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Comprehensive gene expression profile of human activated T_h1- and T_h2-polarized cells

Shigenori Nagai, Shin-ichi Hashimoto, Taro Yamashita, Nobuaki Toyoda, Taku Satoh, Takuji Suzuki and **Kouji Matsushima**

Department of Molecular Preventive Medicine, School of Medicine and CREST, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

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Abstract

In response to antigen stimulation, T_h cells differentiate into two types of effector cells, T_h1 and T_h2. T_h1 cells predominantly mediate cellular immunity, whereas T_h2 cells induce humoral allergic responses. We have conducted here serial analysis of gene expression (SAGE) in human activated T_h1- and T_h2-polarized cells from cord blood. SAGE analysis of 64,510 tags (32,219 and 32,291 tags from T_h1 and T_h2 cells respectively) allowed identification of 22,096 different transcripts. In activated T_h1 cells, many of the known genes (12 genes, $P < 0.01$; 56 genes, $P < 0.05$), including genes encoding IFN-γ, lymphotactin, osteopontin, MIP-1α, MIP-1β, perforin, β-catenin and CD55, are highly expressed. On the other hand, in activated T_h2 cells rather limited numbers of known genes (four genes, $P < 0.01$; 10 genes; $P < 0.05$), such as genes encoding FUS, ILF-2, IL-13 and E2-EPF, are found to be selectively expressed. The comprehensive identification of genes selectively expressed in human activated T_h1 or T_h2 cells should contribute to our understanding of the molecular basis of T_h1/T_h2-dominated human diseases and may provide genetic information to diagnose these diseases.

Introduction

T_h lymphocytes are classified into two subsets based on their cytokine production profile (1). T_h1 cells produce a large amount of IFN-γ, whereas T_h2 cells produce IL-4, IL-5, IL-10 and IL-13. Development of T_h1 cells is driven by IL-12 produced by macrophages and dendritic cells via the transcription factor Stat4 signaling. On the other hand, commitment to the T_h2 lineage is induced by IL-4 via the Stat6 signaling pathway. T_h1 cells mediate delayed-type hypersensitivity responses, and provide protection against intracellular pathogens and viruses, whereas T_h2 cells promote B cells to produce IgE and contribute to the eradication of extracellular parasites, but also induce atopic reaction.

Significant progress has been made in identifying transcription factors that control the transition of naive T cells to the T_h1 or T_h2 lineage. c-Maf induces endogenous IL-4 production in non-T lineage cells (2) and GATA-3 promotes the expression of a broad spectrum of T_h2-specific cytokines, even in developing and committed T_h1 cells (3). In contrast, T-bet, which is one of the T-box family of transcription factors, has been recently identified as a key molecule in T_h1 differentiation, which converts even committed T_h2 cells into T_h1 cells (4).

Furthermore, hematopoietic prostaglandin D synthase, which is well known as a key enzyme involved in prostanoid production by allergen-provoked mast cells, is preferentially produced in T_h2 clones (5) and chemoattractant receptor CRTH2 is selectively expressed on the cell surface of T_h2 cells (6). In contrast, the ligands of E-selectin and P-selectin are selectively expressed on the surface of T_h1 cells (7).

With regard to chemokine receptors, CXCR3 and CCR5 are preferentially expressed on human T_h1 cells. On the other hand, CCR4 is preferentially expressed on T_h2 cells (8,9). The differential expression of chemokine receptors on each type of T_h cell is thought to be critical to the selective cell migration of these cells into the particular immune/inflammatory sites. $CCR5^+$ $CD4^+$ T cells of the T_h1 phenotype selectively accumulate in inflamed joints of rheumatoid arthritis (10), and CCR4-bearing T_h2 cells are recruited by CC chemokines, thymus and activation-regulated chemokine (TARC), and macrophage-derived chemokine (MDC) (11), and the size of this population is much increased in human atopic diseases (K. Kurashima *et al*. and T. Miyawaki *et al*., unpublished observations). With regard to chemokines, preferential or high expression of macrophage inflammatory protein (MIP)-1α, MIP-1β, and regulated upon activation,

Correspondence to: K. Matsushima

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normal T cell expressed and presumably secreted (RANTES) in T_h1 cells has been reported (12,13).

Serial analysis of gene expression (SAGE) allows for the establishment of both a representative and comprehensive different gene expression profile in various cell types and organs under physiological and pathological states (14). Since each template contains identifiable tags corresponding to many genes, this method allows global gene expression profiling including unknown genes. In this study, we have analyzed here the expression profiles in activated T_h1- and T_h2-polarized cells using SAGE, and newly identified numerous genes for which expression is selective in either population. We have also compared our SAGE data with a recently published gene chip analysis of human T_h1 and T_h2 cells (15).

Methods

Medium

The culture medium used throughout was RPMI 1640 supplemented with 2 mM L-glutamine, 1% non-essential amino acids, 1% pyruvate, 5×10^{-5} M 2-mercaptoethanol (Gibco/BRL, Gaithersburg, MD) and 10% FBS (JRH Biosciences, Lenexa, KS).

Generation of T_h1 and T_h2 cells from cord blood leukocytes

Human neonatal leukocytes were isolated from freshly collected, heparinized, neonatal cord blood by density-gradient centrifugation using Lymphoprep (density 1.077; Nycomed, Oslo, Norway). T_h1- and T_h2-polarized cells were generated by stimulating cord blood leukocytes with 1 μg/ml phytohemagglutinin (Gibco/BRL) in the presence of 2 ng/ml IL-12 (Genzyme Techne, Minneapolis, MN) and 200 ng/ml neutralizing anti-IL-4 antibody (34019.111; R & D Systems; Minneapolis, MN) for T_h1 cultures, and 200 U/ml IL-4 (provided by Ono Pharmaceutical, Osaka, Japan) and 2 μg/ml neutralizing anti-IL-12 antibody (C8.6; Genzyme, Cambridge, MA) for T_h2 cultures. Cells were washed on day 3 and expanded in each medium containing 4 ng/ml IL-2. At days 12–14, leukocytes were incubated with anti-CD4 mAb-coated magnetic beads and $CD4^+$ cells were isolated by passing the cultured cells through a MACS system (Miltenyi Biotec, Bergish Gladbach, Germany).

Single-cell analysis of cytokine production

Cord blood-derived T_h1 and T_h2 cells were collected after 3 days of $CD4^+$ cell separation, washed and re-stimulated with 50 ng/ml of phorbol myristate acetate (PMA) and 1 μg/ml of ionomycin (Sigma, St Louis, MO) for 4 h; 10 μg/ml of Brefeldin A (Sigma) was added during the last 2 h of the culture. Then the cells were fixed and permeabilized with IntraPrep permeabilization reagent (Immunotech, Marseille, France) according to the manufacturer's protocol. Fixed cells were stained with FITC-labeled anti-IFN-γ(4S.B3) and phycoerythrin (PE)-labeled anti-IL-4 (MP4-25D2) mAb (PharMingen, San Diego, CA), and analyzed by an Epics XL (Coulter Electronics, Hialeah, FL). The software program System II (Coulter) was used on an Epics XL.

FACS analysis

T_h1- and T_h2-polarized cells were incubated with optimal concentrations of FITC-labeled anti-CCR4 mAb (11) and PE-labeled anti-CD45RO (UCHL1) mAb (PharMingen). After staining and fixation, analysis was performed using an Epics XL (Coulter Electronics).

SAGE protocol

mRNAs of T_h1 and T_h2 cells were purified from a mixture of total RNA from four donors. Poly(A)$^+$ mRNA was isolated using the μMACS mRNA isolation kit (Miltenyi Biotec) according to the manufacturer's instructions. SAGE libraries were generated using 2.5 μg poly(A)$^+$ mRNA and were converted to cDNA with a BRL synthesis kit (Gibco/BRL) following the manufacturer's protocol, with the inclusion of primer biotin–5′-T18-3′. The outline of the SAGE protocol has been described in a previous report (16). Briefly, the cDNA was cleaved with *Nla*III and the 3′-terminal cDNA fragments were bound to Dynabeads M-280–streptavidin (Dynal, Oslo, Norway). After ligation of the oligonucleotides containing recognition sites for *Bsm*F1, the bound cDNA was released from the beads by digestion with *Bsm*F1. SAGE tag overhangs were filled in with Klenow, and tags from the two pools were combined and ligated to each other. The ligation product was amplified with PCR, concatemerized and cloned into the *Sph*I site of pZero-1 (Invitrogen, Carlsbad, CA). Samples were sequenced with the BigDye terminator kit and analyzed using a 96-lane 377 ABI automated sequencer (Perkin-Elmer, Branchberg, NJ).

Sequence files were analyzed by means of the SAGE program group and DNAsis software (Takara, Osaka, Japan). After correcting sequencing mistakes, a total of 64,510 tags representing 32,219 and 32,291 tags from T_h1 and T_h2 cells respectively were analyzed.

RT-PCR

The RNA was reverse transcribed in 50 μl of 10 mM Tris–HCl (pH 8.3), 6.5 mM $MgCl_2$, 50 mM KCl, 10 mM DTT, 1 mM of each dNTP, 2 μM random hexamer and 2.4 U/μl of Molony murine leukemia virus reverse transcriptase for 1 h at 42°C. The conditions for PCR were as follows: in a 50 μl reaction, 15 μM of each primer, 125 μM each of dNTP mixture (Toyobo, Osaka, Japan), 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$ and AmplyTaq (Perkin-Elmer). Primers used were as follows. Osteopontin: sense 5′-TGGCTAAACCC-TGACCCATCT-3′, antisense 5′-TGGATGTCAGGTCTGCG-AAA-3′; GAPDH: sense 5′-CCTTCATTGACCTCAACTAC-3′, antisense 5′-ACCACAGTCCATGCCATCACT-3′; FUS: sense 5′-AACGGGACAGCCCATGATT-3′, antisense 5′-GGGCCTTA-CACTGGTTGCATT-3′; IFN-γ: sense 5′-CTGTTACTGCCAG-GACCCATATGTAAAAG-3′, antisense 5′-CAACCATTACTG-GGATGCTCTTCGACCTTG-3′; MIP-1β: sense 5′-CCGCCT-GCTGCTTTTCTTAC-3′, antisense 5′-TGACAGTGGACCATC-CATAGGG-3′; IL-13: sense 5′-TCAATCCTCTCCTGTTGGC-AC-3′, antisense 5′-CGTCCCTCGCGAAAAAGTTT-3′; lympho-tactin: sense 5′-AGACTTCTCATCCTGGCCCT-3′, antisense: 5′-GCCAGAGACTACTAGCCAGTCA-3′; IL enhancer binding factor (ILF)-2: sense 5′-TTCCTTCAGTGAGGCCTTGCT-3′, antisense 5′-GAAGATTGGGTGGCACTGTTG-3′.

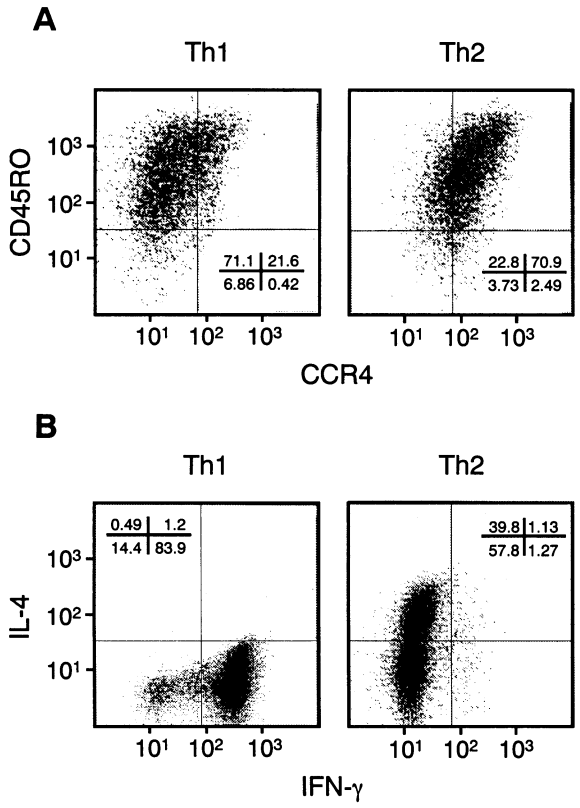

Fig. 1. Polarization of T_h1 and T_h2 cells from cord blood cells. (A) Surface CCR4 expression on T_h1- and T_h2-polarized cells. Both cells were memory ($CD45RO^+$) subsets and T_h2-polarized cells were preferentially expressed in CCR4. (B) Cytokine expression profile of T_h1- and T_h2-polarized cells stimulated with PMA and ionomycin.

Reaction mixtures were incubated in a Perkin-Elmer DNA thermal cycler for 25–35 cycles (denaturation for 30 s at 94°C, annealing for 60 s at 59°C and extension for 60 s at 72°C).

Statistical analysis

Statistical significance between samples was calculated using the equation:

$$(N_1 - kN_1^{1/2}) - (N_2 + kN_2^{1/2})$$

where N_1 and N_2 represent the larger and smaller of the two numbers respectively, and k is the degree of confidence; $P = 0.05$ ($k = 1.96$) and $P = 0.01$ ($k = 2.58$). Positive values derived from the equation were deemed statistically significant at the respective confidence intervals (17).

Results and discussion

SAGE technology can provide quantitative and simultaneous analysis of large numbers of transcripts. In this study, we investigated human activated T_h1- and T_h2-polarized cells. Surface CCR4 expression and the cytokine expression profile were analyzed on these two subsets (Fig. 1A and B). CCR4 has been reported to be selectively expressed on T_h2 cells (11). Our results confirmed that the lymphocytes derived from cord blood cells were well differentiated into T_h1 or T_h2 cells. The mRNAs were prepared from these cells after stimulation with PMA and ionomycin for 6 h, and were processed to SAGE analysis. A total of 64,510 tags, including 32,219 and 32,291 tags from activated T_h1 and T_h2 cells respectively, allowed identification of 22,096 different transcripts. The expressed genes were searched for through the GenBank database to identify individual genes. The top 50 transcripts in two subsets are listed in Table 1. Twenty-four and 25 out of the top 50 transcripts were ribosomal proteins in activated T_h1 and T_h2 cells respectively. However, many genes identical to the cDNAs of secreted proteins were also identified. A T_h1 cytokine, IFN-γ, was greatly expressed in activated T_h1 cells, whose expression frequency is 5.78%. High expression of genes encoding granulocyte macrophage colony stimulating factor (GM-CSF) (0.84%), MIP-1β (0.83%), IL-2 (0.82%), IL-3 (0.74%), TNF-α (0.42%), lymphotactin (0.38%), granzyme B (0.36%) and MIP-1α (0.28%) were also observed in activated T_h1 cells. On the other hand, the genes encoding IL-2 (0.37%), GM-CSF (0.36%), TNF-α (0.29%), IL-3 (0.28%), granzyme B (0.27%) and IL-13 (0.22%) were detected in activated T_h2 cells. Unlike monocyte-derived macrophages and dendritic cells (18,19), the transcripts related to cytoskeleton or cell structure are not highly expressed. This may reflect minimal morphological changes of T cells even after activation.

Expressed genes between activated T_h1 and T_h2 cells were compared, and are shown in Fig. 2. Each dot represents a gene expressed in these two subsets and the expression levels of most of the transcripts between these two subsets were very similar. However, the expression profiles also showed significant difference in many transcripts of these cells.

Tables 2 and 3 show the genes selectively expressed in activated T_h1 or T_h2 cells ($T_h1 > T_h2$, 68 genes; $P < 0.05$; $T_h1 < T_h2$, 14 genes; $P < 0.05$). The genes in the EST database or unidentified in the GenBank database are excluded from the tables and are available at http://www.prevent.m.u-tokyo.ac.jp/SAGE.html. Among cytokine and chemokine genes, IFN-γ was expressed 49.2-fold higher in activated T_h1 cells than in activated T_h2 cells. Furthermore, we identified numerous T_h1-predominantly expressed genes such as osteopontin (19.0-fold), MIP-1β (15.5-fold), lymphotactin (12.5-fold), perforin (11.0-fold), MIP-1α (6.6-fold), RANTES (6.0-fold), lymphotoxin α (5.0-fold), IL-3 (2.7-fold), GM-CSF (2.3-fold), NK enhancing factor (NKEF) (2.3-fold) and IL-2 (2.2-fold).

It has been reported that MIP-1 and RANTES, which are ligands of CCR5, chemoattract the cells related to T_h1-dominated cellular immunity (8,20,21). Interestingly, lymphotactin, expressed in activated T_h1 cells, has chemotactic activity to $CD8^+$ and NK cells (22). Furthermore, NKEF has an ability to enhance NK cytotoxicity (23). Thus, it suggests that once T_h1 cells are activated, effector cells in cellular immunity are recruited around T_h1 cells and then accelerate immune response against invading microorganisms or neoplasm. Osteopontin is not only an adhesive matrix protein (24) but also a chemotactic molecule for smooth muscle cells and T cells (25,26). The granulomatous responses in sarcoidosis and tuberculosis have been reported to be associated with high expression of osteopontin (27,28).

Only IL-13 and IL-9 were cytokines predominantly expressed in activated T_h2 cells, which are related to asthma

Table 1. Transcript profile in activated T_h1 and T_h2-polarized cells[a]

Activated Th1			Activated Th2		
Abundance (%)	Tag Sequence	GenBank match (Accession No.)	Abundance (%)	Tag Sequence	GenBank Match (Accession No.)
5.78	CCTGGTGCTT	IFN-γ (X13274)	1.07	GTGAAACCCC	multiple matches
0.89	TGTGTTGAGA	translation elongation factor 1 α1 (NM_001402)	0.92	TGTGTTGAGA	translation elongation factor 1 α1 (NM_001402)
0.83	GATAACACAT	MIP-1β (M23502)	0.87	CCTGTAATCC	multiple matches
0.84	GCAGAAGAAT	GM-CSF (M10663)	0.67	TTGGTCCTCT	ribosomal protein L41 (Z12962)
0.82	TGTGAATATG	IL-2 (V00564)	0.61	GTTGTGGTTA	β2-microglobulin (AB021288)
0.75	GTGAAACCCC	multiple matches	0.54	CAAGCATCCC	multiple matches
0.74	TTCATTTGTA	IL-3 (M17115)	0.54	CCACTGCACT	ribosomal protein L10 (M64241)
0.69	GTTGTGGTTA	β2-microglobulin (AB021288)	0.52	GCCGTGTCCG	ribosomal protein S6 (J03537)
0.66	TTGGTCCTCT	ribosomal protein L41 (Z12962)	0.51	TAGGTTGTCT	tumor protein, translationally -controlled 1 (X16064)
0.64	CCTGTAATCC	multiple matches			
0.56	AAAACATTCT	multiple matches	0.49	CCCGTCCGGA	ribosomal protein L13 (X64707)
0.52	CACAAACGGT	ribosomal protein S27 (L19739)	0.49	GCCGAGGAAG	ribosomal protein S12 (X53505)
0.50	CCCGTCCGGA	ribosomal protein L13 (X64707)	0.46	TGCACGTTTT	ribosomal protein L32 (X03342)
0.49	TAGGTTGTCT	tumor protein, translationally -controlled 1 (X16064)	0.44	GTGAAACCCT	multiple matches
			0.37	TGTGAATATG	IL-2 (V00564)
0.46	GCCGAGGAAG	ribosomal protein S12 (X53505)	0.37	CACAAACGGT	ribosomal protein S27 (L19739)
0.45	ATAATTCTTT	ribosomal protein S29 (U1497)	0.37	CCAGAACAGA	multiple matches
0.45	CCACTGCACT	ribosomal protein L10 (M64241)	0.36	GCAGAAGAAT	GM-CSF (M10663)
0.45	GCCGTGTCCG	ribosomal protein S6 (J03537)	0.36	ATAATTCTTT	ribosomal protein S29 (L31610)
0.45	TGGTGTTGAG	ribosomal protein S18 (X69150)	0.35	AAAACATTCT	ribosomal protein S6 (J03537)
0.42	CTGGGTTAAT	ribosomal protein S19 (M81757)	0.31	TGGTGTTGAG	ribosomal protein S18 (X69150)
0.42	TAGCCCCCTG	TNFα (M10988)	0.30	CGCCGGAACA	ribosomal protein L4 (D23660)
0.42	TGCACGTTTT	ribosomal protein L32 (X03342)	0.29	CGCCGCCGGC	ribosomal protein L35 (U12465)
0.38	GGATTTGGCC	multiple matches	0.29	TAGCCCCCTG	TNFα (M10988)
0.38	AATAAAATTA	lymphotactin (U23372)	0.29	AAGGTGGAGG	ribosomal protein L18a (X69150)
0.36	AAACGCTACT	granzyme B (J03189)	0.29	CTGGGTTAAT	ribosomal protein S19 (M81757)
0.36	CCGTCCAAGG	ribosomal protein S16 (M60854)	0.28	TTCATTTGTA	IL-3 (M17115)
0.33	CAAGCATCCC	multiple matches	0.28	GGATTTGGCC	multiple matches
0.31	AAGGTGGAGG	ribosomal protein L18a (X69150)	0.27	CGCTGGTTCC	ribosomal protein L11 (L05092)
0.31	CCAGAACAGA	multiple matches	0.27	AAACGCTACT	granzyme B (J03189)
0.30	GTGAAACCCT	multiple matches	0.26	CCGTCCAAGG	ribosomal protein S16 (M60854)
0.30	CGCCGCCGGC	ribosomal protein L35 (U12465)	0.26	GGGCTGGGGT	multiple matches
0.29	AGCTCTCCCT	ribosomal protein L17 (X53777)	0.25	GTGACCACGG	multiple matches
0.28	GCACCAAAGC	MIP-1α (X03754)	0.25	GCCATAAAAT	hematopoetic proteoglycan core protein (X17042)
0.28	GCAGCCATCC	ribosomal protein L28 (U14969)			
0.25	AAGACAGTGG	ribosomal protein L37a (L06499)	0.24	TTGGCCAGGC	multiple matches
0.25	GGCCGCGTTC	ribosomal protein S17 (M13932)	0.23	AGCTCTCCCT	ribosomal protein L17 (X53777)
0.24	CGCCGGAACA	ribosomal protein L4 (D23660)	0.23	AGGCTACGGA	ribosomal protein L13a (X56932)
0.24	GGGCTGGGGT	multiple matches	0.22	GGCCGCGTTC	ribosomal protein S17 (M13932)
0.23	ATGGCTGGTA	ribosomal protein S2 (X17206)	0.22	GACGACACGA	ribosomal protein S28 (U14969)
0.22	CGCTGGTTCC	ribosomal protein L11 (L05092)	0.22	GCAGCCATCC	ribosomal protein L28 (U14969)
0.22	GCCATAAAAT	hematopoetic proteoglycan core protein (X17042)	0.22	GGCTGGTCTG	ESTs
			0.22	TGGTGGGACA	IL-13 (L06801)
0.22	CTGACCTGTG	MHC class I B (D87665)	0.21	CCCCAGCCAG	ribosomal protein S3 (U14990)
0.22	GAGAGTGTCT	TIMP-1 (X03124)	0.21	GAGGGAGTTT	ribosomal protein L27a (U14968)
0.21	CTAAGACTTC	multiple matches	0.20	ATGGCTGGTA	ribosomal protein S2 (X17206)
0.21	CCCCAGCCAG	ribosomal protein S3 (U14990)	0.20	CTGACCTGTG	MHC class I B (D87665)
0.20	ATTCTCCAGT	ribosomal protein L23 (X52839)	0.20	GTTCCCTGGC	ribosomal protein S30 (X65923)
0.20	GGCAAGCCCC	multiple matches	0.20	AAAAAAAAAA	multiple matches
0.20	GACGACACGA	ribosomal protein S28 (U14969)	0.20	TCAGACGCAG	prothymosin α (M14483)
0.20	AGGCTACGGA	ribosomal protein L13a (X56932)	0.19	CTAAGACTTC	multiple matches
0.20	AGAACAAAAC	NKEF (X67951)	0.19	TGATTTCACT	multiple matches

[a]The top 50 transcripts expressed in activated T_h1 and T_h2 cells are listed. The tag sequence represents the 10 bp SAGE tag. Probable GenBank matches are listed.

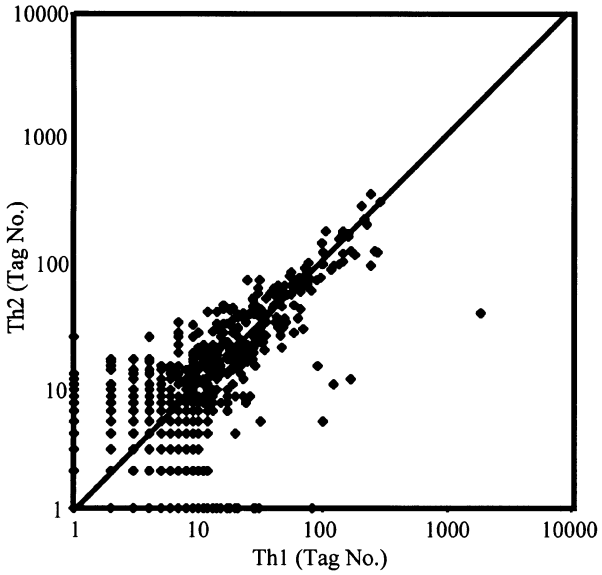

Fig. 2. Distribution of the different tags from T_h1 and T_h2 cells. The number of times each unique SAGE tag appeared was plotted on a logarithmic scale, using a total of 32,219 tags from activated T_h1 cells (*x*-axis) versus 32,291 tags from activated T_h2 cells (*y*-axis). To avoid division by 0, we used a tag value of 1 for any tag that was not detectable in one sample and the tag populations were normalized. The line with slope of a unity through the center predicts equal gene expression in the two subsets.

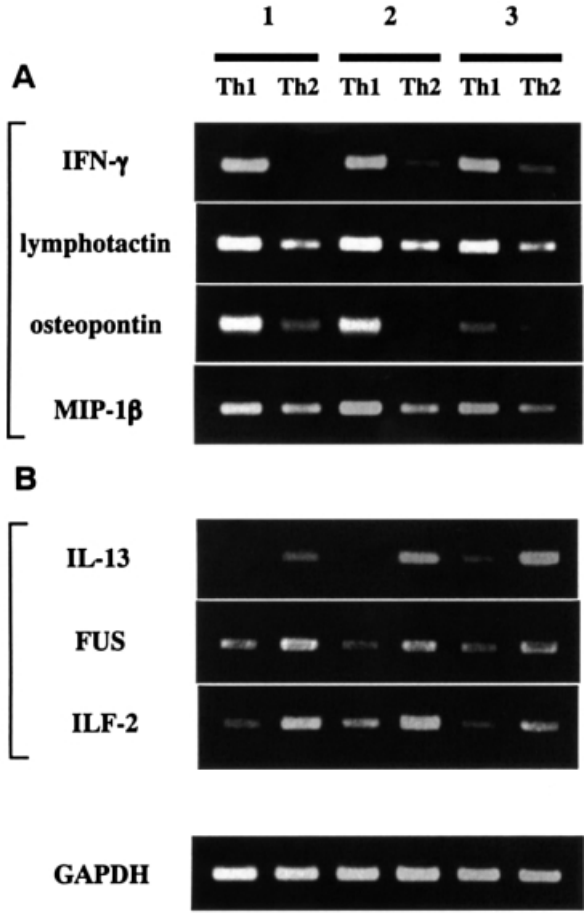

Fig. 3. RT-PCR analysis of genes expressed differently in activated T_h1 and T_h2 cells. RT-PCR was performed on total RNA isolated from both cells. Genes preferentially expressed in (A) activated T_h1 cells and (B) activated T_h2 cells.

or atopic dermatitis (29–31). The mRNA expression of IL-4 and IL-10 was not significantly different between T_h1 and T_h2 cells, and the tag of IL-5 was not detected in either library (Table 3). However, we could confirm the expression of mRNAs of these T_h2-related cytokines in activated T_h2 cells by RT-PCR (data not shown). This might be due to too low expression of these molecules to be analyzed by SAGE.

T_h1/T_h2 differentiation-regulatory genes, such as T-bet, GATA-3 and c-Maf, were not significantly different after activation. However, many immediate-early genes such as c-*jun* and c-*fos*, which propagate the cellular responses to growth stimuli, were highly induced in activated T_h1 cells. NOT (TINUR), which was originally cloned from apoptotic human T lymphoid PEER cells stimulated with PMA and calcium ionophore (32), and TRAIL, which is a member of the TNF family and mediates activation-induced cell death of mature T lymphocytes (33), were highly expressed in T_h1 cells as listed in the 'Apoptosis' category of Table 3. These results may indicate that early-responsive genes in activated T_h1 cells are up-regulated more than in activated T_h2 cells; however, apoptosis-related genes are also induced quickly in activated T_h1 cells in order to eliminate over-activated T_h1 cells. ILF-2 (8.0-fold in activated T_h2 cells) is one of the components of NF-AT (34) and the activation of NF-AT is reported to be involved in the effector function of T_h2 cells (35,36). Thus, it suggests the possibility that ILF-2 is one of the regulators of NF-AT and affects the function of activated T_h2 cells.

All genes categorized in apoptosis and proteolysis were predominantly expressed in activated T_h1 cells. TIMP-1 is an inhibitor of matrix metalloproteinases, which play a crucial role in the infiltration of inflammatory cells and the induction of airway hyper-responsiveness (37). Thus, lower expression of TIMP-1 in the T_h2-dominant condition may contribute to disease onset of some T_h2 diseases, such as asthma.

With regard to enzymes and signaling molecules, the genes encoding kinases and phosphatases are categorized in Table 3. However, JAK and STAT families, which are related to the cytokine signaling pathway (33), were barely detected in either activated T_h1 or T_h2 cells. β-Catenin (T_h1; 7 tags, T_h2; 0 tags) provides a link between cell-surface-expressed cadherins and represents a key molecule connecting cellular adhesion to signal transduction pathways (38). Cadherin expressed on T lymphocytes forms a complex with β-catenin (39), and might be involved in the interactions between activated T cells, especially activated T_h1 cells, and their cellular targets or the extracellular matrix.

Ca^{2+} controls various functions of the cells and is very important for signal transduction. Recently, it has been reported that the rate of Ca^{2+} clearance from the cytosol in T_h2 cells was higher than that in T_h1 cells and the expression of the Ca^{2+}-activated K^+ channel, which controls the membrane potential, is increased in T_h1 cells, and these differences may

Table 2. Differential tag abundance in activated T_h1- and T_h2-polarized cells (1)[a]

A. Th1>Th2

Th1/Th2* (fold)	P value	Th1/Th2 (fold)	Th1	Th2	Tag Sequence	GenBank Match
		p<0.01				
61.5	0.048	49.2	1770	36	CCTGGTGCTT	IFN-γ [X13274]
		19.0	19	0	AATAGAAATT	osteopontin [X13694]
15.2	0.032	15.5	248	16	GATAACACAT	MIP-1β [M23502]
		12.5	125	10	AATAAAATTA	lymphotactin [U23372]
		8.0	8	0	CTGGCTGCAA	cytochrome c oxidase subunit Vβ [M19961]
		7.0	7	0	TAGCTCTATG	ATPase, Na+/K+ transporting, α1 [U16798]
		7.0	7	0	TTTTTGATCA	β-catenin [X87838]
		7.0	7	0	TAAAACAAGA	CD55 [M31516]
6.1	0.001	6.6	86	13	GCACCAAAGC	MIP-1α [X03754]
7.2	0.021	11.0	11	1	TACATAGCTT	perforin [L40557]
		10.0	10	1	CTATAGCATA	amphiregulin [M30704]
		10.0	10	1	CAGGTGCTGT	KIAA0601 protein [U61836]
		p<0.05				
3.5	0.028	6.5	13	2	TAAATGAAAA	NOT [S77154]
5.8	0.013	6.0	12	2	CCACTACACT	TRAIL [U37518]
		6.0	6	0	CAGAGATGAA	heat shock 70kD protein 1 [NM_005345]
		6.0	6	0	TCTGCAATGA	hypothetical protein [AJ012409]
		6.0	6	0	GCGGTGTACA	natural killer cell group 7 sequence (NKG7) [S69115]
		6.0	6	0	AAAAATCGGC	RANTES [M21121]
		5.0	20	4	TGAAAGTGTG	heat shock 105kD α [D86956]
2.3	0.036	5.0	5	0	GTGTTTTTAT	apoptosis inhibitor 2 (IAP homolog C) [U37546]
		5.0	5	0	TTAGTTAAGC	archain 1 [X81198]
		5.0	5	0	AGACTGGAAG	CD3D antigen [NM_000732]
		5.0	5	0	AAAACAAAAA	cDNA DKFZp586H021 [AL110196]
		5.0	5	0	AGGGATCACA	lymphotoxin α [X01393]
		5.0	5	0	TGAGAGGAGA	NADH dehydrogenase (ubiquinone) 1β subcomplex 5 [AF047181]
		5.0	5	0	AGGAACCAGA	phosphatase A2 inhibitor [U51924]
		5.0	5	0	ATCAGTGGCT	proteasome subunit, β type 4 [D26600]
		5.0	5	0	AGACAAGCTG	splicing factor, arginine/serine-rich 5 [AF070562]
		5.0	5	0	TTGGCCAGAA	transcription factor CA150 [AF017789]
		5.0	5	0	TCCTGGCTCT	ubiquitin specific protease 12 [AF022789]
		4.0	4	0	CTGTTATAGG	ATP-dependent metalloprotease YME1L [AJ132637]
		4.0	4	0	TGGAAAGTGA	c-fos [NM_005252]
		4.0	4	0	CTAACGCAGC	c-jun [NM_002228]
		4.0	4	0	AGCCTGCTCA	CD73 [X55740]
		4.0	4	0	TAGCAGCAAT	cDNA DKFZp564A132 [AL049963]
		4.0	4	0	CACCAGCATT	chromosome 15 open reading frame 3 [AL109701]
		4.0	4	0	TACGTTGCAG	GC20 [AF064607]
		4.0	4	0	CACGGACACG	GOT1 [M37400]
		4.0	4	0	TACTAGTCCT	heat shock protein 90 [X15183]
		4.0	4	0	AGCTGTTCTG	heterogeneous nuclear ribonucleoprotein A1 [X79536]
		4.0	4	0	GATGAACTGA	htra2-β [U61267]
		4.0	4	0	CAGGAACGGG	MEK2 [L11285]
		4.0	4	0	TTCTATTTCA	moesin [M69066]

Table 2. *Continued*

		4.0	4	0	GCTCTTCTGC	NF-ATc [U08015]
		4.0	4	0	GCGGACGAGG	PDCD5 [AF014955]
		4.0	4	0	CTCTGCTCGG	peflin [AB018357]
		4.0	4	0	CCCTGCTCCT	phenylalanyl-tRNA synthetase β-subunit [AF042346]
		4.0	4	0	CACGCCAGCC	phosphatidylethanolamine N-methyltransferase [AB029821]
		4.0	4	0	GCTTTGCAGT	potassium channel, subfamily K, member 3 (TASK) [AF006823]
		4.0	4	0	AGTTTTACAA	proteasome subunit, ATPase, 4 [AF038965]
		4.0	4	0	ACAGATGTTG	proteasome subunit, p42 [D78275]
		4.0	4	0	ACTTAAGTAC	protein phosphatase 1 regulatory subunit 2 (PPP1R2) [X78873]
		4.0	4	0	CTGGATGCCG	RD protein [L03411]
		4.0	4	0	TTTCTTAAAG	RNA binding protein [AB016092]
		4.0	4	0	CTGTTTAGTG	serine/threonine protein kinase PRK [U56998]
		4.0	4	0	GACCAGGAGA	SGN3 [AF031647]
		4.0	4	0	CTTAAATATC	SID6-8061 mRNA for pyrophosphatase [AB026723]
		4.0	4	0	TTATGGGGAG	stress-induced-phosphoprotein 1 [M86752]
		4.0	4	0	TCATAACTGT	succinate dehydrogenase flavoprotein subunit (SDH)
		4.0	4	0	GGTGTGTCCG	ubiquitin specific protease 7 [Z72499]
		4.0	4	0	TGGGCGCCTT	uroporphyrinogen decarboxylase [M14016]
		4.0	4	0	GACAGTGACG	zinc finger protein [D45213]
		2.7	69	26	GAGAGTGTCT	TIMP-1 [X03124]
		2.7	248	93	TTCATTTGTA	IL-3 [M17115]
		2.4	46	19	GCCTGTATGA	ribosomal protein S24
		2.3	253	110	GCAGAAGAAT	GM-CSF [M10663]
		2.3	57	25	AGAACAAAAC	NKEF [X67951]
		2.2	255	114	TGTGAATATG	IL-2 [V00564]

B. Th1<Th2

Th2/Th1* (fold)	P value	Th1/Th2 (fold)	pTh1	pTh2	Tag Sequence	GenBank Match
		p<0.01				
		9.0	0	9	GGGGGTAACT	fusion, derived from t(12;16) malignant liposarcoma [X71428]
		8.0	0	8	GTGACAGACA	interleukin enhancer binding factor 2, 45kD [U10323]
		7.0	0	7	CTGGCGAGCG	Human ubiquitin carrier protein (E2-EPF) mRNA [M91670]
		2.5	26	65	TGGTGGGACA	IL-13 [L06801]
		p<0.05				
		11.0	1	11	GATCACAGTT	lactate dehydrogenase B [Y00711]
		10.0	1	10	TTTGGGGCTG	proton-ATPase-like protein [D89052]
		5.0	3	15	CAGCTGGGGC	PTB-2 [X65371]
		5.0	0	5	ACCAAGCTGG	tyrosyl-tRNA synthetase [Y89436]
		5.0	0	5	TCACCTGTAG	kinase A anchor protein [X97335]
		5.0	0	5	GGGGCTTCCA	KIAA0239 [D87076]
		4.0	0	4	CCCCTGGCTG	modulator recognition factor I [M62324]
		4.0	0	4	CAACCAAACC	IL-9 [M30134]
		3.1	9	28	GTGCAGGCTC	TAP-1 [X57522]
3.2	0.017	2.6	15	39	GGCTCAGACC	CD6 [X60992]

[a]The 68 and 14 transcripts displaying the preferentially expressed in T_h1 and T_h2 cells respectively are listed. The tag sequences represent the 10 bp tag. Probable GenBank matches are listed.

*These values are cited from (15).

Table 3. Differential tag abundance in activated T_h1- and T_h2-polarized cells (2)[a]

Cytokines, Chemokines, and Receptors	Th1	Th2
IFN-γ [X13274]	1770	36
IL-3 [M17115]	248	93
GM-CSF [M10663]	253	110
IL-2 [V00564]	255	114
lymphotoxin α [X01393]	5	0
IL-9 [M30134]	0	4
IL-13 [L06801]	26	65
*(IL-4	1	3)
*(IL-5	0	0)
*(IL-10	1	4)
*(IL-11	1	3)
*(IL-2Rα	2	0)
*(IL-2Rβ	1	0)
*(IL-2Rγ	3	0)
*(IL-4R	4	1)
osteopontin [X13694]	19	0
lymphotactin [U23372]	125	10
MIP-1β [M23502]	248	16
MIP-1α [X03754]	86	13
RANTES [M21121]	6	0
*(LARC	4	2)
*(IL-8	13	15)
*(TARC	0	1)
*(MDC	0	1)
*(CCR4	0	0)
*(CCR5	0	0)
*(CCR6	25	46)
*(CCR7	3	0)
*(CXCR3	0	0)
*(CXCR4	2	0)

transcriptional regulation	Th1	Th2
NOT [S77154]	13	2
transcription factor CA150 [AF017789]	5	0
c-fos [NM_005252]	4	0
c-jun [NM_002228]	4	0
heterogeneous nuclear ribonucleoprotein A1 [X79536]	4	0
NF-ATc [U08015]	4	0
zinc finger protein [D45213]	4	0
fusion, derived from t(12;16) malignant liposarcoma [X71428]	0	9
interleukin enhancer binding factor 2, 45kD [U10323]	0	8
PTB-2 [X65371]	3	15
*(T-bet	1	1)
*(GATA-3	1	0)
*(c-Maf	0	0)

Apoptosis & Proteolysis	Th1	Th2
perforin [L40557]	11	1
TRAIL [U37518]	12	2
apoptosis inhibitor 2 (IAP homolog C) [U37546]	5	0
ubiquitin specific protease 12 [AF022789]	5	0
ATP-dependent metalloprotease YME1L [AJ132637]	4	0
PDCD5 [AF014955]	4	0
peflin [AB018357]	4	0
ubiquitin specific protease 7 [Z72499]	4	0
proteasome subunit, ATPase, 4 [AF038965]	4	0
htra2-β [U61267]	4	0
proteasome subunit, p42 [D78275]	4	0
TIMP-1 [X03124]	69	26

Enzymes & Signaling Molecules	Th1	Th2
β-catenin [X87838]	7	0
natural killer cell group 7 sequence (NKG7) [S69115]	6	0
CD3D antigen [NM_000732]	5	0
phosphatase A2 inhibitor [U51924]	5	0
proteasome subunit, β type 4 [D26600]	5	0
GOT1 [M37400]	4	0
MEK2 [L11285]	4	0
phenylalanyl-tRNA synthetase β-subunit [AF042346]	4	0
phosphatidylethanolamine N-methyltransferase [AB029821]	4	0
serine/threonine protein kinase PRK [U56998]	4	0
SGN3 [AF031647]	4	0
SID6-8061 mRNA for pyrophosphatase [AB026723]	4	0
kinase A anchor protein [X97335]	0	5
tyrosyl-tRNA synthetase [Y89436]	0	5
*(JAK1	0	0)
*(JAK2	0	0)
*(JAK3	0	0)
*(STAT1	0	0)
*(STAT2	0	0)
*(STAT3	0	2)
*(STAT4	1	2)
*(STAT5A	0	0)
*(STAT6	2	4)

Ion Channel & Transporter	Th1	Th2
ATPase, Na+/K+ transporting, α1 [U16798]	7	0
archain 1 [X81198]	5	0
potassium channel, subfamily K, member 3 (TASK) [AF006823]	4	0
Src-like-adapter [AF006823]	4	0
proton-ATPase-like protein [D89052]	1	10
TAP-1 [X57522]	9	28

Metabolic Pathways	Th1	Th2
cytochrome c oxidase subunit Vβ [M19961]	8	0
NADH dehydrogenase (ubiquinone) 1β subcomplex 5 [AF047181]	5	0
protein phosphatase 1 regulatory subunit 2 (PPP1R2) [X78873]	4	0
succinate dehydrogenase flavoprotein subunit (SDH)	4	0
uroporphyrinogen decarboxylase [M14016]	4	0
lactate dehydrogenase B [Y00711]	1	11

Adhesion	Th1	Th2
CD73 [X55740]	4	0
CD6 [X60992]	15	39

Growth Factors	Th1	Th2
NKEF [X67951]	57	25
amphiregulin [M30704]	10	1

Others	Th1	Th2
CD55 [M31516]	7	0
KIAA0601 protein [U61836]	10	1
heat shock 70kD protein 1 [NM_005345]	6	0
hypothetical protein [AJ012409]	6	0
heat shock 105kD α [D86956]	20	4
cDNA DKFZp586H021 [AL110196]	5	0
splicing factor, arginine/serine-rich 5 [AF070562]	5	0
cDNA DKFZp564A132 [AL049963]	4	0
chromosome 15 open reading frame 3 [AL109701]	4	0
GC20 [AF064607]	4	0
heat shock protein 90 [X15183]	4	0
RD protein [L03411]	4	0
RNA binding protein [AB016092]	4	0
stress-induced-phosphoprotein 1 [M86752]	4	0
moesin [M69066]	4	0
ribosomal protein S24	46	19
Human ubiquitin carrier protein (E2-EPF) mRNA [M91670]	0	7
KIAA0239 [D87076]	0	5
modulator recognition factor 1 [M62324]	0	4

[a]Each number of tags is normalized to 32,000.
[b]Not significant.

affect the production of different cytokines between T_h1 and T_h2 cells (40). Thus, a gene encoding TASK, which is one of the potassium channels and preferentially expressed in activated T_h1 cells, might be also related to preferential cytokine gene expression in T_h1 and T_h2 cells by controlling the membrane potential.

Furthermore, stress-induced transcripts such as some of the heat shock proteins were markedly expressed in activated T_h1 cells. On the other hand, the gene encoding fusion, derived from t(12;16) malignant liposarcoma (FUS) is preferentially expressed in activated T_h2 cells. FUS protein contains an RNA-recognition motif and is a component of nuclear riboprotein, which is related to not only cell proliferation but also cell differentiation (41). Interestingly, Hicks *et al.* reported that disruption of FUS had an effect on B cell development and activation in the cause of the defect of accessory cells (42). Thus, in type 2 immunity (humoral immunity), FUS in activated T_h2 cells may have an important role in generating the specific antibodies in B lymphocytes.

Although we obtained cord blood from a minimum of four healthy volunteers to find the average gene expression, there could be differences in gene expression between individual donor-derived cells. To justify the SAGE results, we picked up seven genes of which expression is distinct between activated T_h1 and T_h2 cells, and analyzed their expression by RT-PCR (Fig. 3). The PCR results validated the SAGE data.

Very recently, transcript imaging of human T_h1/T_h2 cells using oligonucleotide arrays of ~6000 genes was reported (15). When our SAGE data was compared with the result of oligonucleotide arrays (part of their results is cited in Table 2), it was noticed that very limited numbers of genes were examined by an oligonucleotide array by the other group. The number of human genes has been estimated to be in the range from ~35,000 to 120,000 (43–45). The SAGE data in this study includes >22,000 transcripts; however, comprehensive gene expression profile analysis using the DNA arrays lifted only 6000 genes, which is definitely too limited. Furthermore, they did not describe the abundance of these genes, either. Among the genes preferentially expressed in either cell type, the expression of genes encoding IFN-γ [SAGE, 49.2-fold (T_h1, 1770 tags; T_h2, 36 tags); array, 61.5-fold], MIP-1β [SAGE, 15.5-fold (T_h1, 248 tags; T_h2, 16 tags); array, 15.2-fold], MIP-1α [SAGE, 6.6-fold (T_h1, 86 tags; T_h2, 13 tags); array, 6.1-fold], perforin [SAGE, 11.0-fold (T_h1, 11 tags; T_h2, 0 tags); array, 7.2-fold], NOT [SAGE, 6.5-fold (T_h1, 13 tags; T_h2, 2 tags); array, 3.5-fold], TRAIL [SAGE, 6.0-fold (T_h1, 12 tags; T_h2, 2 tags); array, 5.8-fold] and IAP homolog C [SAGE, 5.0-fold (T_h1, 5 tags; T_h2, 0 tags); array, 2.3-fold] in activated T_h1 cells, and CD6 [SAGE, 2.6-fold (T_h1, 15 tags; T_h2, 39 tags); array, 3.2-fold] in activated T_h2 cells is more or less similar, but most of the genes shown to be differentially expressed are different from their results. The most probable reason for this discrepancy is that they generated T_h1- and T_h2-polarized cells for array hybridization analyses at an early stage (3 days) of the differentiation, whereas we prepared the RNAs from well-differentiated (2 weeks) T_h cells.

In conclusion, thorough identification of the genes selectively expressed in human activated T_h1 and T_h2 cells should provide useful information to clarify the functions of these cells. In the future, by combining with a DNA microarray system, the data presented in this report should be very informative to diagnose various human T_h1/T_h2-dominated immune diseases. Furthermore, cloning of numerous unknown genes identified only in the EST database should provide further understanding of molecular pathogenesis of T_h1/T_h2 dominated human diseases and may provide novel therapeutic targets for the treatment of these diseases.

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Abbreviations

FUS	fusion, derived from t(12;16) malignant liposarcoma
GM-CSF	granulocyte macrophage colony stimulating factor
ILF	IL enhancer binding factor
MDC	macrophage-derived chemokine
MIP	macrophage inflammatory protein
NKEF	NK enhancing factor
PE	phycoerythrin
PMA	phorbol myristate acetate
RANTES	regulated upon activation, normal T cell expressed and presumably secreted
SAGE	serial analysis of gene expression
TARC	thymus and activation-regulated chemokine

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