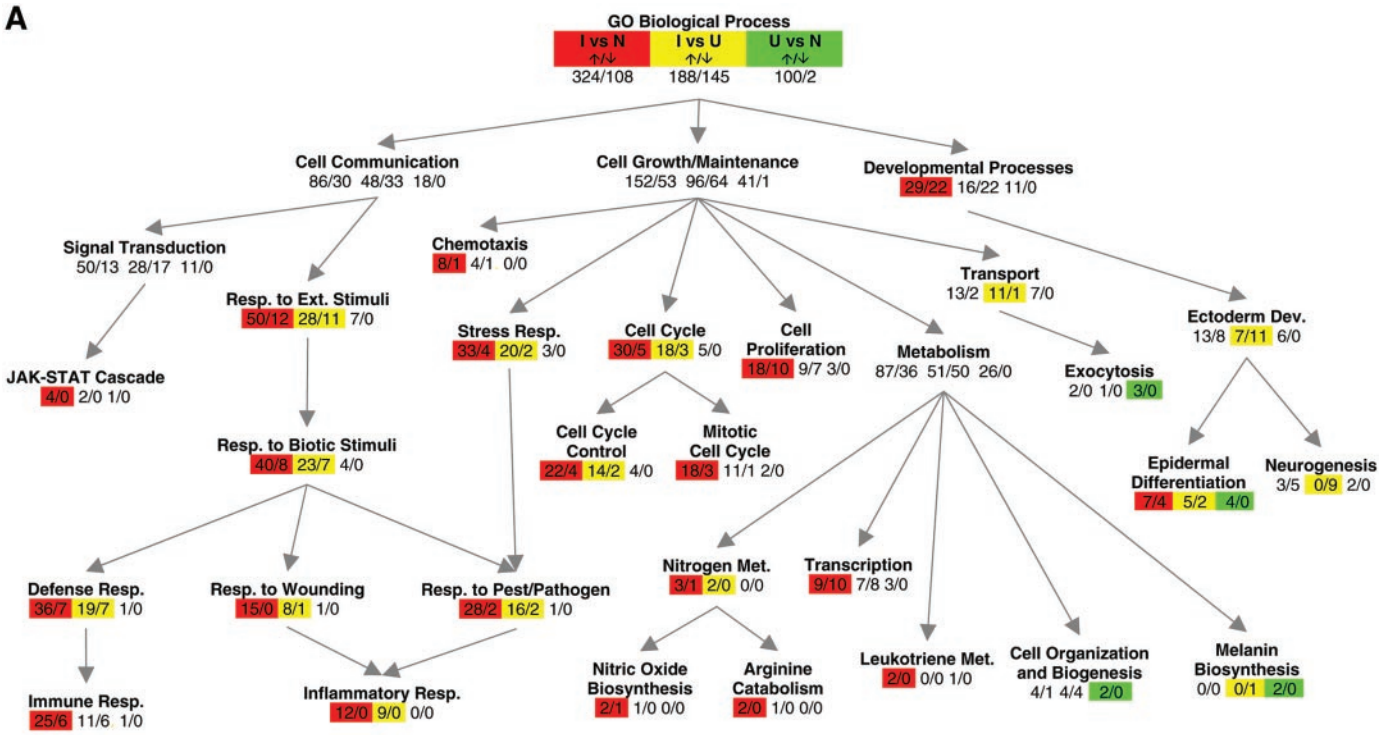


Fig. 1. Venn diagram representing the overlapping and non-overlapping expression alteration among the 3 skin groups. The diagram shows the number of genes (number of upregulated genes/number of downregulated genes) with the indicated expression patterns.

Alterations in Immune Signaling in Psoriasis

Components of immune signaling cascades, such as adhesion receptors, cytokines, chemokines, their receptors, and immune-regulated transcription factors, have been shown to play fundamental roles in the pathogenesis of psoriasis. From current GO annotations and lists compiled from the literature, we identified 131 genes involved in immune signaling that showed mean expression fold changes >1.2 with P values <0.05 between any 2 of the 3 sample groups. We clustered them into groups based on their expression profiles (Fig. 3) to identify those with potentially cooperative roles.

Interleukin-1 cluster of genes. Our expression studies suggest complex interactions between interleukin-1 (IL-1) gene family products in psoriasis. Prior studies have described elevated expression of IL1RA, in involved skin (27). IL-1 receptor activation is also implied by our prior Affymetrix study, in which we observed elevated expression of the IL-1 receptor associated kinase, IRAK1 (34). In the current study we also observed increased expression of IRAK2. In the hierarchical clustering tree, one prominent cluster of genes that were upregulated in involved vs. normal skin included three IL-1 receptor antagonists: IL1RN, IL1HY1, and the putative IL-1 receptor antagonist IL1H1 (fold changes of 2.3, 21.4, and 6.2, respectively). Only IL1RN has previously been reported to be upregulated in psoriasis (23). To confirm these findings, we performed real-time (TaqMan) RT-PCR on both IL1HY1 and IL1H1 mRNA and obtained mean expression fold changes between involved and normal skin of 16.4 and 11.0, respectively. We also observed high concordance between the microarray data and the RT-PCR measurements across samples, with Pearson's correlation coefficient 0.93 for IL1H1 and 0.92 for IL1HY1. This suggests that our microarray study accurately gauged differential expression and confirms the involvement of these two IL-1 receptor antagonists in psoriasis. Several IL1R agonists, such as IL-1α and IL-1β, were also previously documented to show expression alterations in psoriasis (23). In our study,



B

Process	I vs. N	I vs. U	U vs. N	Genes
Arg. Cat.	↑	↑		ARG1
	↑			ARG2
Cell Organization and Biogenesis	↑	↑		ECGF1, SMC4L1
	↑			KNSL1, PRC1
		↑		CSPG6, TOP1
			↑	DNM1L, LOC56993
	↓	↓		PXF
		↓		CBX4, PEX11A, SCA7
Cell Proliferation	↑	↑		CDKN2B, CKS2, HBP17, IL8, IL8RB, KRT16, LAMP3, MAD, PBEF
	↑			AREG, CDK5R1, GSPT1, IFITM1, MCT-1, MYC, PCNA, SEI1, UHRF1
			↑	CTBP2, DAB2, MKPX
	↓	↓		BTC, CRIP1, CTNNBIP1, FABP7, INSIG1
	↓			CDKN1C, GNRH1, LMO1, RARRES1, SFN
		↓		IRS2, SCYA14
Epidermal Differentiation	↑	↑	↑	S100A7
	↑	↑		ALOX12B, FABP5, KRT16, SPRR1B
	↑		↑	DSP
	↑			ATP2A2
			↑	CTGF
		↓	↑	DCT
	↓	↓		ALDH3A2
	↓			KRT15, LAMC2, SCEL
Exocytosis	↑	↑		LMAN1
	↑		↑	SNAP23
			↑	PRO1912, SEDL
Immune Resp.	↑	↑	↑	TRIM22
	↑	↑		CD24, DEFB2, FCGR3B, IL4R, IL7R, IL8RB, RGS1, SCYA20, SERPINA3
	↑			ARHGDIB, C1QB, C1R, CCR1, CD53, CD83, CTSC, LGALS3BP, MICB, MNDA, PX19, SCYA18, SCYA2, STAT3, TLR2
		↑		EBI2
	↓	↓		APOE, AQP9, CRIP1, F3, SCYA27
	↓			ALCAM
		↓		ITGB1
Inflamm. Resp.	↑	↑		AOX1, CRHSP-24, HPSE, IL8, IL8RB, NMI, S100A9, SCYA20
	↑			ALOX5AP, CCR1, S100A8, SCYA2
		↑		S100A12
JAK-STAT Cascade	↑	↑	↑	STAT1
	↑	↑		NMI
	↑			SCYA2, STAT3
Leukotriene Met.	↑		↑	CLP
	↑			ALOX5AP
Melanin Biosyn.		↓	↑	DCT
			↑	TYR
Mitotic Cell Cycle	↑	↑		ANLN, CDKN2B, CKS2, ECGF1, HEC, MAD2L1, MPHOSPH6, SMC4L1, ZRF1
	↑		↑	BCCIP
	↑			CDK5R1, GSPT1, KNSL1, KPNA2, PCNA, PRC1, REV1L, SEI1
		↑		CCNE2, CSPG6
			↑	SGT1
	↓	↓		CCND1
	↓			CDKN1C, CHES1
Neurogenesis	↑			GDA, OLFM1, STAT3
			↑	MAFB
		↓	↑	CKTSF1B1
	↓	↓		ALDH3A2, EGR2, FABP7, PCP4
	↓			MJD
		↓		CLN8, DPYSL3, LOC51195, SPON2
NO Biosyn.	↑	↑		GCH1
	↑			ARG2
	↓			GCHFR
Transcription	↑	↑	↑	STAT1, TRIM22
	↑	↑		ELL2, HIF1A, NMI, PTTG1
	↑			MYC, STAT3, UHRF1
		↑		TMF1
			↑	PREB
	↓	↓		CTNNBIP1, GATA3, ID4,PPARG, PPARGC1, THRSP
	↓			RBBP2, SOX5, SOX9, TAF6L
		↓		CBX4, GTF3A

Fig. 2. Comparison of biological processes differing between the 3 skin groups. *A*: GeneOntology (GO) annotations of differentially expressed genes were collected from LocusLink (**http://www.ncbi.nlm.nih.gov/LocusLink**), the European Bioinformatics Institute (EBI; **http://www.ebi.ac.uk**), and TIGR (**http://www.tigr.org**). Forty percent of all differentially expressed genes matched at least one process ontology term corresponding to a node (biological process category) on the GO hierarchy. For each of the three differentially expressed gene sets [involved vs. normal skin (I vs. N), involved vs. uninvolved skin (I vs. U), and uninvolved vs. normal skin (U vs. N)], the numbers of genes involved in each biological process are shown (number of upregulated genes/number of downregulated genes). We further assessed the statistical significance of any dependence of the gene expression alteration on each GO process category with a generalized hypergeometric model for 2-by-3 contingency tables. Those GO nodes passing the cutoff *P* value of 0.05 are in red (I vs. N), yellow (I vs. U), or green (U vs. N). *B*: given a biological process, genes were cross-classified in the columns according to their 3 possible expression alterations (upregulated, downregulated, or no significant change) and in the rows according to whether it is involved in the given process. Differentially expressed genes involved in biological processes that were significantly perturbed in at least one pairwise skin group comparison were listed. The "↑" symbols denote overexpression; "↓" symbols denote underexpression (see text for criteria); Biosyn, biosynthesis; Cat, catabolism; Inflamm, inflammatory; Met, metabolism; NO, nitric oxide; and Resp, response.

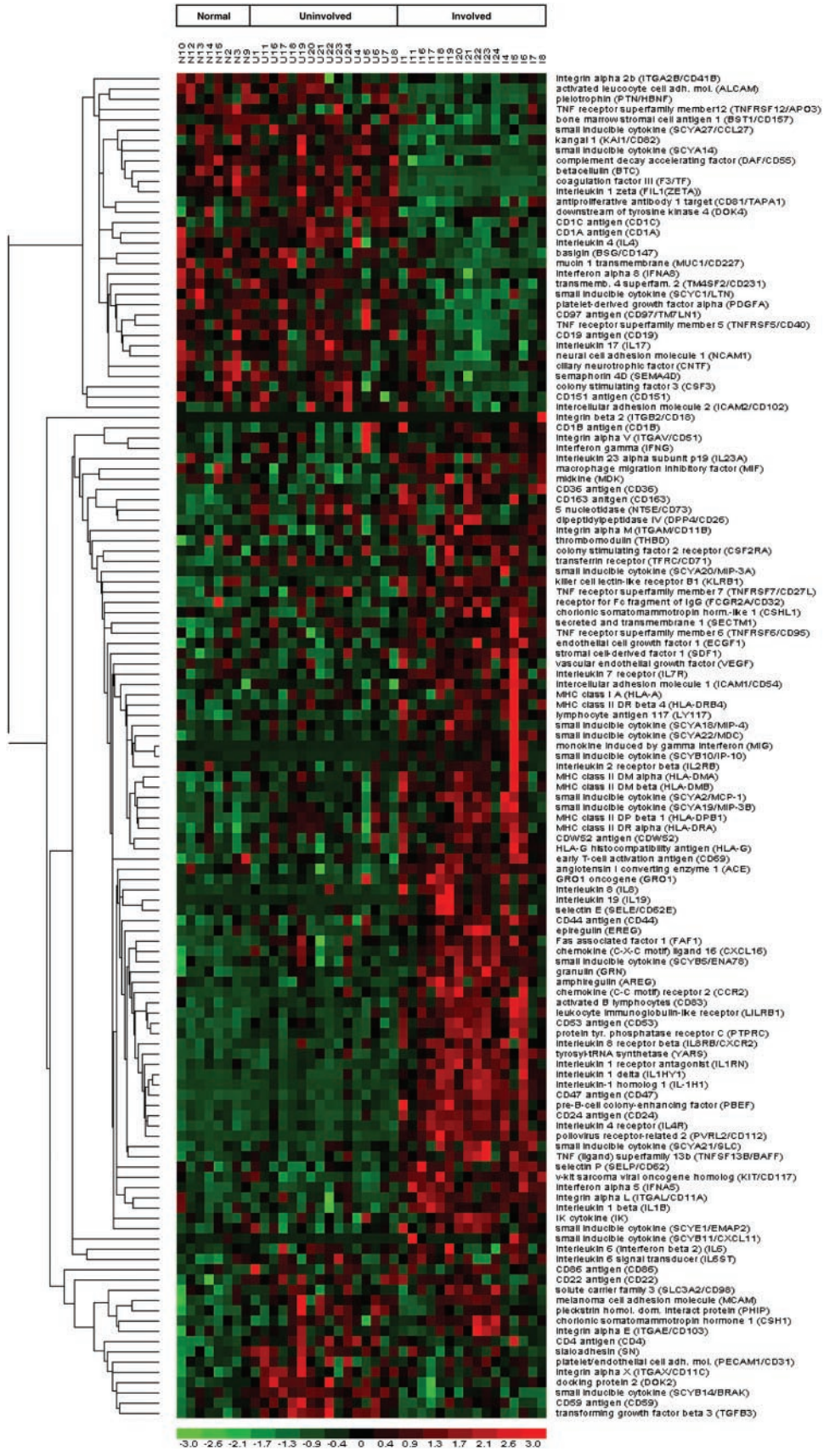


Fig. 3. Clustering of expression profiles of immune signaling molecules; 131 immune signaling genes were selected that were significantly differentially expressed ($P < 0.05$ and fold changes > 1.2) in at least one pairwise comparison among the 3 skin groups. The tissue types are presented on the *top*: normal skin (N), psoriasis-uninvolved skin (U), and psoriasis-involved skin (I). The normalized expression level for each gene (rows) in each sample (columns) is indicated by a color code (see the expression index bar at the *bottom*). Gene names from the UniGene database as well as common synonyms are indicated.

another IL1R agonist, IL-1ζ, also showed a pattern of differential expression. Most of these IL-1 cytokines receptor antagonists were discovered in the last few years.

T-cell and DC activation. The middle section of Fig. 3 shows genes that were upregulated in involved vs. normal skin. It thus reflects an immune profile of psoriatic lesions. It is known that psoriasis lesions contain marked increases in various classes of infiltrating leukocytes, including macrophages, CD11c+ DCs, CD83+ DCs (probably derived from activated Langerhans cells in situ), CD4+ T cells, CD8+ T cells, CD103+ T cells, and neutrophils (27). Each of these leukocytes subsets can be characterized by the upregulation of activation-related proteins and strong expression of lineage-related gene products. We previously reported upregulation of IL-8, CD24, CD25, and CD47 in involved skin (10, 38). However, upregulation of most of the genes shown in Fig. 3 has not been described before in psoriasis. This upregulation could be due in part to persistent activation of T lymphocytes and DCs in the skin, or de novo activation of memory T cells and DCs in the skin. Upregulation of CD47, a general lineage marker for blood-derived cells is very strong across all samples. This probably reflects various leukocyte subsets infiltrating psoriasis lesions. T cells in lesions are activated as evidenced by the upregulation of IL-2Rα and IL-2Rβ subunits, CD71, CD69, and IL-7R.

Trafficking of leukocytes into peripheral tissues is strongly regulated by "leukocyte integrins." Both the major T-cell integrin CD11a/CD18 and the second leukocyte integrin CD11b/CD18 are known to be expressed in high levels on monocytes/DCs and neutrophils (28). All genes encoding these subunits were upregulated in involved skin. The upregulation of adhesion molecules (E-selectin, P-selectin, and ICAM) would support leukocyte infiltration into inflamed skin compartments, as well as T-cell adhesion to DCs.

CD163, CD32, major histocompatibility complex (MHC) class I, MHC class II, CD83, CD53, and CD24 are overexpressed in involved skin. The products of these genes are associated with monocytes/DCs and antigen presentation.

CD44 is a receptor for hyaluronic acid. By FACS (BD Biosciences) staining, we have found upregulation of CD44 on lesional T cells (unpublished), while immunostaining shows diffuse expression on various cells in psoriasis lesions. Therefore, we suggest CD44 as the best candidate for expression of an adhesion molecule that would promote T-cell or DC retention in inflamed skin. The infiltration of neutrophils is reflected by the overexpression of IL-8 along with its receptor CXCR2.

The bottom of Fig. 3 shows genes with expression changes that differ in uninvolved vs. normal skin. CD4 antigen, expressed by CD4+ T cells and by dendritic antigen-presenting cells, is increased in uninvolved skin, as is CD11c, which is known to be strongly expressed by some DCs. While psoriatic lesions exhibit a very large increase in CD11c+ DCs (2), we have seen an increase in these cells in the early stages of eruptive psoriasis lesions (unpublished results). Hence, the upregulation of CD11c in uninvolved skin in the current study implies either activation of gene expression in endogenous DCs or the beginning of infiltration by exogenous cells. We note that DCs endogenous to the skin can be activated by processing of an antigen or by reaction to cytokines such as TNF. Since we found putative binding sites for NFκB, STAT1, and IRF-1 in the promoter of the CD11c gene, its expression may be upregulated in response to increases in circulating TNF or γ-interferon (IFN-γ) in patients with active disease. An increase in DC activation could also explain the increased expression of CD86, a major costimulatory molecule that is upregulated by the activation process. Another gene that is elevated is CD103. Its expression is restricted to a group of epithelial homing CD8+ T cells that were first identified in gut tissue (12). In a previous study we found increased CD103+ CD8+ T cells in psoriatic lesional epidermis (37). Other studies (36) suggest that TGFβ is a maturing or inducing factor for this integrin, probably in the cutaneous microenvironment, and in fact TGFβ3 clusters with these genes that are upregulated in uninvolved vs. normal skin. SCYB14/BRAK is a chemokine that promotes trafficking of activated monocytes into skin as part of normal immune surveillance, and is upregulated in uninvolved vs. normal skin. With its increased expression, there may be more monocyte trafficking and even some differentiation of monocytes into macrophages or DCs. In conclusion, gene expression in uninvolved skin suggests a low level of immune activation involving both DCs and T cells.

A type-1 bias to activated T cells is indicated by the overexpression of IFN-γ. Orchestrating a wide range of diverse cellular programs, IFN-γ controls the expressions of many genes. Interestingly, the most highly upregulated transcription factors in involved skin were TRIM22 (upregulated 12.3-fold) and STAT1 (upregulated 5.3-fold), both of which are IFN-γ inducible. While STAT1 is a primary response gene to IFN-γ, the transcription regulation of TRIM22 in IFN-γ response remains to be determined.

Chemokines in psoriasis. Chemokines play a complex role in lymphocyte trafficking in psoriasis (27). Table 1 lists the 19 chemokines that were determined to be differentially expressed in this study. Eleven of these have not been previously described in psoriasis. SCYA19/CCL19, SCYA21/CCL21/SLC, and SDF-1/CXCL12 were all upregulated in involved skin. We investigated SCYA2/CCR7, the receptor for SCYA19, with flow cytometry and found evidence for its expression in T cells and DCs of psoriasis lesions (results not shown). The combination of adhesion receptor (L-selectin) and chemokine receptor is normally found on node-homing T cells. CLA+ and CLA− cells both express CCR7, suggesting entry of cells into psoriasis lesions which are not specifically differentiated for skin homing and would otherwise be expected to home to lymph nodes or other "formal" lymphoid tissues. Since there are very few naïve T cells in involved skin (measured by CD45RA and CD45RA/CD27 staining of CD4+ and

Table 1. *Chemokines differentially expressed among psoriatic involved vs. normal skin (fold change > 1.2 and P value < 0.05)*

Canonical Gene Name	Other Names	Expression Deviation	Receptor	Prior report of involvement in Psoriasis	Function
SCYA19	CCL19/MIP3β	↑	CCR7	No	Regulate T-cell trafficking to lymph nodes and/or other lymphoid tissues. May promote the formation of secondary lymphoid tissue.
SCYA21	SLC	↑	CCR7	No	Regulate T-cell trafficking to lymph nodes and/or other lymphoid tissues. May promote the formation of secondary lymphoid tissue.
SDF-1	CXCL12	↑	CXCR4	No	Suggested role in the control of DC positioning in both nonlymphoid and lymphoid tissue. May promote the formation of secondary lymphoid tissue.
SCYA18	CCL18/PARC/ DC-CK1/MIP-4	↑		No	Made by macrophages or DCs in lymph nodes. Chemoattractant for naïve and memory CD4+ and CD8+ T cells.
CXCL16	Bonzo receptor ligand	↑	CXCR6/ Bonzo	No	Made by DCs in T-cell zones of lymphoid organs. May attract/organize CD8+ T cells (CD8-selective) and DCs. Membrane expression on CD11C+ DCs.
SCYA20	MIP-3α	↑	CCR6	Yes (39)	Attraction of LCs or DCs into inflamed skin in psoriasis.
SCYA2	MCP-1	↑	CCR1, CCR2	Yes (18)	Recruitment of DCs and Langerhans cells into skin. Made by keratinocytes in lesions.
SCYB9	MIG/CXCL9	↑	CXCR3	Yes (19)	Mainly T-cell recruitment. Proposed role monocyte recruitment.
SCYB10	IP-10/CXCL10	↑	CXCR3	Yes (16)	Mainly T-cell recruitment. May affect monocyte recruitment.
SCYB5	ENA-78/CXCL5	↑	CXCR2	No	Mainly neutrophil recruitment.
IL-8		↑	CXCR1 CXCR2	Yes (17)	Mainly neutrophil recruitment.
GRO1	CXCL1	↑	CXCR2	Yes (17)	Mainly neutrophil recruitment
SCYA22	CCL22/MDC	↑	CCR4	Yes (37)	Upregulated in psoriasis (TARC also upregulated and binds to CCR4)
SCYE1	EMAPII	↑		No	Isolated from (apoptosis-induced) tumor cells. Pro-inflammatory. Affects endothelial cells, monocytes, and PMNs. Upregulates TNF-R1 but not R2. Expressed in some experimental autoimmune diseases.
SCYB11	CXCL11/I-TAC	↑	CXCR3	No*	One of the most potent chemoattractants of normal human blood CD4+ and CD8+ effector memory T cells. It is likely to be a major mediator of T cell migration into peripheral sites of inflammation.
SCYA27	CTACK	↓		Yes (24)	Selectively expressed in skin tissue. Recruits skin-homing T cells. Proposed immune surveillance function. One prior report has decrease in psoriasis lesions.
SCYA14	CCL14α/HCC-1	↓	CCR1, CCR5	No	One of several chemokine genes on chromosome 17q11. Chemotactic for T cells, monocytes, DCs, and neutrophils.

DC, dendritic cell; LC, lymphocyte. *W. Lew, E. Lee, and J. Krieger, unpublished observations.

CD8+ cells, respectively; data not shown), our conclusion is that CCR7 is expressed mainly on central memory T cells (T_{cm}) in psoriasis lesions.

A dense T-cell infiltrate around dermal blood vessels, that contain peripheral node addressin+ (PNAd+) endothelial cells in high endothelial venules has been previously described in psoriasis (35). The perivascular T cells are intermixed with many CD11c+ and CD83+ DCs (2). The increased expression of lymphoid tissue chemokines (CCL19, CCL21, and SDF-1) may complete a cellular and biochemical environment that is similar to paracortical (T-cell rich) regions of lymph nodes. Potentially, psoriatic involved skin could function as secondary lymphoid tissue. The presence of many CCR7+ T cells in psoriatic lesions also supports the hypothesis implied in part by the gene expression findings mentioned above, that the spatial organization of T cells and DCs, along with the production of many regulating cytokines and chemokines, could sustain chronic T-cell activation and its persistence within focal skin regions. Hence, increased expression of genes for chemokines, cytokines, receptors, and DC/T-cell adhesion molecules could all contribute to chronic disease activity in psoriasis by providing the ongoing molecular interactions to sustain T-cell trafficking, activation, and effector immune functions. We note that ectopic expression of CCL19, CCL21, or SDF-1 in experimental systems has been sufficient to induce T-cell

Table 2. *Potential TFBS motifs for genes of tight expression clusters*

TFBS Motif	Functions of Individual TFs	Synexpression Genes
[Ikaros3]	hematopoietic differentiation	SLC6A14, PBEF*, GLUL, EVA1*
[c-Ets-2]	proliferation, differentiation, development, transformation, apoptosis	S100A7, S100A8*, S100A9*, KRT6A
[NFκB]	inflammation, immune response	LDLR*, LAMP3, MYC *[NFκB(8)]*, HBP17*
[AP1]	hematopoietic differentiation, immune response, apoptosis	TGM3*, AKR1B10*, EVA1*, GJB6*, GJB2†, CPT2*
[RORα1]	neural development, bone metabolism	TNFSF10, RASGRP1*, DNASE1L3*, KPNA2*, MYO1B*, GK
[VDR/RXR]-[HSF1]	[VDV/RXR]: differentiation and apoptosis of monocytes [HSF1]: stress response	WNT5A*-*, GBP2, CASP1
[PPAR]-[OCT1]	[PPAR]: inflammation, lipid metabolism, epidermal differentiation [OCT1]: Constitutive	ISG15[*-*], TRIM22[*-*], MX1†-*, FLJ20637†-*
[GATA-1]-[EVI1]	[GATA-1]: hematopoietic differentiation, angiogenesis [EVA1]: hematopoietic differentiation	KYNU[*-*], S100A12, FLJ21343[*-*]
[NF1]-[Creb1]	[NF1]: proliferation, epidermal differentiation [Creb1]: cAMP response	TRB@ *[NF1(9)],* FLJ22662*-*, POLB *[CREBP1 (33)]*-†
[IRF1]-[E2F]	[IRF1]: antiviral response [E2F]: proliferation	FLJ10134[*-*], FLJ22408, AI968669[*-*]
[NF-Y]-[Sp1]	[NF-Y]: cellular senescence [Sp1]: constitutive, immune response	‡TOP2A *[NF-Y(3)][Sp1(41)]*-†, TRA1*-*, EIF5*-*, APG-1†-*
[IRF2]-[ISRE]	[IRF2]: immune response [ISRE]: immune response	OAS1[*-*], OAS2[*-*], ISG15[*-*], Clorf29*-*
[Ikaros1]-[STAT1]	[Ikaros1]: hematopoietic differentiation [STAT1]: immune response	LOC84518[*-*], AMD1*-†, TGM3[*-*]

Transcription factor binding sites (TFBS) were identified by searching for overrepresentation of particular promoter elements or their combinations in the upstream sequences of genes in the same expression clusters generated based on highly correlated gene expression profiles with a constrained recursive K-means clustering approach. The distance between paired TFBSs is constrained between 20-80 bp. For those genes marked with * or †, their mouse orthologs can be identified; */† denotes the presence/absence of the same TFBS motif in the upstream sequences of the mouse orthologs. For paired TFBS motifs, *-* denotes that the mouse ortholog contains both of the motifs identified in its human counterpart. In addition, [*-*] denotes that the distance in the paired motif in the mouse ortholog is between 20-80 bp and in the same order. Likewise, for other combinations of * and †. Literature support for specific TFBSs of our predictions is noted with underscore. ‡The upstream sequence of the mouse TOP2A is unsatisfactory, due to the existence of large gaps in the Celera mouse genome assembly.

infiltrates and organized lymphoid tissues in other organs (30), while the expression of CCL19 and CCL21 by endothelial cells in early lesions of experimental autoimmune encephalomyelitis has been suggested to be a key inducer of this disease (5).

SCYA18/CCL18, also upregulated in psoriatic skin, could play an additional role in this type of inflammatory reaction, since it might recruit naïve T cells to skin-draining lymph nodes. Another upregulated gene, CXCL16, is expressed on the surface of CD11c+ DCs. This suggests a link with the increased abundance of CD11c+ DCs in psoriatic tissue. In addition, we note that CXCL16 selectively recruits of CD8+ T cells, which are particularly increased in the epidermis of involved skin.

The IFN-γ-regulated chemokines IP-10 and MIG form a tightly upregulated cluster in involved skin in Fig. 3. These two chemokines, together with I-TAC, are ligands of G protein-coupled receptor 9 (CXCR3). These findings are in concert with observed infiltration of CXCR3+ T cells in psoriatic skin (37). Clustered together with IP-10 and MIG are MDC and MIP-4, both of which are expressed in normal human keratinocytes activated by Th1-derived supernatant (4). MIP-3α, proposed as a major regulator of DC migration into skin (13), was also overexpressed in lesional skin.

Large-scale Promoter Analysis

The expression clustering of psoriasis-related genes over the whole genome enabled us to perform large-scale promoter analysis of coexpressed genes. We present in Table 2 thirteen such coexpressed gene clusters and their shared TFBS motifs which are in addition strongly supported by mouse orthologous data.

As an interesting example, we identified the TFBS motif [IRF2]-[ISRE] in four of seven genes in an expression cluster. The transcription factor IRF2 (interferon response factor 2) can be induced by IFN-γ (1), and ISRE is the interferon-stimulated response element. Notably, three of the four genes identified with this motif are IFN inducible: 2,5-oligoadenylate synthetase 1 (OAS1), 2,5-oligoadenylate synthetase 2 (OAS2), and interferon-stimulated protein (ISG15). Although the function of the remaining gene C1orf29 is unknown, based on its expression pattern and its promoter organization, it is likely to also be IFN inducible and to play a part in inflammation and antiviral response. In fact, its mouse ortholog is a minor histocompatibility antigen. The mouse orthologs of all four genes contain the same paired [IRF2]-[ISRE] motif.

The TFBS pair [NF-Y]-[Sp1] is identified in the upstream sequences of four genes in an eight-gene ex-

pression cluster. The four genes, topoisomerase IIα (TOP2A), tumor rejection antigen 1 (TRA1), eukaryotic translation initiation factor 5 (EIF5), heat shock protein (APG-1), are all involved in cell proliferation and stress response. The proliferation-dependent expression of topoisomerase IIα has been experimentally shown to require both NF-Y (3) and Sp1 (41). Other studies have shown that these two transcription factors synergistically stimulate the transcription of some cell-cycle-dependent genes (42). In fact, in an independent study their binding to adjacent sites has been shown to be cooperative (40). Our results suggest that this cooperativity may be more prevalent than previously known.

As another example, we identified TFBSs for NFκB in four clustered genes: DC lysosome-associated membrane protein (LAMP3), c-myc (MYC), heparin-binding growth factor binding protein (HBP17), and low-density lipoprotein receptor (LDLR). The first three are involved in cell proliferation, while the last supports cell proliferation in lymphocytes (31). Except for LAMP3, we were able to obtain their mouse orthologs, all of which contained NFκB TFBSs in their upstream sequences. In addition, the induction of expression of c-myc by NFκB has been experimentally determined (8).

Conclusion

This is the most comprehensive analysis of the human transcriptome to date. We have generated a list of 1,338 genes that are potentially psoriasis-related, and 60% of these encode newly discovered proteins. Some of these genes are likely to be potential targets for new therapeutic strategies (21).

In the context of the whole transcriptome, we surveyed the complete GeneOntology hierarchy and identified a wide range of biological processes that were significantly perturbed in psoriasis. The genes involved in immune signaling pathways were of particular interest and suggest de novo activation of T cells and DCs in the skin or novel methods for maintaining their activation.

Large-scale gene expression analysis also facilitated the discovery of regulatory mechanisms that govern the differential expression of genes. By focusing on tightly coregulated genes and by combining both expression data and mouse orthologous promoter sequences, we identified several individual and paired potential TFBSs in the upstream regions of psoriasis-related genes. Many of these motifs are biologically plausible and are related to epidermal differentiation, immune response, or proliferation. An important role is proposed in psoriasis for STAT1 and TRIM22 transcription factors, primary response genes in the IFN-γ pathway.

This study also provides an explanation for the efficacy of numerous biologic agents currently under evaluation. For example, efalizumab, a humanized antibody to CD11a, downregulates and blocks LFA on T cells (20), and data presented here argue for the upregulation and potential importance of the leukocyte integrins. The therapeutic agent CTLA4Ig binds CD80 and CD86 and targets activated DCs. Its therapeutic responses have been linked to decreased tissue infiltration of CD11c + DCs, CD83 + DCs, and T cells (2). The impressive response of psoriasis to TNF inhibitors is likely to be reflected in alterations in the expression of genes with NFκB sites, possibly combined with GAS or ISRE elements.

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