Activation changes the spectrum but not the diversity of genes expressed by T cells

T. Kent Teague*†, David Hildeman†‡, Ross M. Kedl†‡, Tom Mitchell*†, William Rees*, Brian C. Schaefer‡, Jeremy Bender*, John Kappler‡§¶‖, and Philippa Marrack‡§¶**††

‡Howard Hughes Medical Institute, *Department of Medicine, National Jewish Medical and Research Center, Denver, CO 80206; and Departments of **Biochemistry and Molecular Biology, §Immunology, ‖Pharmacology, and ¶Medicine, University of Colorado Health Sciences Center, Denver CO 80206

Contributed by Philippa Marrack, August 25, 1999

During activation T cells are thought to change their patterns of gene expression dramatically. To find out whether this is true for T cells activated in animals, the patterns of genes expressed in resting T cells and T cells 8 and 48 hr after activation were examined by using Affymetrix gene arrays. Gene arrays gave accurate comparisons of gene expression in the different cell types because the expression of genes known to vary during activation changed as expected. Of the approximately 6,300 genes assessed by the arrays, about one-third were expressed to appreciable extents in any of the T cells tested. Thus, resting T cells express a surprisingly large diversity of genes. The patterns of gene expression changed considerably within 8 hr of T cell activation but returned to a disposition more like that of resting T cells within 48 hr of exposure to antigen. Not unexpectedly, the activated T cells expressed genes associated with cell division at higher levels than resting T cells. The resting T cells expressed a number of cytokine receptor genes and some genes thought to suppress cell division, suggesting that the state of resting T cells is not a passive failure to respond to extant external stimuli.

activated | resting | gene array

A good deal of attention has been paid to gene expression in differentiated T cells of various types. Subtraction techniques, differential display, and gene array analysis all have been used to investigate differences in gene expression between different kinds of T cells (1–6). Most of these experiments have been performed by using established T cell lines, or at least T cells that have been withdrawn from their host and cultured for some time. Relatively few experiments have addressed the question of overall gene expression in T cells in animals. Because of this there are significant holes in our knowledge of these cells. For example, little is known about gene expression in resting T cells. Gene expression in resting T cells is not, however, an uninteresting problem. Although for many years it was thought that resting T cells were in a quiescent stage, existing independently of their surroundings, recent experiments have shown that this is far from true. The survival of small, naïve resting T cells in animals depends on receipt of many life-preserving factors from their environment. For example, the life expectancy of these cells is known to be dependent on low-affinity engagement of their antigen receptors by the MHC protein and probably the peptide that drove their selection in the thymus (7–10). For their survival, small, resting T cells in animals probably also depend on engagement of cytokines such as IL-6 and IL-7 (11–15).

The appearance of antigen or superantigen in animals leads to the rapid activation of specific T cells. These activated cells divide rapidly and then many of the progeny of the dividing cells die within a few days (16, 17). This phenomenon does not occur if the T cells are activated *in vitro*. Therefore, an understanding of the ways in which activated T cells die in animals depends on activating the cells *in vivo*, not *in vitro*.

This paper describes our attempts to deal with these deficiencies in our knowledge. mRNA was purified from resting T cells, or T cells that recently had been activated. Analysis was performed by using Affymetrix gene arrays (Santa Clara, CA). The results showed that resting T cells expressed a surprisingly large diversity of different mRNAs. Within 8 hr of activation *in vivo* the range of genes expressed by the T cells changed dramatically, although the total number of genes expressed did not change. Two days after activation, the spectrum of genes expressed by the activated cells was much more like that of resting T cells. However, again, the total number of different genes expressed was still about the same, amounting to about one-third of all the genes on the array. Comparison of the genes expressed by resting T cells with T cells 48 hr after activation showed that the latter cells expressed, not surprisingly, many genes associated with cell division. Among the genes expressed at higher levels by resting cells were those coding for a number of cytokine receptors and for several genes thought to inhibit cell division. Hence, the state of resting T cells may depend on active processes that allow the cell to be nurtured by its environment and actively prevent it from entering cell division.

Materials and Methods

Mice and Cells. T cells were activated in C57BL/10 mice (The Jackson Laboratory) by i.v. injection of 150 μg of the Vβ8.x-specific superantigen, staphylococcal enterotoxin B (SEB; Sigma). This procedure generates T cells bearing Vβ8.x that are fully activated as judged by the fact that, 8 hr later, they all bear CD69 and, 18 hr after injection of SEB, all the Vβ8.x$^+$ T cells begin to divide (ref. 17; unpublished observations). One group of mice was sacrificed 8 hr after injection of SEB. Another group of mice was sacrificed 48 hr after injection of SEB, at the time when the SEB-stimulated T cells had reached their maximum numbers and just before they were to start to die (17). Lymph nodes were harvested from these animals and from control, untreated mice, and T cells prepared by passage through nylon wool columns (18). To purify the T cells more thoroughly, the cell preparations were stained and sorted by using a MoFlo Instrument (Cytomation, Fort Collins, CO). Normal resting T cells were isolated from nonimmunized mice after staining with phycoerythrin (PE)-labeled anti-Cβ, CyChrome-labeled anti-CD4 and anti-CD8, and fluorescein (FL)-labeled anti-IA^b and anti-CD69 (PharMingen). Sorting gates were set to collect small Cβ^+ cells bearing CD4 or CD8 and to exclude cells bearing IA^b or CD69. Sorted cells were analyzed on a FACScan cytofluorograph (Becton Dickinson) to assess their purity.

Activated T cells were isolated from mice previously injected with SEB. Lymph node cells from these animals were passed over nylon wool columns, and these T cell-enriched preparations

Abbreviation: SEB, staphylococcal enterotoxin B.

†K.T., D.H., R.K., and T.M. contributed equally to this work.

††To whom reprint requests should be addressed at: Howard Hughes Medical Institute, Department of Medicine, National Jewish Medical and Research Center, 1400 Jackson Street, Denver, CO 80206. E-mail: marrackp@njc.org.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Table 1. Characteristics of materials used in gene array experiments

T cell type	No. of cells sorted	% cells bearing Cβ or Vβ8.x	% cells bearing IA^b	Yield RNA, μg	μg RNA/10^7 cells
Resting	13.9×10^7	99.6	0.4	115	8.2
8-hr activated	2.7×10^7	98.6	0.7	38	14.1
48-hr activated	6.9×10^7	97.4	0.5	136	20.0

were sorted to isolate the activated, Vβ8.x-bearing cells after staining with PE-anti Vβ8.x, CyChrome anti-CD4 and anti-CD8, and FL-anti-IA^b. Sorting gates were set to include $V\beta8.x^+$ cells bearing CD4 or CD8 and to exclude cells bearing IA^b. This procedure thus isolated SEB-stimulated $V\beta8.x^+$ T cells and excluded resting T cells, B cells, and dendritic cells. As above, the effectiveness of the procedure was checked by analysis of the staining profile of the sorted cells. The characteristics of the various sorted cell populations are shown in Table 1. More than 97% of the cells in all three populations bore Cβ (resting T cells) or Vβ8.x. The populations were contaminated with 0.7% or less B cells, dendritic cells, and class II^+ macrophages. Residual cells in the SEB-activated populations were resting T cells that bore β other than Vβ8.x (data not shown).

RNA was isolated from these cells by using rapid total RNA isolation kits (5 Prime → 3 Prime). Between 8.2 and 20 μg of total RNA was isolated per 10^7 cells from the various populations (Table 1). Poly(A)$^+$ mRNA was purified from each of the preparations by using Oligotex mRNA minikits (Qiagen). The quality of the poly(A)$^+$ mRNA was evaluated on the Affymetrix Gene Arrays as described below.

Preparation of cRNA and Gene Chip Hybridization. cDNA was synthesized from the poly(A)+ mRNA by using SuperScript Choice kits (GIBCO/BRL) and nucleotide primers that contained a sequence recognized by T7 RNA polymerase. cRNA was prepared in an *in vitro* transcription reaction by using T7 polymerase (MegaScript T7 kit; Ambion, Austin, TX). The quality of the cRNA prepared from the cells was evaluated by control hybridizations with probes built to match the 5′, middle, and 3′ sequences of β actin, glyceraldehyde phosphate dehydrogenase, and 18S RNA. For all three preparations of cRNA the signals obtained for different regions of the same gene were about the same, i.e., the cRNA contained intact coding sequences. Also, the signals obtained by hybridization to 18S RNA were comparatively low, demonstrating that the poly(A)$^+$ mRNA from which the cRNAs were prepared were relatively pure (data not shown). The cRNAs were not significantly contaminated with the products of B cells, dendritic cells, or macrophages because their cRNAs gave little or no signal with probes for Ig, class II MHC, or macrophage-specific proteins, with the exception of class II IEβ (http://www.kmlab.njc.org).

Results

Normalization of the Measurement of Gene Expression in Resting and Activated T Cells. Both 8- and 48-hr activated T cells contained about twice as much total RNA, and probably about the same amount more poly(A)$^+$ mRNA, than resting T cells (Table 1). However, activated T cells are much larger than resting T cells; therefore, a doubling in the amount of a particular mRNA does not indicate a doubling in the concentration of that mRNA, or the protein it codes for, in the activated cell. For many proteins, concentration is probably more significant than total number of molecules per cell. Therefore, in the discussions below, we chose not to consider the fact that the bulk amount of RNA was increased between resting and activated cells. Rather, we evaluated the concentration of a given mRNA in a sample relative to the entire pool of poly(A)$^+$ transcripts in that pool. To accomplish this, the gene chip signals were normalized to an overall signal, the average of signals for each cRNA preparation on each chip, before analysis.

Anaylsis of Overall Gene Expression in Resting and Activated T Cells. RNA transcript levels for different genes were assessed by using Affymetrix software. The relative abundance of a particular mRNA was expressed as the "average difference." This is calculated from the difference in fluorescence intensity given by a labeled RNA sample when hybridized to oligos built to match a particular gene sequence vs. when hybridized to oligos mismatched by one base. To get an overall impression of the differences in gene expression between the different types of T cell we plotted the values for average differences obtained for each gene in each type of T cell against their values in the other cells (Fig. 1). Gene expression between resting and 8-hr-activated T cells was quite different as indicated by the scatter in the points on Fig. 1*A*. Differences in average differences of more than 2-fold for a particular gene between two samples of RNA from different cells are, in general, likely to reflect real differences in gene expression (Affymetrix). Many genes in resting and 8-hr-activated T cells differed by at least this much as indicated in Fig. 1*A* by the points that lie outside the lines drawn to show 2-fold differences in level. The overall correlation coefficient for Fig. 1*A* is low, at less than 0.79. Interestingly, the number of genes whose expression decreased upon activation was as large as the number of genes whose expression increased. Hence, transcriptional inhibition in activated T cells was unexpectedly frequent (30).

Comparison of overall gene expression between resting T cells and T cells 48 hr after activation with SEB in mice showed fewer differences. Fewer genes differed in average differences by more than 2-fold, and the correlation coefficient was higher, at greater than 0.92. Again, some genes were expressed at higher levels in 48-hr-activated cells than in resting T cells and *vice versa* (Fig. 1*B*).

Not surprisingly, given the data in Fig. 1 *A* and *B*, T cell gene expression also was quite different when data from cells 8 hr after activation and 48 hr after activation were compared (Fig. 1*C*). The significance of differences in gene expression in two samples of mRNA was calculated by Affymetrix software by using a combination of actual values of the average differences for that gene in the two samples and the value of the subtraction of the average differences. The parameter thus derived is called the sort score.

To get an overview of the differences in gene expression between resting and activated T cells, we counted the numbers of genes in each comparison that had sort scores greater than 2 in such comparisons and an average difference value in the higher-expressing tissue greater than 100. This cutoff gave a conservative estimate of the numbers of genes that actually changed their expression levels between resting and activated T cells. The results are shown in Table 2. Also shown in Table 2 is the number of RNAs in each sample that had average difference scores of greater than 100.

These data confirm the impression given by Fig. 1. All three types of T cells expressed about the same number of RNAs with average difference scores of greater than 100. RNA expression

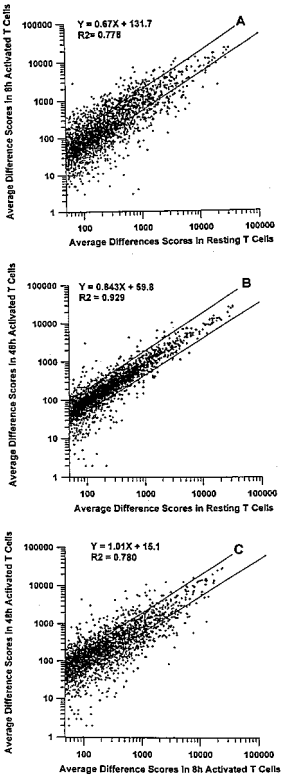

Fig. 1. Comparison of gene expression in resting and activated T cells. Poly(A) RNA was prepared and converted into fluorescent-labeled cRNA as described in *Materials and Methods*. The levels of cRNAs derived from different genes were measured by using Affymetrix Gene Arrays and expressed as average differences. The plots compare genes that had average differences greater than 50 in the T cell type against which the comparisons were made. Genes with average differences ≤0 in the index T cell type were omitted from the analyses. These were 57 genes in *A*, 53 genes in *B*, and 78 genes in *C*. The lines drawn on the graphs represent differences in average difference of 2-fold between the two samples considered. (*A*) Comparison of average differences for gene expression in T cells 8 hr after activation and resting T cells, with 2,758 genes considered. (*B*) Comparison of average differences for gene expression in T cells 48 hr after activation and resting T cells, with 2,762 genes considered. (*C*) Comparison of average differences for gene expression in T cells 48 hr after activation and 8 hr after activation, with 2,592 genes considered.

between resting and 8-hr-activated cells was quite different, with about 280 of the 6,319 genes evaluated as being significantly differently expressed, some expressed more highly in activated cells, and some expressed at greater levels in resting cells. There was much less difference between 48-hr-activated and resting T cells with only 51 genes differing, at this level of sensitivity, in their level of expression. Thus, shortly after exposure to antigen in animals, target T cells dramatically change their mRNA composition. At later times this pattern returns to a composition that is much closer to, but not the same as, that of resting T cells.

Table 2. Activation changes the spectrum but not the diversity of genes expressed in T cells

	T cell type		
	Resting	8-hr activated	48-hr activated
No. genes with average differences >100	2,057	1,852	2,056
No. genes increased*		143	36
No. genes decreased*		139	15

*Genes noted had average differences in the higher-expressing T cells >2 and sort scores ≥2 and are compared with their level in resting T cells.

Comparison of Individual Gene Expression in Resting and Activated T Cells. Individual genes were evaluated to find out whether changes in gene expression were consistent with expectations. Signals for housekeeping genes such as HPRT and β-tubulin were unaffected by activation. The levels of CD3, CD4, and CD8 proteins in T cells are known to be unchanged by activation, and the expression of their genes likewise was unaffected. On the other hand, expression of the α-chains for both the IL-6 receptor and IL-7 receptors was reduced at the protein level by activation, and the levels of expression of the genes for these proteins, as detected by the Gene Arrays, also was reduced in activated samples. Finally, surface expression of the α-chain of the IL-2 receptor was transiently increased and that of CD62L was transiently decreased during activation, a result that mirrored the RNA expression data (http://www.kmlab.njc.org; and data not shown).

Occasionally there were discrepancies between the protein and gene expression data. For example, the Gene Array data indicated that expression of the gene for the IL-2 receptor β-chain increased after activation. Levels of this protein on the surface of T cells did not increase, however, until 48 hr after activation. This discrepancy may be because protein synthesis and expression on the cell surface will always, of course, be delayed by comparison with mRNA induction. Thus, for the IL-2 receptor β-chain, 8 hr of activation may have been early enough to observe mRNA induction but too early to observe increases in surface protein. Similarly, mRNA for CD62L fell precipitously by 8 hr after T cell activation. However, cell surface levels of the protein were only halved at this time, again demonstrating a significant temporal delay in levels of protein consequent to changes in mRNA level (http://www.kmlab.nationaljewish.org; and data not shown).

The results for genes that changed in expression level more than 2-fold and that had average differences of greater than 100 in the higher-expressing tissue, between resting and 48-h-activated T cells, are shown in Tables 3 and 4. Expressed sequence tags (ESTs) were omitted from this list because, in our experience, data from ESTs do not necessarily represent values for the gene to which they are thought to be similar.

Many genes contributing to cell division were expressed at higher levels in activated vs. resting T cells, as expected. These included the genes for DNA polymerase and primase, for the cyclins, and for many of the enzymes involved in synthesis of DNA precursors. Eight-hour-activated T cells contained higher levels of mRNA for cyclins D and G1, presaging their entry into mitosis about 16 hr later. Forty-eight-hour-activated T cells contained elevated levels of mRNA for cyclins A1, B, and E. Also increased in activated cells were mRNAs for Myb and Myb-B, DNA-binding proteins involved in the stimulation of cell division (refs. 19 and 20; Table 3).

Conversely, some genes that are expressed preferentially in nondividing cells and/or whose products are thought to prevent cell division were expressed at higher levels in resting than activated T cells. Included in these were the genes for the retinoid acid receptor RAR, Dyrk, a protein involved in terminal differentiation and cessation of proliferation, and the proliferation inhibitory transcription factors D52 and TSC-22 (refs. 6 and 21–23; Table 4).

There were large changes in expression of other transcription factors. For example, as reported previously, resting T cells expressed the gene for the Kruppel-like transcription factor, LKLF, at higher levels than activated T cells (ref. 24; Table 4). Less expected was the fact that this also applied to other Krueppel-related transcription factors, EZF and BKLF. Also

Table 3. RNAs increased in 48-hr-activated vs. resting T cells

Accession no.	Description	Average differences: Resting	8-hr activated	48-hr activated
Extracellular matrix and cell adhesion				
X16834	Carbohydrate-binding protein, Mac-2	110	71	271
U08020	Alpha-1 Type 1 collagen	60	176	175
U25652	Alpha-1 Type XII collagen	41	44	127
L24430	Osteocalcin precursor, gla protein	103	16	244
D00622	Heparin-binding protein 44	86	41	221
Cell surface receptors/transporters/proteins				
X71788	Blr-1, receptor for chemokine BLC	34	86	123
X85214	Ox40	238	1,481	637
X05719	CTLA-4	831	1,477	3,706
X98113	CD4-like cell surface glycoprotein	4	184	235
X04653	Ly-6E.1	781	5,881	1,951
M99377	Alpha-2 adrenergic receptor	43	18	125
M63436	GABA-A receptor alpha-1 subunit	20	43	119
L01776	Neuronal calcium channel	42	41	132
U65593	K+ channel beta 4 subunit	39	54	147
Cell structure/vesicle movement/secretion				
W29468	Myosin light chain 2	29	178	147
X97650	Myosin 1	103	43	293
W13586	Atrial/fetal myosin light chain	35	2	186
X54511	Mbh1, gelsolin actin-binding protein	190	93	448
M26251	Vimentin	2,172	537	4,810
D12646	kif4, kinesin-like protein	47	37	135
Y09632	Kinesin-like protein 174	33	30	190
M16455	Calpactin-1 light chain (p11)	1,586	336	4,362
D10024	Calpactin-1 heavy chain	143	110	1,086
U66865	Vacuolar protein-sorting hom. (VPS45)	35	58	126
M62418	Clathrin-associated protein 19 (AP19)	143	188	333
L33726	Fascin	17	19	117
W29418	Fast skeletal troponin C	36	32	152
Signal transduction				
U03856	CD45-associated protein (cd45-ap)	1,294	1,304	2,640
U28168	Familial adenomatous polyposis	287	285	677
D00208	S100A4 + Ca^{2+}-binding protein, pEL98	311	61	802
AA120244	S100 Ca^{2+}-binding protein, A13	553	142	1,292
X66449	Calcyclin	−33	−122	338
M19380	Calmodulin (Cam III)	324	389	929
X65138	Eph-related receptor tyr kinase	−23	50	122
U38196	Mpp-1	82	61	218
Chromatin and nuclear structure				
X12944	HMG-17 chromosomal protein	2,015	2,553	4,436
X58069	Histone H3.2-F, H2a.1-F H2b-F	222	319	725
X16705	lamin B	89	75	558
Z46757	High mobility group 2 protein	1,606	1,106	6,829
Cell division				
X62154	P1 protein (P1.m)	45	282	147
D13545	Primase large subunit	−7	61	113
J04620	Primase small subunit	78	290	308
D17384	DNA polymerase α subunit	1	42	105
D12513	DNA topoisomerase IIα	50	55	355
U19604	DNA ligase I	23	75	212
D86726	mMIS5	426	1,113	1,116
D13473	RecA-like protein MmRad51	−3	24	141
L26320	Flap endonuclease-1 (FEN-1)	19	187	141
Z26580	Cyclin A	96	80	561
X64713	Cyclin B	10	17	123
X66032	Cyclin B2	37	19	653
X75888	Cyclin E	105	142	215
U63337	Cyclin-dependent kinase-2α	41	107	185
D26091	mCDC47	572	1,387	1,488
U58633	p34 CDC2 + B68	19	−5	555
U20497	Cdk4 and Cdk6 inhibitor p19	177	−30	491
D21099	Stk-1	10	−12	206
u50378	Ku70	59	281	165
K02927	Ribonucleotide reductase M1	157	323	541
X15666	Ribonucleotide reductase M2	74	27	263
W08120	Thioredoxin	2,142	7,378	6,254
X77731	Deoxycytidine kinase	35	28	178
M68489	Cytosolic thymidine kinase	−79	−61	347
M13019	Thymidylate synthase	−38	132	640
M63445	Methylenetetrahydrofolate DeH	65	234	139
L08266	Fanconi's anemia complementation	23	49	104
X14805	Cytosine-5-methyltransferase	116	146	314
Transcription				
X61385	T cell transcription factor, TSF1	526	730	1,234
Z54283	Oct-binding factor 1	42	46	138
M16449	Myb	338	254	845
X70472	B-Myb	−8	44	158
X72310	DRTF-polypeptide-1	69	425	224
M83380	RelB	86	152	187
U19 799	IkB-β	53	357	125
M36146	Zfp-35	33	14	110
X72697	Xmr meiosis-regulated protein	179	258	464
U32394	Mad3	59	20	134
U46187	KRAB + Zinc finger protein	1	13	104
U41741	USF	51	94	135
Y07836	Basic helix–loop–helix protein	74	291	225
M13018	Cysteine-rich intestinal protein (CRIP)	90	−28	226
D26090	Hox-3.1, Hox 3.2-Hox-3.1 intergenic region	353	1,107	1,250
M75953	Homeobox+, PMUR10F	28	28	137
M34857	Hox-2.5	17	5	125
U52951	Putative transcrip., reg., mEnx-1	59	349	348

Table 3. (*Continued*)

Accession no.	Description	Average differences: Resting	8-hr activated	48-hr activated
Protein synthesis/degradation				
U39302	26S proteosome sub. 4 ATPase	233	783	484
W11011	Ubiquitin-like protein	−147	172	121
U48830	Subtilisin-like convertase-7	96	44	218
Glycolysis/ATP production				
M32599	Glyceraldehyde-3-phos. DeH	2,571	3,730	5,162
X53333	Triose phosphate isomerase	375	2,723	1,399
AA028501	Cytochrome c oxidase VIII-H	14	−16	105
Redox control/control of oxidation damage				
X82067	Thioredoxin-dep. peroxide red'ase	315	684	657
D49956	8-oxo-dGTPase	88	252	177
X61147	Iron-responsive element-binding protein	52	113	132
M68896	Androgen-regulated protein, arMEP24	44	14	119
U48420	Theta class glutathione transferase type 2	−34	−20	113
Cell life and death				
L16462	A1, Bcl2-like protein	583	510	1,257
U54803	Caspase 3	85	86	719
L37296	BAD	12	54	115
X95591	C1D	65	33	136
X73985	Calretinin	122	141	331
Secreted products				
X86374	TAG7, TNF-like cytokine	147	35	335
X04072	Granzyme B	12	3,561	238
M13226	Granzyme A	88	336	763
X53257	Neurotrophin 3	23	−2	171
AA124831	Eosinophil second. gran. protein (mEAR-2)	80	80	198
X04573	Preproelastase	67	60	140
X04574	Preprotrypsin	61	34	213
D00466	Apoplipoprotein E	78	98	160
J02644	Type 1 epidermal keratin	88	83	186
Miscellaneous				
M23236	Proline-rich protein (MP-2)	55	135	148
L21027	A10	209	1,307	463
U69488	Viral envelop-like protein (G7e)	80	48	415
M34897	Ecotropic viral integration site 2 ORF	39	−103	121
M21332	RNA-binding protein	1	196	212
M26270	Stearoyl-CoA desaturase (SCD2)	94	311	437
U42385	FGF-inducible gene 16 (FIN16)	34	87	195
D21099	Putative ser/thre kinase, Stk1	10	−12	206
U10484	Lymphoid membrane protein, Jaw1	46	220	938
W13002	β-galactosidase-binding protein	526	91	4,460
X82786	Ki-67 (MIB1)	36	21	895
L42293	Acyl CoA:cholesterol acetyltrans'ase	72	95	152
U13837	Vacuolar ATPase subunit A	38	80	101
U232332	p13MTPC1	−7	12	134
X58523	MIPP	45	28	105
X06917	Aspartate aminotransferase	250	700	547
J03857	B 29	56	23	114
L09192	Pyruvate carboxylase homo. protein	1	70	139

noteworthy was the increased expression of RelB (19, 25) and IkB-β and several Hox genes in activated cells.

Many papers have shown that members of the Fos/Jun family are very important inducers of gene expression in activated T cells (26). Others have shown that expression of these genes is changed during T cell development, increased upon T cell activation, and decreased in anergic cells (27–29). The analysis in this paper and in a previous report (30) showed a dramatic lowering in levels of mRNA for proteins of the Fos/Jun family and for one of the kinases upstream of activation of these proteins, SEK1. These results suggest that the function of this branch of the MAP kinase signaling pathway may be significantly curtailed in activated T cells.

mRNAs coding for some of the secreted products of T cells, such as the granzymes, increased as the cells were activated. Surprisingly, however, it appeared that resting T cells also make transcripts for some secreted proteins. Overall, the decrease in expression of one of the integrins and a ligand for E-selectin and the increase in expression of enzymes such as trypsin and elastase, which could be involved in tissue penetration, gave the impression that, as is known to occur, the activated T cells were preparing themselves for greater mobility than their resting precursors.

Finally, there were several examples in which expression of a gene in resting T cells appeared to be replaced by expression of a related gene in activated T cells. For example, activated T cells

Table 4. RNAs decreased in 48-hr-activated vs. resting T cells

Accession no.	Description	Average differences: Resting	8-hr activated	48-hr activated
Extracellular matrix and cell adhesion				
U12236	α M290 integrin, binds β7 integrin	530	53	253
X64550	RHAMM, hyaluronan receptor	111	66	40
X84037	E selectin ligand-1	119	107	54
X58251	Pro-alpha-2(1) collagen	174	11	52
D00613	Matrix Gla protein	108	38	23
Cell surface receptors/transporters/proteins				
M27960	IL-4 receptor	1,634	519	559
M29697	IL-7 receptor α-chain	897	83	228
X53802	IL-6 receptor	549	70	271
U69599	IFNγ receptor second chain, ifngr2	383	203	104
D63679	Decay accelerating factor	255	28	91
U36757	Thrombin receptor	120	96	56
X62701	Urokinase-type plasminogen acti R	111	180	17
X99581	Leucocyte 7 transmembrane R	1,411	557	396
X15643	β-2-Adrenergic receptor	139	33	42
X62600	α-1-Acid glycoprotein, AGP/EB	318	157	27
X61433	Na/K ATPase β-subunit	649	184	241
D78572	LIG-1	145	77	60
D83206	p24	164	70	70
U49720	Blue cone pigment	246	90	39
X81582	IGF binding protein 4	1,077	1,029	−54
U35836	Tumor-ass. glycoprotein E4, Tage4	111	14	−25
X85992	Semaphorin C	134	40	55
Cell structure/vesicle movement/secretion				
AA123361	Rab6/rab5-ass. protein, rab6	2,940	961	1,205
M13444	α-Tubulin isotype M-α-4	1,315	1,448	629
Signal transduction				
X02452	Ki-ras	368	120	142
M63630	GTP-binding protein, IRG-47	292	462	112
U19119	G protein-like LRG-47	221	284	66
U15636	U2, T cell GTP-binding protein	915	605	155
X51829	MyD116	2,998	924	1,286
Y08361	RIL	175	7	−12
U38252	Proline-rich RING finger protein	926	631	330
X63039	RSP-1	257	138	114
U58497	Mnb protein kinase, Dyrk	183	59	80
U58885	SH3-containing protein SH3P8	131	125	26
U58882	SH3 domain-containing protein, Lasp-1	133	−45	28
U58512	Rho-associated protein kinase	108	44	45
U56909	Tousled-like kinase	123	85	49
L01695	Calmodumin-dep. p.diesterase, PDE1B	254	73	54
M96163	Serum-inducible kinase, SNK	130	33	39
U18310	SEK1	123	70	34
Chromatin and nuclear structure				
X70887	Protein like transition protein 2, TP2	752	1,597	349
U62673	Histones H2a(A)-613, H2a(B)-613, H2b-613	103	87	31
J03482	Histone H1	273	55	130
U40796	DNA repair enzyme, ERCC5	224	34	80
Cell cycle				
U44426	D52, cell cycle inhibitor	161	180	80
Z14986	S-Admethionine decarbox'ase	1,314	728	601
Transcription				
U25096	Kruppel-like factor LKLF	5,727	583	1,754
U70662	Kruppel-like factor EZF, Zie	708	529	155
U36340	BKLF	389	101	184
U06924	STAT1	1,468	873	437
J03236	junB	11,687	2,121	5,821
J04115	c-Jun	234	25	−25
X14897	FosB	514	235	151
X98096	Transcription factor BFCOL1	1,195	286	567
X62940	TSC-22	237	50	68
M58564	TIS11	552	311	241
U73329	Dix7, Distal less homeobox gene	158	60	60
M82974	Hen1	127	59	37
U28071	Hoxc-5	105	20	36
U20282	Stromelysin PDGF-resp. elem binding ?	277	121	94
X61753	Heat shock transcription factor 1	140	350	14
U13878	Neural-restrictive silencer factor	174	60	85
M34476	Retenoic acid receptor gamma-A	198	61	82
Protein production and degradation				
W13646	Polyubiquitin, TI-225	3,991	12,610	1,170
Z19579	slah-1A	314	81	52
L40406	Heat shock protein 105 kD β	1,343	2,599	451
U63323	Translation init. factor, Eif4g2	1,532	1,187	726
U70674	B2 element and 18S RNA seq.	122	7	2
U16162	Prolyl-4-hydroxylase alpha-1	143	204	65
U35646	Aminopeptidase	171	35	75
Cell life and death				
U43678	Ataxia telangiectasia gene	106	76	21
Intermediary metabolism				
X85983	Camitine acetyl transferase	138	67	64
U53142	Constitutive nitric oxide synthase	113	−10	−36
X51905	Lactate dehydrogenase-B	141	55	57
U00978	Type 1 inosine monophosphate DeH	261	596	130
Secreted products				
L38580	Galanin	106	55	14
M11943	Wnt-1	2,334	145	107
X96618	Stromal cell protein-inducing RAG	431	184	181
Miscellaneous				
M64292	TIS21	1,623	317	305
V00727	Replication-defective murine sarcoma virus	3,956	643	1,209
U34072	Steroid DeH, Ke 6, Ke 6a, Ke 6b	343	24	110
U43085	Glucocorticoid-atten. resp. gene 39, GARG-39	197	30	54
D30785	Neuropsin, serine protease	575	146	276
x96639	EXT1, analog of human multiple exostosis	173	46	33
M29011	Immunoglobulin α-chain switch region	123	−36	27
D87744	Atrophin 1 (DRPLA)	157	48	73
U42386	FIN14, FGF-inducible gene	1,259	564	565
X59379	Nexin II, amyloid beta precursor	102	16	13
X67140	SR calcium ATPase	227	175	110
M21532	PCD-5	188	65	79
X57199	Lysosomal acid phosphatase	429	206	82
Z46720	Perinuclear-bind. protein, PICK-1	159	−26	18
M10021	Cytochrome P1-450	113	22	−8

contained more mRNA for Alpha 2 type I collagen and for the transcription factor BFCOL1, which activates this gene (31), and less mRNA for Alpha 1 types I and XII collagen than resting T cells. Likewise, expression of genes related to the basement membrane-binding proteins osteocalcin and matrix Gla protein seesawed in the two types of T cells, as did genes for some of the Hox proteins.

Discussion

Recently, immunologists' view of the resting T cell has undergone a revolution. Our data and other recent reports have demonstrated that the resting T cell is constantly receiving signals from its environment in the animal. These signals help to keep the cell alive and to guide the cell to various locations (7–14, 32). Given this new appreciation of the activity of resting T cells it perhaps is not surprising to find that mRNAs for many different proteins are expressed at detectable levels in these cells, a result that has been suggested before (30).

Among the factors that are detected by resting T cells, and that help to keep them alive, are IL-4 and IL-7 (12–14). IL-4 also can stimulate the proliferation of activated T cells. However, it is not a good proliferative factor for resting cells. Perhaps the failure of resting T cells to divide in response to IL-4 is because resting cells express proteins such as Dyrk, D52, TSC-22, and LKLF, which may be inhibitors of cell division (21–24). If so, cell division by resting T cells may be, in a sense, actively inhibited by factors within the cell.

Activation caused an approximate doubling in the amount of total RNA per cell, and it is interesting to notice that expression of many of the RNAs in T cells increased concordantly when the cells were activated. This was very striking 48 hr after activation, a time when analysis showed that, in spite of the increase in RNA per cell, once the amounts of RNA per cell had been normalized, the levels of expression of many different genes in resting and activated T cells were the same. Such a result suggests that a global mechanism of transcription regulation was induced by T cell activation, increasing expression of many genes similarly. Such a global mechanism could involve components of the transcription apparatus, p53, or other proteins that concordantly regulate many populations of genes and/or mRNA stability (33, 34).

It is not surprising that many genes change in their level of expression after T cells had been activated for only 8 hr in animals. Perhaps this rapid response is, in part, due to the nature of the antigen we used. Superantigen does not need to be processed by antigen-presenting cells before it can interact with T cell receptors, and, therefore, T cell responses to such material probably will be a few hours faster than that of T cells to conventional protein antigens.

We were surprised to find that, although many genes increase in expression immediately after T cell activation, approximately the same number decrease. Thus, the variety of mRNAs in resting and activated T cells, as previously suggested (30), is about the same in magnitude. Again, such a result may reflect the recently appreciated high receptivity of resting T cells.

We did not expect to find that the mRNA content of T cells 48 hr after exposure to superantigen in animals would be more similar to that of resting T cells than to that of recently (8-hr) activated cells. Forty-eight hours after injection of superantigen, target T cells are still dividing vigorously, although they will stop dividing very shortly thereafter (data not shown). At 48 hr, the activated T cells are also about to die by apoptosis (16, 17), an event that we thought might involve induction of quite a few new genes. We have shown that exposure to reactive oxygen species is the major cause of the death of these cells (35). Nevertheless, we did not find increases in expression of any of the genes on the microarrays that code for proteins that might cause increases in reactive oxygen species concentrations.

There is the question of whether the kinetics and nature of the changes in gene expression that we report here, during T cell responses to superantigen, reflect the kinds of change that take place during T cell responses to conventional peptide antigens and/or infectious organisms. The kinetics of T cell responses to peptides and proteins administered in the absence of adjuvants are very similar to those of T cells responding to superantigens. Therefore, we believe that such responses will prove to be very similar to those described in this paper. Under nonlaboratory conditions, however, T cells usually encounter superantigens and conventional antigens in the presence of infections. Infectious organisms and laboratory adjuvants induce components of innate immunity. Such components directly or indirectly affect T cell responses such that the responses are larger in magnitude and the responding T cells are more long-lived (17, 36). Infectious agents therefore change gene expression in activated T cells, and the pattern of gene expression in activated T cells described in this paper thus probably is not identical to that which will be found in T cells activated in the presence of bacterial or viral products.

We thank Drs. Clive Slaughter and Steven Madden, University of Texas Southwestern Medical Center, Dallas, for their help in preparation of the cRNA samples, for conducting the Affymetrix gene array hybridizations, and for their patient advice afterward. We thank Drs. Louis Staudt, Gary Johnson, and James Hagman for reading the manuscript and for their very helpful suggestions. We also thank Bill Townend at the National Jewish Medical and Research Center for his help with cell sorting. This work was supported by Public Health Service Grants AI-17134, AI-18785, and AI-22295.

1. Zipfel, P. F., Irving, S. G., Kelly, K. & Siebenlist, U. (1989) *Mol. Cell. Biol.* **9,** 1041–1048.
2. Zheng, W. & Flavell, R. A. (1997) *Cell* **89,** 587–596.
3. Choi, J. W., Lee, S. Y. & Choi, Y. (1996) *Cell. Immunol.* **168,** 78–84.
4. Liu, A. Y., Torchia, B. S., Migeon, B. R. & Siliciano, R. F. (1997) *Genomics* **39,** 171–184.
5. Renner, C., Pfitzenmeier, J. P., Gerlach, K., Held, G., Ohnesorge, S., Sahin, U., Bauer, S. & Pfreundschuh, M. (1997) *J. Immunol.* **159,** 1276–1283.
6. Ishaq, M., Zhang, Y. M. & Natarajan, V. (1998) *J. Biol. Chem.* **273,** 21210–21216.
7. Kirberg, J., Berns, A. & von Boehmer, H. (1997) *J. Exp. Med.* **186,** 1269–1275.
8. Rooke, R., Waltzinger, C., Benoist, C. & Mathis, D. (1997) *Immunity* **7,** 123–134.
9. Tanchot, C., Lemonnier, F. A., Perarnau, B., Freitas, A. A. & Rocha, B. (1997) *Science* **276,** 2057–2062.
10. Bender, J., Mitchell, T., Kappler, J. & Marrack, P. (1999) *J. Exp. Med.* **190,** 367–374.
11. Teague, T. K., Marrack, P., Kappler, J. & Vella, A. T. (1997) *J. Immunol.* **158,** 5791–5799.
12. Boise, L. H., Minn, A. J., June, C. H., Lindsten, T. & Thompson, C. B. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 5491–5496.
13. Akbar, A. N., Borthwick, N. J., Wickremasinghe, R. G., Panayoitidis, P., Pilling, P., Bonfill, M., Krajewski, S., Reed, J. C. & Salmon, M. (1996) *Eur. J. Immunol.* **26,** 294–299.
14. Vella, A. T., Dow, S., Potter, T. A., Kappler, J. & Marrack, P. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 3810–3815.
15. Boursalian, T. E. & Bottomly, K. (1999) *J. Immunol.* **162,** 3795–3801.
16. Kawabe, Y. & Ochi, A. (1991) *Nature (London)* **349,** 245–248.
17. Vella, A. T., McCormack, J. E., Linsley, P. S., Kappler, J. W. & Marrack, P. (1995) *Immunity* **2,** 261–270.
18. Julius, M. H., Simpson, E. & Herzenberg, L. (1973) *Eur. J. Immunol.* **3,** 645–649.
19. Kelly, K. & Siebenlist, U. (1988) *J. Biol. Chem.* **263,** 4828–4831.
20. Torelli, G., Selleri, L., Donelli, A., Ferrari, S., Emilia, G., Venturelli, D., Moretti, L. & Torelli, U. (1985) *Mol. Cell. Biol.* **5,** 2874–2877.
21. Becker, W. & Joost, H. G. (1999) *Prog. Nucleic Acid Res. Mol. Biol.* **62,** 1–17.
22. Byrne, J. A., Nourse, C. R., Basset, P. & Gunning, P. (1998) *Oncogene* **16,** 873–881.
23. Kawamata, H., Nakashiro, K., Uchida, D., Hino, S., Omotehara, F., Yoshida, H. & Sato, M. (1998) *Brit. J. Cancer* **77,** 71–80.
24. Kuo, C. T., Veselits, M. L. & Leiden, J. M. (1997) *Science* **277,** 1986–1990.
25. Kahn-Perles, B., Lipcey, C., Lecine, P., Olive, D. & Imbert, J. (1997) *J. Biol. Chem.* **272,** 21774–21783.
26. Jain, J., Valge-Archer, V. E. & Rao, A. (1992) *J. Immunol.* **148,** 1240–1250.
27. Chen, F., Chen, D. & Rothenberg, E. (1999) *Int. Immunol.* **11,** 677–688.
28. Shin, H. M. & Han, T. M. (1999) *Mol. Immunol.* **36,** 197–203.
29. Sundstedt, A. & Dohlsten, M. (1998) *J. Immunol.* **161,** 5930–5936.
30. Alizadeh, A., Eisen, M., Botstein, D., Brown, P. O. & Staudt, L. M. (1998) *J. Clin. Immunol.* **18,** 373–379.
31. Hasegawa, T., Takeuchi, A., Miyaishi, O., Isobe, K. & de Crombrugghe, B. (1997) *J. Biol. Chem.* **272,** 4915–4923.
32. Jung, T. M., Gallatin, W. M., Weissman, I. L. & Dailey, M. O. (1988) *J. Immunol.* **141,** 4110–4117.
33. Sauer, F. & Tijan, R. (1997) *Curr. Opin. Genet. Dev.* **7,** 176–181.
34. el-Deiry, W. S. (1998) *Semin. Cancer Biol.* **8,** 3445–3457.
35. Hildeman, D. A., Mitchell, T. C., Teague, T. K., Henson, P., Day, B. J., Kappler, J. & Marrack, P. (1999) *Immunity* **10,** 735–744.
36. Mitchell, T., Kappler, J. & Marrack, P. (1999) *J. Immunol.* **162,** 4527–4535.