Cellular Genomic Maps Help Dissect Pathology in Human Skin Disease

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Genomic signature maps of different cell types can aid in the interpretation of genomic data of specimens collected during disease states. We have defined "lineage-specific" genes, as well as "activation" genes, for cellular components of the skin: keratinocytes, fibroblasts, macrophages, monocytes, T cells, immature, and mature dendritic cells (DCs). Re-analysis of a previously published gene set of psoriasis then provided a model for assessing the usefulness of these maps. We were able to ascribe over 90% of these genes to specific cell types, and there was a surprisingly large contribution from DCs. This shows the utility of such cellular gene maps.

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INTRODUCTION

Array-based techniques that profile genes expressed in inflammatory and neoplastic skin diseases have become important methods to characterize molecular alterations associated with cutaneous pathology (Blumenberg, 2006). With this genomic information, functional pathways that are activated in diseased tissue can be delineated and dissected. However, this objective requires prior classification of genes into known categories. This may be based on a primary knowledge of genes activated in signaling cascades, for example a well-documented sequence of genes activated by IFN-γ signaling (Boehm *et al.*, 1997). Alternatively, bioinformatics programs can interrogate complex expression data sets, for example Gene Ontology or pathway-organizing computer programs.

Psoriasis vulgaris is one of the first skin diseases to be classified through such expression profiling (Oestreicher *et al.*, 2001). Current whole-genome maps indicate that $>$1,300 genes are expressed differently in skin with psoriasis, compared with the normal skin or non-lesional skin of the same patient (Zhou *et al.*, 2003; Haider *et al.*, 2006). Within the set of genes expressed in psoriasis, a strong signature exists for IFN-γ-regulated gene products ($\sim$63 genes) (Lew *et al.*, 2004). Many other cytokines and inflammatory products can be identified and put into provisional networks (Liu *et al.*, 2007). A broader analysis of the expressed genes using Gene Ontology annotations enables researchers to classify the functional categories of genes (Zhou *et al.*, 2003); however, this provides no information on the associated cell types.

Interpretation of complex genomic sets derived by array-based methods is currently limited because signatures of specific cell sets that make-up resident or infiltrating populations are mostly unknown. In this study we used a single-gene expression platform (Affymetrix U95Av2 arrays) to derive cell-specific gene lineage maps for several types of cells that are found in the skin during cutaneous inflammatory processes. We have developed such maps for keratinocytes, fibroblasts, macrophages, monocytes, T cells, and immature and mature dendritic cells (DCs). Peripheral blood mononuclear cells (PBMCs) were also included as some subsets of infiltrating or resident leukocytes might not have been represented in this list. In addition, gene sets, which were associated with the activation of T cells and PBMCs through *CD3/CD28* ligation or with the activation of keratinocytes in the presence of cytokines, were also codified.

In the case of psoriasis, the growth and differentiation of resident cells is altered, in addition, the skin is heavily infiltrated by T cells, *CD11c*$^+$ myeloid DCs, and by smaller populations of other types of leukocytes (Krueger, 2002; Lowes *et al.*, 2005). Earlier work with arrays or expression tags has identified many genes, which are altered by the activation of T cells (Diehn *et al.*, 2002) or by the progressive differentiation of myeloid DCs (Teague *et al.*, 1999; Hashimoto *et al.*, 2000), but gene sets that are unique or classify diverse cell lineages are largely unavailable. We illustrate how the use of these gene array-derived cell-specific maps can be used to further classify the disease-related gene set in psoriasis. Using our lineage and activation genomic maps, we can now assess $>$90% of expression changes in psoriasis to specific cell sets or to activated cells. These new

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Abbreviations: DCs, dendritic cells; LAMP, lysosomal-associated membrane protein; LTB, lymphotoxin beta; PBMCs, peripheral blood mononuclear cells; RLB, Rneasy lysis buffer; TNF, tumor necrosis factor

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data significantly advance our understanding of the biologic processes in psoriasis, and can be applied to other cutaneous diseases. Overall, our approach argues for developing yet more extensive and more complete genomic maps to classify biologic processes in skin diseases in a comprehensive fashion.

RESULTS

Defining genes expressed in keratinocytes, fibroblasts, macrophages, monocytes, T cells, and immature and mature DCs

Psoriasis vulgaris, atopic dermatitis, and other inflammatory skin diseases are characterized by significant increases in infiltrating leukocytes such as T cells and DCs, as well as altered growth of resident skin cells. In this study, we elected to study T cells, several types of myeloid leukocytes, for example monocytes, macrophages, and DCs, as well as keratinocytes and fibroblasts, for patterns of gene expression. Using Affymetrix U95Av2 GeneChips, we quantified the expressions of ~12,000 known genes using several independent isolates of each cell type for all the analyses. All the genes that were expressed in at least one cell type were codified and relative levels of gene expression were compared using analysis of variance with a correction for multiple comparisons. Genes in which expression was different ($P<0.05$) from at least one other cell ''lineage'' were selected by this method. We then assembled the genes into cell sets and recognized that some genes were highly expressed only in one cell type, whereas other genes were expressed in two or more cell types. Genes with expression above our selection threshold (see Materials and Methods) in only one cell type were classified as cell-specific genes for that cell type, whereas other genes were classified into two or more cell types. A set of genes common to myeloid leukocytes was also identified and these genes can be considered to be lineage-related for myeloid cells.

With this approach we defined broad sets of genes with restricted expression in one cell type only: 513 genes in keratinocytes, 633 genes in fibroblasts, 286 genes in macrophages, 602 genes in monocytes, 770 genes in T cells, and 377 genes in DCs (Figure 1a). A complete list of all the genes in each cell type is available in Tables S1A–6. Of the 377 genes in DCs, 59 genes were common to both immature and mature DCs, 95 genes were specific to immature DCs, and 223 genes had higher expression in mature DCs (Figure 1b). These gene lists are listed in Table S6A. To confirm that these lists were applicable to each specific cell type, we evaluated the level of expression of known (based on the literature) genes in each cell type. The blue areas in Figure 1c highlight this expression, showing that known representative genes are indeed increased in each cell type compared with other types of cells.

Common genes in cell groups

We also detected groups of genes with higher expression levels in more than one cell type (Figure 2a), and these could be combined into biologically relevant groups of cells. We considered these in four groups: (a) genes commonly expressed in myeloid cells (547 genes); (b) genes in skin resident cells (fibroblasts and keratinocytes; 385 genes); (c) leukocyte signature genes (681 genes), and (d) genes in multiple cells (744 cells). Complete lists of genes are available in Tables S7A–S10. The blue areas in Figure 2b highlight the expression of genes known to be present in these groups of cells, and help validate the genes that we were able to define with this approach. The majority of increased genes had not been previously associated with these cell families.

Defining cell activation genes

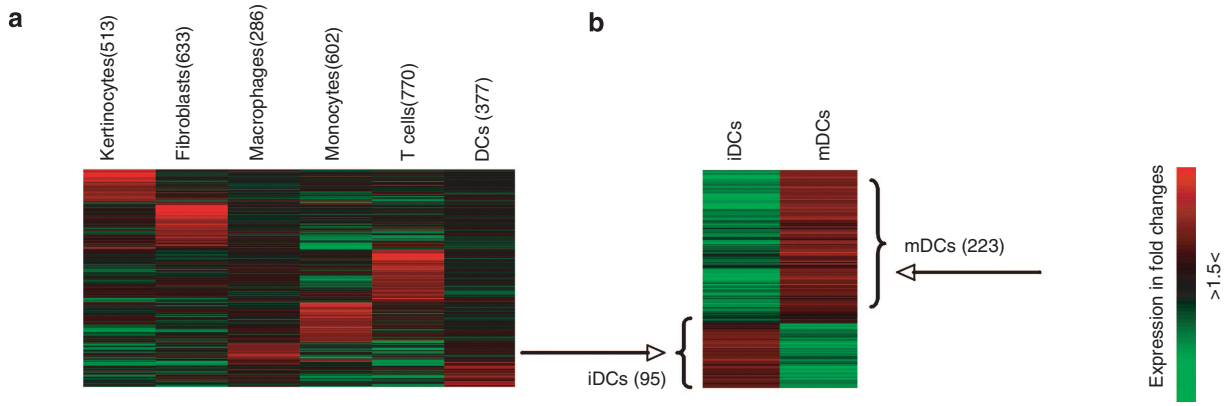
To understand the contribution that activated cells play in inflammatory dermatoses, we developed maps of CD3/CD28-activated T cells and PBMCs for 24 hours (Figure 3a). Activated PBMCs were included as they might be more representative of the mixtures of cells infiltrating the skin during inflammation. As the selection of activation genes required a comparison of two different conditions within the same cell type, we selected activation genes with 1.2-fold higher expression when compared with control. We detected a set of >1,100 genes in activated isolated T cells, and >1,600 genes in activated PBMCs. There were 735 genes commonly activated in the two populations of cells (Table S12A for gene lists). A subset of these genes is listed in Figure 3c. As expected, activated PBMCs contain a mixture of genes, which were not detected in purified T cell preparations. Several of the highly upregulated genes unique to activated PBMCs, for example *MMP-12*, *CCL8*, *CD1B*, and *CCL18*, were gene products detected only in myeloid DCs and were not typically associated with T cells.

To confirm this concept of lineage- and activation-specific genes, we performed RT-PCR on these populations for selected genes (Figure 4). The expression of *CD3D* was stable during activation, whereas those of lymphotoxin-α and *IFN-*γ were clearly increased. Similarly, *CD11c* was consistently expressed in the common myeloid cell group, but DCs-lysosomal-associated membrane protein (*LAMP*) and indoleamine-pyrrole 2,3-dioxygenase were markers for mature/activated DCs.

Using published gene sets (Banno *et al.*, 2003, 2004), we reanalyzed the gene expression in keratinocytes in response to *IFN-*γ and tumor necrosis factor (*TNF*) cytokines at 1, 4, 24, and 48 hours (Table S13A–15). There were 606 genes upregulated by *IFN-*γ, the majority of which were upregulated at 24–48 hours. In response to *TNF*, 258 genes were upregulated in keratinocytes. We found 112 genes that were commonly regulated by the two cytokines, whereas 494 genes were uniquely regulated by *IFN-*γ and 146 uniquely to *TNF*.

Re-classification of a common inflammatory dermatosis using cell-specific gene maps: psoriasis vulgaris

Psoriasis is an ideal disease to evaluate the usefulness of these cellular genomic maps, as it is one of the few inflammatory dermatoses with published gene sets. Earlier genomic analyses of psoriasis used Gene Ontology annotation to identify the function of ~425 genes out of the 1,300



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Cell types	Keratinocytes	Fibroblasts	Macrophages	Monocytes	T cells	iDCs	mDCs	Symbol	Gene product
Keratinocytes	275.7	0.738	0.97	1.3	1.03	0.863	1.02	KRT14	50 kDa epidermal keratin
	226	0.698	0.96	1.01	0.87	1.036	0.96	KRT5	keratin 5
Fibroblasts	1.044	279.8	1.05	0.91	0.96	1.072	1.11	COL1A2	Human procollagen type 1 alpha-2 chain
	0.951	121.7	0.98	0.95	1.05	1.142	1	CDH11	cadherin 11, type 2, OB-cadherin (osteoblast)
Macrophages	0.994	0.96	29.3	1.07	0.9	0.984	0.98	CAMP	cathelicidin antimicrobial peptide
	1.039	1	22.8	0.79	0.82	0.769	1.43	ADAMDEC1	ADAM-like, decysin 1
Monocytes	1.171	0.81	1.01	46.9	0.99	0.869	0.98	S100A12	S100 calcium binding protein A12 (calgranulin C)
	0.709	0.64	0.99	3.25	0.71	0.972	0.99	TLR6	Toll like receptor 6
T cells	0.852	0.75	1.09	1.32	26.4	1.028	1.04	CD2	CD2 antigen (p50), sheep red blood cell receptor
	1.01	0.783	1.21	1.5	117	0.941	0.98	TRA@	T cell receptor alpha locus
DCs	0.8	0.859	0.75	0.91	0.77	20.83	2.75	ALOX15	arachidonate 15-lipoxygenease
	0.822	0.692	1.01	0.98	0.73	6.441	3.05	CD1B	CD1B antigen, b polypeptide
iDCs	0.986	0.64	0.99	1.02	0.8	3.676	1.34	LY64	lymphocyte antigen 64 homolog
	0.388	0.215	1.22	1.16	0.27	2.113	1.26	LGALS9	lactin, galactoside-binding
mDCs	1.237	0.843	0.94	1.16	0.9	0.943	11.6	IL12B	interleukin 12B
	1.006	0.868	1.11	0.98	1.26	0.908	85.4	INDO	indoleamine-pyrrole 2,3 dioxygenase

Figure 1. Defining genes expressed in keratinocytes, fibroblasts, macrophages, monocytes, T cells, and immature and mature DCs. mRNA from cultured cells was hybridized to individual oligonucleotide arrays containing ~12,000 human genes (Affymetrix HG-U95Av2 chips). Heatmaps show unsupervised hierarchical clusters of upregulated genes in individual cell types (>1.5-folds compared with other cell types; $P<0.05$, $n=2$–4) using the similarity measure: Pearson's correlation. Areas of clusters in red for individual cell types define the number of upregulated cell lineage genes (number shown in parenthesis), compared with green areas with lower expressions of same genes (horizontally) in other cell types. (**a**) Lineage genes in keratinocytes, fibroblasts, macrophages, monocytes, T cells, and DCs. (**b**) DCs cluster: lineage DCs genes before (immature DCs) or after maturation (mature mDCs). (**c**) Examples of normalized gene expression corresponding to the cell types described in (**a**) and (**b**). The expressions of the representative cell lineage genes are 1.5-fold greater than in all other cell types (blue areas) (Tables S1A–6 list all genes).

regulated genes (Zhou *et al.*, 2003), leaving >800 genes with unknown function. With the cell genomic maps presented in this study, it is now possible to associate ~90% of the psoriasis genes with different cell types. In this study we analyzed untreated psoriasis lesions of eight patients using U95Av2 GeneChips and found that 1,210 genes were upregulated in psoriasis. The statistical analysis of this data set has been published previously (Haider *et al.*, 2006). Table 1 gives a quantitative overview of the number of genes that were present in psoriasis and can be associated with different cell types.

We classified the genes upregulated in psoriasis into two categories: lineage genes that were expressed in resting cells (Table 1a), and genes that were upregulated after the activation of cells in the presence of anti-*CD3/CD28* antibodies and cytokines such as *IFN-γ* and *TNF*, or after activation due to the maturation of DCs (Table 1b). Some of the genes that were expressed in resting cells were also expressed in activated cells. Thus, we further defined genes that were uniquely expressed in activated cells and not expressed in resting cells. Of the 1,210 genes that were upregulated in psoriasis, we were able to classify >90% of

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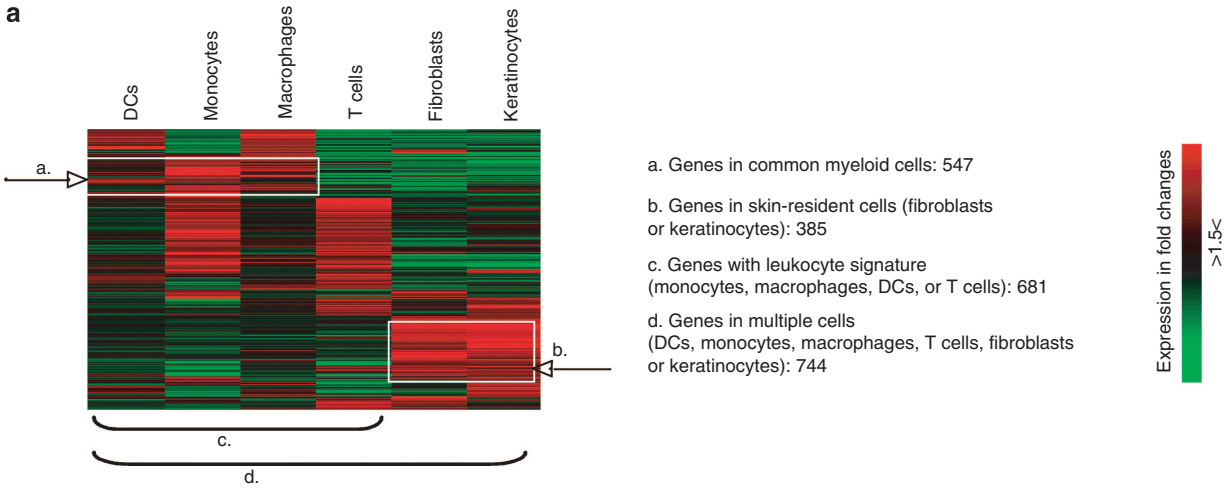

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Cell groups	iDCs	mDCs	Monocytes	Macrophages	T cells	Fibroblasts	Keratinocytes	Symbols	Gene produtcs
a. Common myeloid cells	0.9	1.9	10.9	26.2	0.6	0.9	0.6	CD14	Human gene for CD14 differentiation antigen.
	2.8	3.9	1.6	1.0	0.1	0.1	0.1	CD86	CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)
b. Skin-resident cells	1.0	1.0	0.9	0.8	0.9	10.3	6.4	IGFBP5	insulin-like growth factor binding protein 5
	0.8	1.0	0.6	0.9	1.4	73.5	17.2	SERPINE2	serine (or cysteine) proteinase inhibitor, member 2
c. Leukocytes	0.2	7.2	8.3	2.9	2.3	0.2	0.2	IL-8	Human interleukin 8 (IL8) gene, complete cds.
	2.7	4.8	1.9	0.8	37.9	0.6	0.8	CCL5	chemokine (C-C motif) ligand 5
d. Multiple cells	0.9	0.7	0.4	1.2	3.8	2.5	1.0	STAT1	Homo sapiens transcription factor ISGF-3
	0.8	4.7	8.3	0.9	4.4	23.2	0.7	IL-6	interleukin 6 (interferon, beta 2)
	2.0	0.7	0.5	13.1	0.4	1.6	0.3	CCL2	Human interferon gamma treatment inducible
	0.4	0.3	0.1	3.2	3.9	0.4	10.9	ITGA6	integrin, alpha 6

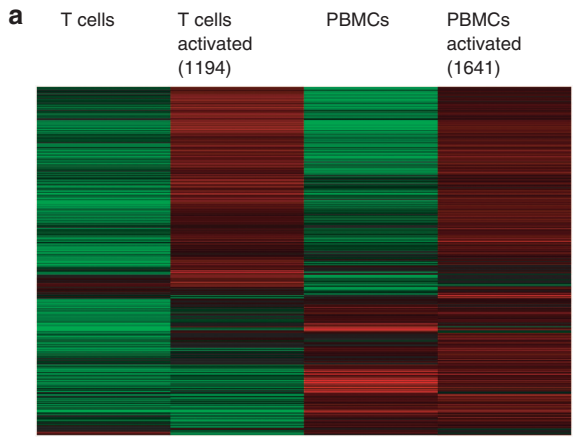
Figure 2. Common genes in cell groups. mRNA from cultured cells was hybridized to individual oligonucleotide arrays containing ~12,000 human genes (Affymetrix HG-U95Av2 chips). Heatmaps show unsupervised hierarchical clusters of upregulated genes in cell groups (>1.5-folds compared with other cell type; $P<0.05$, $n=2$–4) using the similarity measure: Pearson's correlation. Areas of clusters in red for individual cell groups define the number of upregulated cell lineage genes (number shown in parenthesis) compared with green areas with lower expressions of the same genes (horizontally) in other cell types. (**a**) Common myeloid cells (DCs, macrophages, and monocytes), b skin-resident cells (fibroblasts and keratinocytes) (**c**) leukocytes (monocytes, macrophages, DCs, and T cells), and d multiple cells (DCs, monocytes macrophages, T cells, fibroblasts, or keratinocytes). (**b**) Examples of normalized gene expressions of the cell groups described in (**a**). The expressions of the representative group lineage genes are 1.5-fold greater than in other cell types (blue areas) (Tables S7A–10 list all genes).

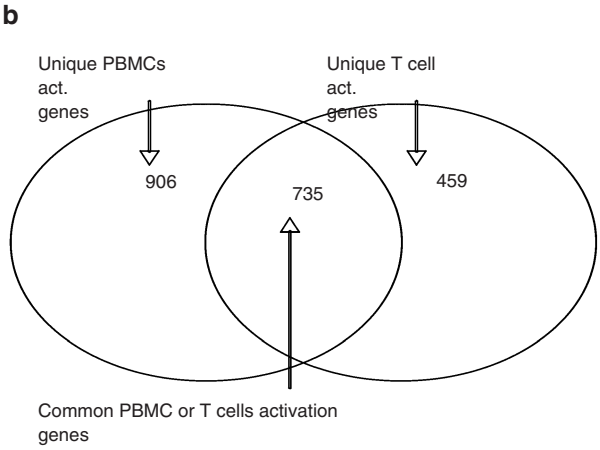
genes: 974 genes were lineage-specific and 121 were uniquely associated with cellular activation (gene lists are available in Tables S1B–2).

We had previously identified ~63 psoriasis genes from the literature as being associated with the *IFN-γ* pathway (Lew *et al.*, 2004). Our current analysis of all the psoriasis genes defined many more *IFN-γ*-associated genes (143) to be uniquely regulated by *IFN-γ*. To our surprise there were fewer psoriasis genes (27) that were regulated either uniquely by TNF or in combination with *IFN-γ* (40).

We further cell-typed other cytokines, chemokines, and proinflammatory genes in psoriasis (Figure 5). Several key observations can be made. A combination of cell types, rather than a single cell type, contributed these genes. The expression of these cytokines was highly represented in the common myeloid cells (~60%). The majority of genes expressed in DCs were associated with mature DCs rather than immature DCs. Approximately 40% of these genes were associated with T cell activation. Many genes in keratinocytes were uniquely expressed in response to *IFN-γ*. Several genes were commonly regulated by cytokines such as *IFN-γ*, *TNF*, or *IL-20*, which suggests an important role of these genes in psoriatic epidermal hyperplasia.

Our analysis revealed that the majority of genes in psoriasis were expressed simultaneously in different cell types. However, we were also able to determine which proinflammatory genes in psoriasis were uniquely expressed in either resting or activated cells. We were able to identify





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	Fold changes: ratio of normalized expression in treated versus control			
	T cell act. /control	PBMC act. /control	Symbol	Gene product
T cell and PBMC	4.2	4.8	IL2	Human interleukin-2 (IL-2) gene and 5′-flanking region
	16.7	5.9	IFNG	Homo sapiens interferon-gamma (IFNG) gene
	4.0	11.2	LTA	Lymphotoxin alpha (TNF superfamily, member 1)
	7.1	2.4	CD69	CD69 antigen (p60, early T cell activation antigen)
PBMC	ND	4.6	IL1B	Interleukin 1, beta
	ND	18.2	MMP12	Matrix metalloproteinase 12 (macrophage elastase)
T cell	5.8	ND	IRF4	Interferon regulatory factor 4
	8.4	ND	XCL2	Homo sapiens DNA for SCM-1beta precursor

Figure 3. Defining cell activation genes. mRNA from cultured cells was hybridized to individual oligonucleotide arrays containing ~12,000 human genes (Affymetrix HG-U95Av2 chips). Heatmaps show unsupervised hierarchical clusters of upregulated genes in activated cells (>1.2-fold compared with control; $P<0.05$, $n=2$) using the similarity measure: Pearson's correlation. Areas of clusters in red for activation genes define the number of upregulated genes (number shown in parenthesis) as compared with green areas with lower expressions of the same genes (horizontally) in controls. (**a**) Genes found in anti-CD3/CD28-treated T cells and PBMCs, (**b**) Venn diagram describes the number of unique and common genes in activated cells. (**c**) Examples of normalized gene expressions in activated T cells and PBMCs. Ratios of gene expressions of selected genes in each activated cell type compared with control are greater than 1.2-fold in treated versus control ($P<0.05, n=2$), ND = not detected. (Table S12A lists all genes).

several cytokines (Figure 5) to be uniquely expressed in fibroblasts (cehmokine (C-X-C-motif) Ligand 12 (*CXCL12*), fibroblast growth factor 2, and nerve growth factor-β), T cells (*IL-4* and LIB), and monocytes *(S100A12)*. We were also able to classify genes like *CXCL1*, *DEFB4*, and *IL-12B* that were expressed uniquely in activated cells like mature DCs, activated T cells and *TNF*, *IFN-γ*, or *IL-20*-induced genes in keratinocytes (gray highlighted genes in Figure 5).

DISCUSSION

In this study, we profiled the genes expressed in multiple cell types that were associated with the skin in a steady state, and also the cell types that infiltrate the skin in inflammatory diseases. This approach leads to the identification of relatively large sets of genes that have their expression restricted to one cell type (lineage genes) or to groups of related cells. Furthermore, this innovative approach gives an overview of the genes, and the associated cell types of a diseased tissue. These gene lists contain not only known genes that have been previously used to classify unique cell lineages, for example expression of *CD3* (T cell receptor) in T cells or keratins in keratinocytes, but also many other genes not previously appreciated as having cellular restriction. In addition, we identified genes that were uniquely associated with specific activation or differentiation states of cells (activation genes), that is leukocyte activation, cytokine-induced transcriptional activation of keratinocytes, and maturation/activation of dendritic antigen-presenting cells. The sum of these data provide a set of cell lineage and activation genomic maps that can be used to help dissect cellular processes, which occur in a wide range of skin diseases. Complete lists of cell lineage and activation gene sets are provided as Supplementary Material in this report, so that researchers working on skin diseases can have ready access to the needed data sets.

Microarrays provide excellent tools to identify genes for many functions: interpreting cell types in a given disease, classification of disease ''signatures'', describing biological

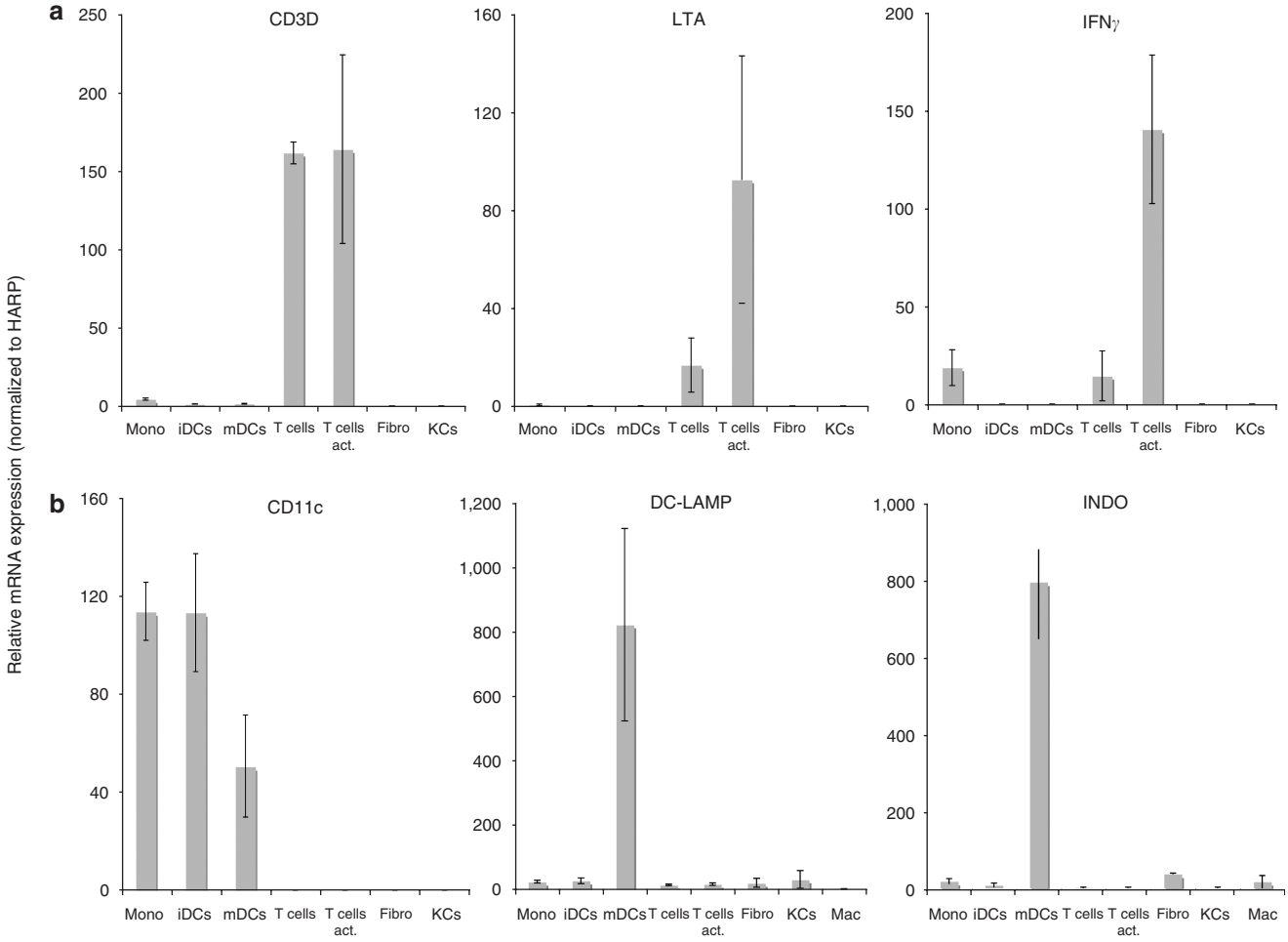


Figure 4. Lineage- and activation-specific genes: mRNA analysis with real-time RT-PCR from cultured monocytes (mono), immature DCs (iDC), mature DCs (mDC), T cells controls and after activation with anti-CD28/CD3 antibodies (T cell act.), fibroblasts (Fibro), keratinocytes (KC), and macrophages (Mac). (**a**) Genes expressed in resting and activated T cells: CD3D, lymphotoxin-α, and IFN-γ. (**b**) Genes expressed in resting and activated myeloid cells: CD11c, DCs-LAMP (LAMP 3) and indoleamine-pyrrole 2,3-dioxygenase (INDO). Mean of relative mRNA expression normalized to human acidic ribosomal protein (HARP) is plotted on column bars: SEM, $n = 2$–4.

pathways, estimating clinical outcomes, and also predicting response to drugs. In a seminal study, Golub *et al.* (1999) predicted the cellular contribution in leukemia as being either myeloid or lymphoid using array data. Cutaneous diseases have also been characterized to determine their ''genomic signature'' such as basal cell carcinoma (O'Driscoll *et al.*, 2006), squamous cell carcinoma (Haider *et al.*, 2006; Nindl *et al.*, 2006), and atopic dermatitis (Nomura *et al.*, 2003). The role of pathways and cytokines such as IFN-γ has been deduced from microarray data (Crow *et al.*, 2003). Predictions of clinical outcomes are becoming possible by meta-analyses of microarray data sets, such as cancer survival (Lyman and Kuderer, 2006). Some efforts have been also made to describe responses to cancer drugs based on array data (Cheok *et al.*, 2003). A database of gene-expression signatures of human diseases and their responses to therapeutic drugs may be a useful tool and should be made available to scientists. Integration of such a database has been conceptualized by Gullans (2006). ''Connectivity maps'' are an example of this approach and contain gene-expression profiles of human cells lines cultured *in vitro* and exposed to different small bioactive molecules (Lamb *et al.*, 2006). These maps led to the proposal of a combination of dexamethasone and sirolimus as an effective approach in the treatment of acute lymphoblastic leukemia. All these uses of microarray data will be enhanced by the application and correlation of our cell maps to array analysis.

In this study, we illustrate how lineage and activation gene maps can be applied to inflammatory dermatoses by re-analyzing genomic data in psoriasis vulgaris. Our expanded analysis produces expected and unexpected results. Given that many different cell types contribute to the histopathology of psoriasis, it is not a major surprise to find that gene products of many different cell types contribute to the summed genomic signature (>1,200 genes), generated from profiling biopsies of full-thickness skin lesions. From the standpoint of cell types that produce cytokines, chemokines, and other inflammatory products, it is also clear that this

Table 1. Number of psoriasis genes in disease-associated cell types

Cell types	Genes upregulated in P/NL (1210)
(a) Lineage genes	
Monocyte	69
Macrophages	35
DC	18
Common myeloid	115
Leukocytes	83
T cells	58
Fibroblasts	44
Keratinocytes	118
Skin-resident cells	64
Multiple cells	115
Housekeeping	255
Total lineage	974
(b) Unique activation	
Cytokine activation (35)	
Unique cytokine (not T cell activation)	12
T cell activation (PBMC or T cell) (93)	
Unique T cell activation (not cytokine activation)	59
Common cytokine, T cell activation, or mDC	38
mDC (40)	
Unique mDC	12
Total unique activation	121
(c) Summary	
Not classified	115
Total psoriasis genes	1,210
% classified	90.5

DC, dendritic cells; mDC, mature mDC; PBMC, peripheral blood mononuclear cells.
Overview of number of genes contributed by different cell types and groups (presented in Figures 1–3) that are associated with psoriasis genes. Complete list of all genes is in Table BS1 and S2.

global inflammatory environment is produced by many different cell types, with a strong likelihood that paracrine effects of these products produce a dense web of cytokine: cell interactions take place in ways that are interdependent and interactive for this specific disease state. At the genomic level, cell lineage gene maps produce a view of molecular pathogenesis that is somewhat different from the view generated by immunohistochemical phenotyping of disease-associated cellular alterations. In terms of infiltrating leukocytes, psoriasis contains quite large populations of both T lymphocytes and *CD11c*$^+$ DCs, with a slight numerical advantage of the T cells (Lowes *et al.*, 2005). At the level of gene expression, the signature of *CD11c*$^+$ myeloid cells and DCs is much more prominent than that of T cells, with about a 5:1 ratio of myeloid versus T cell expressed genes being measured. Clearly, activation products of both T cells and DCs are present, which argues that both cell lineages are intimately involved in molecular pathogenesis. However, the very large number of expressed genes, which are associated with activated/mature DCs, bolsters the emerging view that inflammatory DCs and mature DCs are key cell types in the pathogenesis of psoriasis (Lowes *et al.*, 2005, 2007; Nestle *et al.*, 2005).

Use of our genomic cellular maps helps dissect disease pathology. These must be considered as a starting point to illustrate the principle that having this type of information may be important to understanding the molecular pathology of skin diseases. If progress along this pathway is to continue, then much more work will be needed to develop more accurate and precise maps of all cell types that are found in the skin. In our approach, we analyzed cell types that could be obtained in abundant quantities from a culture of blood or skin *in vitro*. These cell sets are almost certainly different from *in vivo* counterparts in critical ways, for example in the use of selective growth factors or cytokines to obtain the cell populations or in the differentiation states attained. The genes, for example, expressed in cultured keratinocytes will clearly have fewer differentiation-specific genes for terminal stages of corneocyte differentiation. In addition, there are many cell types in the skin that we have not incorporated into this early analysis and with the addition of those cell types, for example endothelial cells, smooth muscle cells, or melanocytes, the lineage gene lists we show are likely to become smaller and more specific. We believe that this line of work should be extended, as we are at a scientific junction where skin diseases may be more accurately defined by genomics than by conventional histopathology and where therapeutic approaches may be guided by the molecular definition of diseases.

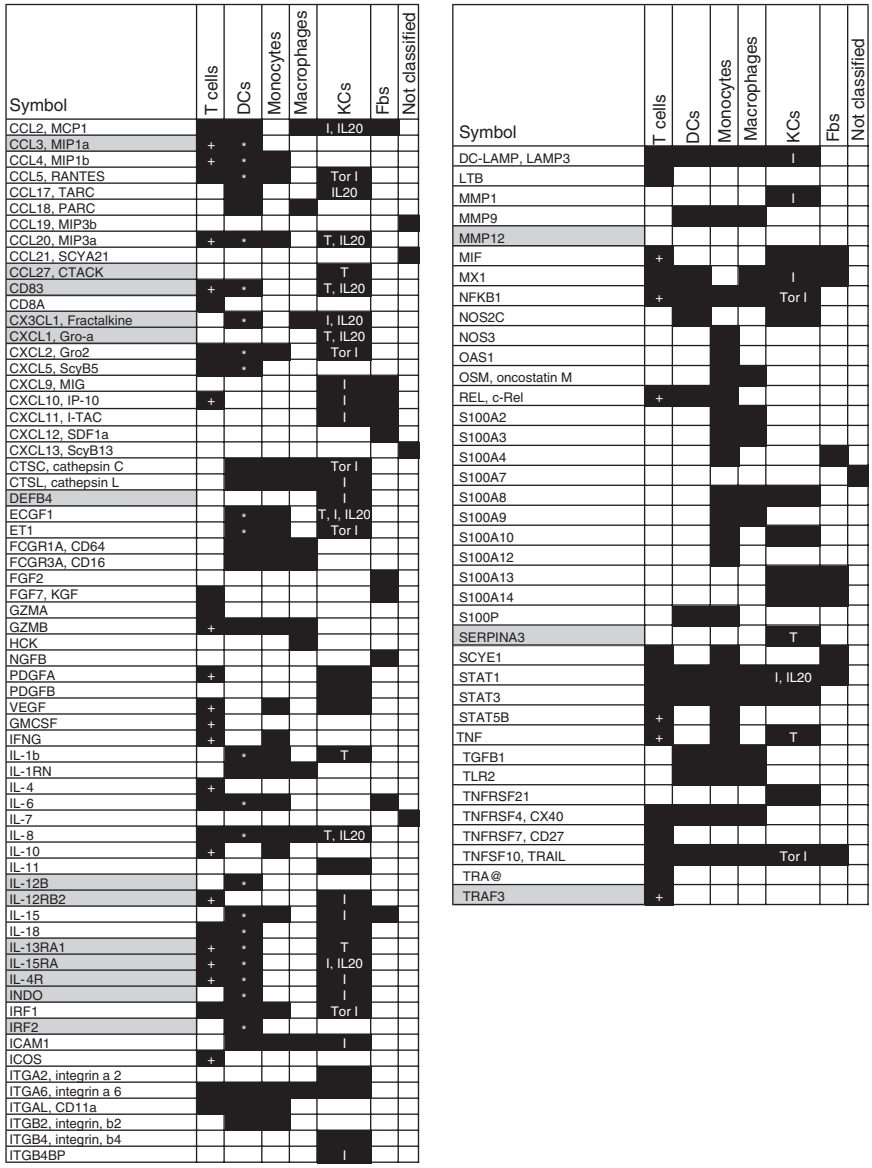
MATERIALS AND METHODS

The Institutional Review Board's approval was obtained before inviting healthy volunteers or patients with psoriasis to participate in the study. Informed consent was obtained from volunteers and patients before their participation, and the study was performed with strict adherence to the Declaration of Helsinki Principles.

Skin samples and cell cultures

Skin punch biopsies from psoriasis lesional and non-lesional skin were obtained from eight patients with psoriasis as described previously (Bowcock *et al.*, 2001). Comparison of these groups with normal skin was beyond the scope of this paper. Major inclusion criteria were: involvement of psoriasis vulgaris of >10% body surface area, no systemic treatment for at least 4 weeks nor topical treatment for at least 2 weeks before entering the study, no significant infections or immune suppression, and no significant renal, hepatic, or other medical disease.

PBMCs and T cells: PBMCs from two donors were isolated from heparinized human blood using Ficoll-Paque Plus according to the standard protocol. T cells were negatively isolated using Dynal®



Symbol	T cells	DCs	Monocytes	Macrophages	KCs	Fbs	Not classified
CCL2, MCP1					I, IL20		
CCL3, MIP1a	+	*					
CCL4, MIP1b	+	*					
CCL5, RANTES		*			Tor I		
CCL17, TARC					IL20		
CCL18, PARC							
CCL19, MIP3b							
CCL20, MIP3a	+	*			T, IL20		
CCL21, SCYA21							
CCL27, CTACK					T		
CD83	+	*			T, IL20		
CD8A							
CX3CL1, Fractalkine		*			I, IL20		
CXCL1, Gro-a					T, IL20		
CXCL2, Gro2		*			Tor I		
CXCL5, ScyB5		*					
CXCL9, MIG					I		
CXCL10, IP-10	+				I		
CXCL11, I-TAC					I		
CXCL12, SDF1a							
CXCL13, ScyB13							
CTSC, cathepsin C					Tor I		
CTSL, cathepsin L					I		
DEFB4					I		
ECGF1		*			T, I, IL20		
ET1		*			Tor I		
FCGR1A, CD64							
FCGR3A, CD16							
FGF2							
FGF7, KGF							
GZMA							
GZMB	+						
HCK							
NGFB							
PDGFA	+						
PDGFB							
VEGF	+						
GMCSF	+						
IFNG	+						
IL-1b		*			T		
IL-1RN							
IL-4	+						
IL-6		*					
IL-7							
IL-8		*			T, IL20		
IL-10	+						
IL-11							
IL-12B		*					
IL-12RB2	+				I		
IL-15		*			I		
IL-18		*					
IL-13RA1	+	*			T		
IL-15RA	+	*			I, IL20		
IL-4R	+	*			I		
INDO		*			I		
IRF1					Tor I		
IRF2		*					
ICAM1					I		
ICOS	+						
ITGA2, integrin a 2							
ITGA6, integrin a 6							
ITGAL, CD11a							
ITGB2, integrin, b2							
ITGB4, integrin, b4							
ITGB4BP					I		

Symbol	T cells	DCs	Monocytes	Macrophages	KCs	Fbs	Not classified
DC-LAMP, LAMP3					I		
LTB							
MMP1					I		
MMP9							
MMP12							
MIF	+						
MX1					I		
NFKB1	+				Tor I		
NOS2C							
NOS3							
OAS1							
OSM, oncostatin M							
REL, c-Rel	+						
S100A2							
S100A3							
S100A4							
S100A7							
S100A8							
S100A9							
S100A10							
S100A12							
S100A13							
S100A14							
S100P							
SERPINA3					T		
SCYE1							
STAT1					I, IL20		
STAT3							
STAT5B	+						
TNF	+				T		
TGFB1							
TLR2							
TNFRSF21							
TNFRSF4, CX40							
TNFRSF7, CD27							
TNFSF10, TRAIL					Tor I		
TRA@							
TRAF3	+						

Figure 5. Cytokines, chemokines, and proinflammatory genes in resting and activated cells in psoriasis. Black highlights describe the presence of the gene in the listed cell types. (+): activation genes in T cells (*): genes expressed in mature DCs. T: genes induced by TNF in keratinocytes. I: genes induced by IFN-γ in keratinocytes; IL-20: genes induced by IL-20 in keratinocytes. Grey highlight: genes uniquely expressed in activated cells.

T-Cells Negative Isolation Kit (Dynal Biotech, Invitrogen Corp, Carlsbad, CA, Cat. no. 113–11D) according to the manufacturer's protocol. Non-stimulated PBMCs or T cells served as control to stimulation with anti-*CD3/CD28* using Dynabeads® *CD3/CD28* T Expander (Dynal Biotech) according to the manufacturer's protocol. All reagents were added at time 0 and cells and supernatant were collected at 24 hours after stimulation. Activated PBMCs and T cell phenotype were confirmed by flow cytometry (*CD69* expression on $CD3^+$ cells). PBMCs or T cells were isolated and stored at −80°C until required, as described previously (Chamian *et al.*, 2005).

Monocytes: Monocytes were obtained from three donors as described previously (Wang *et al.*, 2006). Leukocyte-enriched blood was obtained from the New York Blood Bank, and processed as above to obtain PBMCs. Monocytes were isolated from PBMCs using a Monocyte Negative Isolation Kit (Dynal Biotech). Monocyte phenotype was confirmed by flow cytometry (*CD14* expression).

DCs: *In vitro*-derived DCs from three donors were generated as described previously (Lee *et al.*, 2002). Immature and mature DCs phenotype was confirmed by flow cytometry ($CD86^+CD83^{lo}$ and $CD86^+CD83^{hi}$, respectively).

Macrophages: *In vitro*-derived macrophages from two donors (in duplicates) were generated from monocytes by a standard method (Davis and Gordon, 2005). Macrophage phenotype was confirmed by flow cytometry ($CD11b^+$, $CD11c^+$, $CD68^+$, $HLA\text{-}DR^+$, $CD86^+$, $C83^-$).

Fibroblasts: Fibroblasts from two donors were grown from isolated foreskin and cultured in Dulbecco's modified Eagle's medium (Gibco-BRL Life Technologies, Gaithersburg, MD) + 10% heat-inactivated human serum (Hyclone, Invitrogen Corp,

SH30071.03,) and PenStrep (Penicillin 10 kU/ml, Streptomycin 10 mg/ml) (Invitrogen Corp. Carlsbad, CA, Cat. no. 15140–148). Cultures were set up in 100 ml/dish (Corning, NY, Cat. no. 25020) until confluent.

The cultured cells were lysed with 2 ml per dish of Rneasy lysis buffer (RLT) buffer (Qiagen, Valencia, CA; plus10 μl/ml 2-mercapto ethanol), and stored at -80°C for later RNA preparation.

Cell phenotypes

PBMCs and T cell activation, monocytes, DCs, macrophage.

Cells were stained for 15 minutes at room temperature with the following antibodies to phenotype: monocytes, *CD14*; PBMCs and T-cell activation, *CD69 and CD3* (Becton Dickinson, San Jose, CA); DCs, *CD86* (Becton Dickinson) and *CD83* (Immunetech, Westbrook, ME); macrophages, *CD86* (Becton Dickinson), *CD83* (Immunetech), *CD11b* (Becton Dickinson), *CD11c* (BioLegend, San Diego, CA), *CD68* (DakoCytomation, Dako, Carpinteria, CA) and *HLA-DR* (Becton Dickinson) conjugated to either phycoerythrin, peridinin-chlorophyll-protein, or allophycocyanin (Becton Dickinson) as described previously (Chamian *et al.*, 2005).

Microarray analysis

RNA was extracted from the samples as published previously (Haider *et al.*, 2006). U95Av2-set GeneChip probe microarrays (Affymetrix Inc., Santa Clara, CA) were used containing probe sets representing approximately 12,000 genes. Fragmentation and array hybridization were carried out according to the manufacturer's instructions (Affymetrix Inc.). The raw data of control and cytokine-activated (*TNF and IFN-γ*) keratinocytes microarray was used from a previous publication (Banno *et al.*, 2003, 2004). The raw GeneChip data for psoriasis has also been published previously (Haider *et al.*, 2006). Scanning and quality control, GeneChip expression value analysis, hierarchical clustering, and heatmaps were performed as described previously (Haider *et al.*, 2006). A heatmap of the computed tree represents unsupervised hierarchical clustering and is presented by red and green lines. Each line presents genes with relatively upregulated (red) or downregulated (green) expression value in fold changes. Gene Ontology annotations of differentially expressed genes were collected from LocusLink (http://www.ncbi.nlm.nih.gov/LocusLink).

Cell lineage classification and statistical analysis

The determination of the cell lineage maps involved two steps. First, expression values were analyzed with a one-way analysis of variance using cell types as factors. *P*-values were adjusted for multiple hypothesis testing using the Benjamini–Hoechberg procedure. Genes with false discovery rate <0.05 were considered for further analysis. These sets of genes (7,909 genes) were the ones where the mean expression values differ in at least one of the factors and was further subjected to the second step: selection of genes that defined lineage characteristic of a cell type or cell group. This was achieved by arbitrarily taking 1.5 as the normalized mean expression value or as a cutoff number such that any gene that was expressed at 1.5 or higher value in a cell type or cell group compared with each and all other cell types was defined as lineage gene.

The following conditions were defined:

(1) *Lineage genes in the following cell types*: keratinocytes, fibroblasts, macrophages, monocytes, T cells, and immature and mature DCs.

(2) *Common genes in the following cell groups*:

a. common myeloid cells (DCs, macrophages, and monocytes),
b. skin-resident cells (fibroblasts and keratinocytes)
c. leukocytes (monocytes, macrophages, DCs, and T cells)
d. multiple cells (DCs, monocytes macrophages, T cells, fibroblasts, and keratinocytes).

Housekeeping: gene expressed in lower than the 1.5 normalized expression value in all cell types.

Gene lists for these analyses are available in Table S1.

Cell activation classification

To look for genes that were significantly different between activated cells and controls, a one-way analysis of variance was used. *P*-values were adjusted for multiple hypothesis testing using the Benjamini–Hoechberg procedure and genes with false discovery rate <0.05 and fold change of 1.2 or above were considered.

The following conditions were defined:

(1) *T cell and* PBMCs *activation genes*: genes expressed in anti-*CD3/CD28*-treated T cells and PBMCs were compared with those in untreated controls.
(2) *Cytokine activation of keratinocytes*: genes expressed in *TNF* and *IFN-γ*-treated keratinocytes were compared with those in untreated controls.

Gene lists for these analyses are available in Table S1A.

Re-classification of a common inflammatory dermatosis using cell-specific gene maps: psoriasis vulgaris

Genes upregulated in psoriasis lesional versus non-lesional were classified into lineage genes, as obtained from the analysis above.

Gene lists are available in Table S1B.

Expression changes in mRNA with real-time RT-PCR analysis

The primers and probes for these genes for the TaqMan RT-PCR assays were generated with the Primer Express algorithm, version 1.0, using published genetic sequences (NCBI-PubMed) for each gene. The human acidic ribosomal protein (hARP) gene, a housekeeping gene, was used to normalize the expression of each gene. The primer sequences for *hARP* and *IFN-γ* have been published (Chamian *et al.*, 2005). The primers for certain genes were designed by Applied Biosystems (Foster City, CA): *CD3D* (assay ID Hs00174158_m1) and indoleamine-pyrrole 2,3-dioxygenase (assay ID Hs00158027_m1). All primers were purchased from Applied Biosystems: lymphotoxin-α forward, TTGGCCTCACACCTTCAGCT; lymphotoxin-α reverse, TGCTGTGGGCAAGATGCA; lymphotoxin-α probe, 6FAM-TAMRAAGACTGCCCGTCAGCACCCCAATAMRA (GeneBank accession no. NM_000660); *CD11c* forward, GCCACCGCCATC CAAAA; CD11c reverse, TCCCTACGGGCCCCATAT; *CD11c* probe, 6FAM-TCGTGCACCGATTGTTCCATGCCT-TAMRA (GeneBank accession no. NM_021334); *DC-LAMP/LAMP3/CD208* forward, TTGTCACCCAGGCTGGAGTAC; DCs-LAMP reverse, AATCACTT GAACCCGGGAGG; DC-LAMP probe, 6FAM-TGGCACGATCTC GGCTTATGGCAA-TAMRA (GeneBank accession no. AJ005766). The RT-PCR reaction was performed using EZ PCR Core Reagents (Applied Biosystems) according to the manufacturer's directions and as described previously (Haider *et al.*, 2006).

Tables with all gene lists can be downloaded from http://www.rockefeller.edu/labheads/krueger/supplemental.php

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SUPPLEMENTARY MATERIAL

Table A1S. Genes expressed in keratinocytes.

Table A2S. Genes expressed in fibroblasts.

Table A3S. Genes expressed in macrophages.

Table A4S. Genes expressed in monocytes.

Table A5S. Genes expressed in T cells.

Table A6S. Genes expressed in iDCs, mDCs or both.

Table A7S. Genes expressed commonly in myeloid derived cells (monocytes, macrophages, iDCs and mDCs).

Table A8S. Genes expressed in skin-resident cells (fibroblasts and keratinocytes).

Table A9S. Genes expressed in leukocytes (T cells, monocytes, macrophages, iDCs and mDCs).

Table A10S. Genes expressed in multiple cells (more than one cell type).

Table A11S. Housekeeping genes: expressed with <1.5 expression levels in all cell types.

Table A12S. Activation genes in PBMCs as compared to T cells.

Table A 13–15 S. Genes induced by IFN-γ and TNF in keratinocytes at 1, 4, 24 or 48-hours post treatment.

Table B1S. Genes upregulated in psoriasis and cell typed.

Table B2S. Unique activation genes upregulated in psoriasis.

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