Multiple differences in gene expression in regulatory Vα24JαQ T cells from identical twins discordant for type I diabetes

S. Brian Wilson*†, Sally C. Kent‡, Heidi F. Horton§, Andrew A. Hill§, Paul L. Bollyky¶, David A. Hafler‡, Jack L. Strominger‖, and Michael C. Byrne§†

*Cancer Immunology and AIDS, Dana Farber Cancer Institute, Boston, MA 02115; ‡Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, MA 02115; §Genetics Institute, Cambridge, MA 02140; ¶Harvard Medical School, Boston, MA 02115; and ‖Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138

Contributed by Jack L. Strominger, April 10, 2000

Quantitative and qualitative defects in CD1d-restricted T cells have been demonstrated in human and murine autoimmune diseases. To investigate the transcriptional consequences of T cell receptor activation in human Vα24JαQ T cell clones, DNA microarrays were used to quantitate changes in mRNA levels after anti-CD3 stimulation of clones derived from identical twins discordant for type 1 diabetes and IL-4 secretion. Activation resulted in significant modulation of 226 transcripts in the IL-4 secreting clone and 86 in the IL-4-null clone. Only 28 of these genes were in common. The differences observed suggest both ineffective differentiation of diabetic Vα24JαQ T cells and a role for invariant T cells in the recruitment and activation of cells from the myeloid lineage.

Invariant CD161+ T cells are reported to be important in the regulation of T helper cell (Th) Th1/Th2 bias (1). In several murine models of autoimmunity, CD161+ Vα14Jα281 T cells were shown to be present in diminished numbers and to further decrease in frequency before the onset of disease (2–4). When this population of cells was transferred from either nonobese nondiabetic (NOD) or nonobese diabetic/Vα14Jα281-transgenic donors to prediabetic animals, the recipients were protected from diabetes (4, 5). This transfer of protection was significantly inhibited by the coadministration of anti-IL-4 antibodies (6).

Humans have a homologous invariant (i.e., with no N region additions) CD161+Vα24JαQ T cell population whose restriction element, like that for the murine CD161+Vα14Jα281 T cells, is the nonpolymorphic class Ib molecule CD1d (7). We recently demonstrated that in five sets of monozygotic twins and triplets discordant for type 1 diabetes, invariant Vα24JαQ T cells were present at significantly higher frequencies in the nondiabetic siblings (8). Moreover, Vα24JαQ T-cell clones from the nondiabetic siblings secreted both IL-4 and IFN-γ, whereas those derived from the diabetic siblings had an extreme impairment in the ability to secrete IL-4. To delineate differences in gene expression that might account for the discordant phenotype and to ask whether the loss of IL-4 secretion was the only defect, a comprehensive analysis of T cell activation was undertaken in a representative clone pair derived from these disease-discordant twins.

Methods

Antibodies. Anti-Vα24, -Vβ11, and -$\alpha\beta$TCR were purchased from Immunotech (Westbrook, ME). Anti-CD4, -CD8, and -CD161 were purchased from PharMingen. Anti-CD3, clone UCHT1, was purchased from Ancell (Bayport, MN), and IgG1 control was purchased from Sigma.

Flow Cytometry. Stained cells were analyzed on a FACScan cytometer (Beckton Dickinson), and single-cell sorting and calcium flux determination was performed by using a MoFlo cytometer (Cytomation, Fort Collins, NJ) as described (8).

Cell Culture. Single Vα24-positive, CD4/8-negative single-cell sorts were grown on irradiated allogeneic feeders at 50,000 cells per well with 5,000 cells per well irradiated (5,000 rads) 721.221 lymphoblastoid cells with 1 μg/ml PHA-P, IL-2, and IL-7 each at 10 units/ml (Boehringer Mannheim) and propagated as described (8). Clones positive for Vα24 and NKR-P1A by flow cytometry and a Vα24JαQ CDR3 T cell antigen receptor sequence were assayed for cytokine secretion in C1R/CD1d restriction experiments. For cytokine secretion and inhibitor studies, Vα24JαQ T cell clones GW4 (nondiabetic) and ME10 (diabetic) at 5×10^4 cells per well were activated with plate-bound anti-CD3 or Ig control at 1 μg/ml. Secreted IL-4 and IFN-γ were assayed by ELISA after 4 h of activation as described (8). Optimal concentrations of inhibitors previously were determined by inhibitor dose–response experiments. The concentrations of inhibitors used were 10 nM wortmannin; 10 μM LY294002; 50 μM PD98059, a mitogen-activated protein kinase kinase inhibitor; and 50 μM SB203580, a p38 kinase inhibitor. The concentrations of phorbol ester and calcium ionophore used were 1 ng/ml phorbol 12-myristate 13-acetate (PMA) and 1 μg/ml ionomycin. Cyclosporin A (CsA) was used at 5 ng/ml. Calcium flux was determined by loading cells with indo-1 as per the manufacturer's specifications (Molecular Probes), followed by activation with anti-CD3 at 10 μg/ml. Maximal calcium flux was determined by the addition of ionomycin.

Messenger RNA Expression. Vα24JαQ T cell clones GW4 and ME10 (1×10^7 cells) were activated for 4 h with 10 μg/ml soluble anti-CD3 or control IgG. Optimal concentrations of anti-CD3 previously were determined by dose–response experiments measuring cytokine secretion. Total RNA was isolated with Qiagen RNeasy kits. Total RNA then was converted to double-stranded cDNA by priming with an oligo(dT) primer that included a T7 RNA polymerase promoter site at the 5′ end (11). The cDNA was used directly in an *in vitro* transcription reaction in the presence of biotinylated nucleotides to produce labeled cRNA (antisense RNA), which

Abbreviations: Th, T helper; PI3-kinase, phosphoinositide-3-OH kinase; PMA, phorbol 12-myristate 13-acetate.

See commentary on page 6933.

†To whom reprint requests should be addressed. E-mail: mbyrne@genetics.com (M.C.B.) or brian_wilson@dfci.harvard.edu (S.B.W.).

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.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.120161297. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.120161297

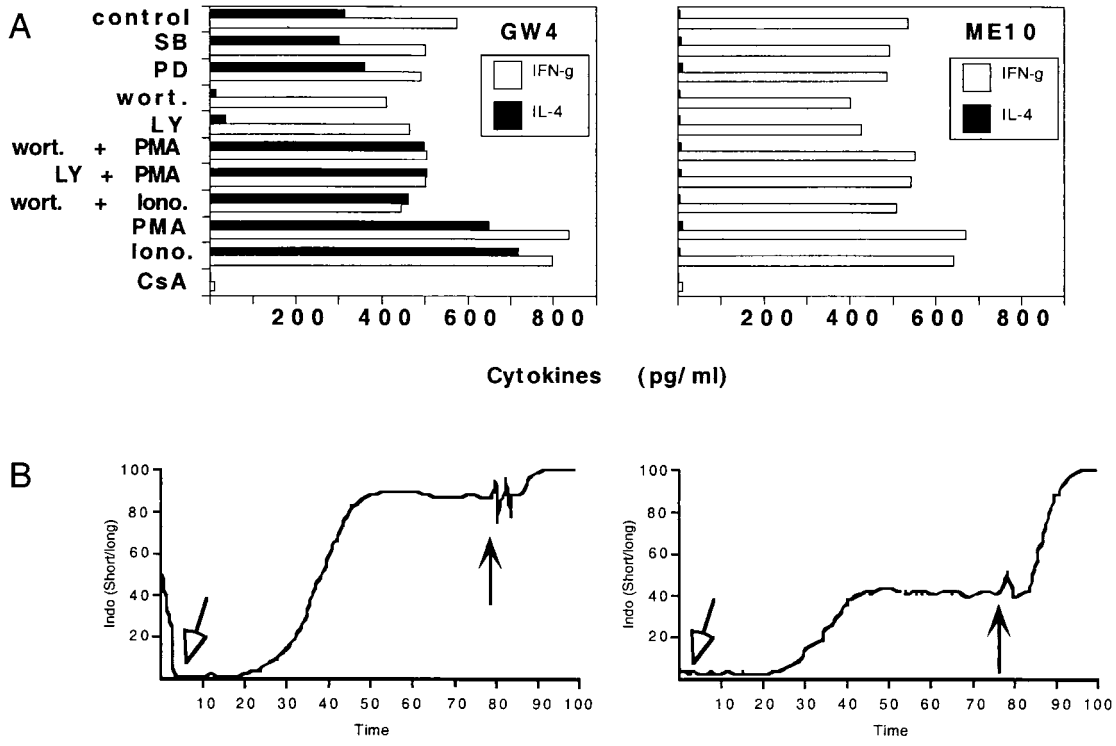

Fig. 1. Discordant expression of PI3-kinase-regulated events differentiates IL-4+ from IL-4-null Vα24JαQ T cell clones. (*A*) Vα24JαQ T cell clones GW4 (nondiabetic) and ME10 (diabetic) were activated with plate-bound anti-CD3 or Ig control. Levels of secreted IL-4 and IFN-γ were assayed by ELISA. The concentrations of inhibitors used were 10 nM wortmannin (wort.); 10 μM LY294002 (LY); 50 μM PD98059 (PD), a mitogen-activated protein kinase kinase inhibitor; and 50 μM SB203580 (SB), a p38 kinase inhibitor. Data points were collected in triplicate, and the Fig. is representative of four independent experiments. The concentrations of phorbol ester and calcium ionophore used were 1 ng/ml PMA and 1 μg/ml ionomycin (Iono). Cyclosporin A (CsA) was used at 5 ng/ml. (*B*) Vα24JαQ T cell clones GW4 (IL-4+) and ME10 (IL-4-null), 1×10^6 cells each, were loaded with Indo-1 (10 μM) for 45 min, then stimulated with 10 μg/ml anti-CD3 (open arrowhead) and analyzed on a Cytomation MoFlo instrument. At the end of the experiment, ionomycin was added to a final concentration of 1 μg/ml (filled arrowhead) to determine maximal flux. The ratio of Indo-1 fluorescence 410/490 nm (410, Ca^{2+}-bound; 490, Ca^{2+}-free) after stimulation in a representative pair of clones is pictured. Control experiments showed no differences in calcium flux in response to thapsigargin treatment (data not shown).

was hybridized overnight to Genechips (Affymetrix, San Jose, CA). After staining with phycoerythrin-streptavidin, the fluorescence of bound RNA was quantitated by using a Genechip Reader (a modified confocal microscope; Affymetrix).

Results and Discussion

To identify which of the known signaling cascades initiated by T cell antigen receptor ligation played a dominant role in IL-4 secretion, a series of inhibitor studies was performed. Inhibitors of specific kinase cascades were used in conjunction with anti-CD3 stimulation. Both phosphoinositide-3-OH kinase (PI3-kinase) inhibitors wortmannin and LY294002 blocked anti-CD3-induced IL-4 secretion from the IL-4+ clone, but had no effect on the secretion of IFN-γ from either the IL-4+ or IL-4-null clones (Fig. 1). In contrast, inhibition of the mitogen-activated protein kinase kinase by PD98059 or the JNK and p38 cascades with SB203580 (9, 10) had no effect. After inhibition of PI3-kinase, IL-4 secretion could be rescued in the IL-4+ clone by the inclusion of the phorbol ester PMA or the calcium ionophore ionomycin. Neither of these substances alone or in combination repaired the defect in IL-4 secretion from the diabetic-derived clone ME10. In addition, Vα24JαQ T cell clones derived from diabetic individuals had a diminished capacity to accumulate intracellular calcium after anti-CD3 stimulation (Fig. 1). These data suggest that the observed discordant IL-4 phenotype seen after T cell antigen receptor ligation cannot simply be located upstream of PI3-kinase, and the differences are likely to include proteins that regulate calcium flux.

Because the defect in IL-4 secretion was likely the result of multiple differences, a representative clone pair was chosen for intensive analysis with DNA microarrays that monitor the expression of $\approx$6,800 genes (Unigene collection; National Center for Biotechnology Information, Bethesda, MD). The DNA microarrays provide a practical and reproducible approach for large-scale study of complex differences in gene expression (11–14). Expression profiles were determined after 4 h of stimulation with anti-CD3 or control IgG. This time point was selected because it was used in a previous analysis of cytokine secretion in clones derived from monozygotic twin pairs discordant for type 1 diabetes (8). The number of genes with detectable expression either before or after stimulation was nearly identical for the IL-4 null and IL-4-secreting clones (1,523 and 1,558, respectively). As expected, the frequency of the majority of transcripts was unchanged. Interestingly, only about 2/3 of this set (988) were shared between the two clones. The number of genes whose expression after anti-CD3 stimulation was found to increase or decrease by at least 2-fold relative to unstimulated genes were 86 (6%) and 226 (15%) in the IL-4-null and IL-4+ clones, respectively.

To more thoroughly analyze the differences in gene expression between the IL-4-null and IL-4-secreting clones, genes were grouped into six distinct expression patterns, by using the Self-Organizing Map algorithm (Fig. 2) (15). All genes modulated at least 2-fold on anti-CD3 stimulation in either the IL-4-secreting or IL-4-null clones were clustered according to the relative behavior of each gene in the two clones. The first

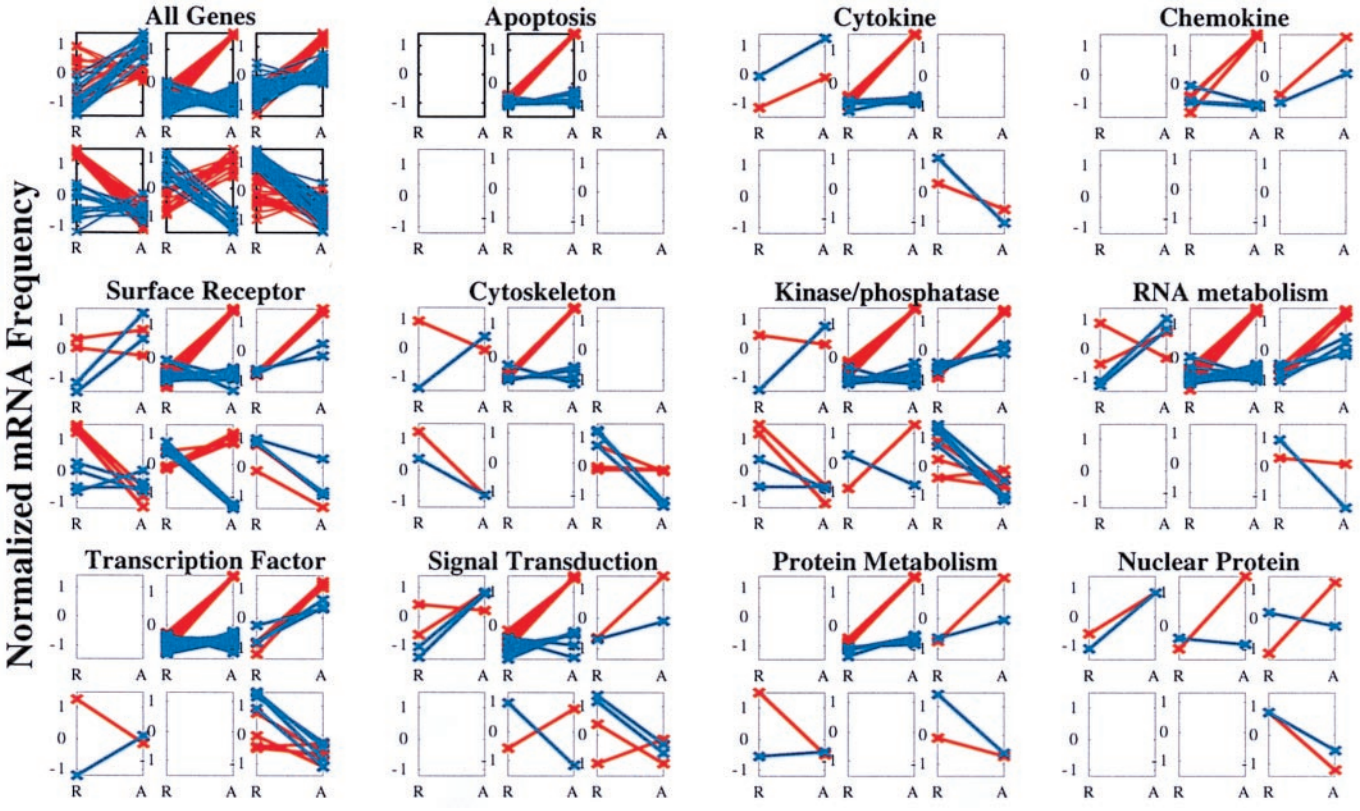

Fig. 2. The fraction of genes in Vα24JαQ T cell clones altered by anti-CD3 treatments. Graphical representation of genes differentially expressed in natural killer T cell clones derived from a diabetic/nondiabetic twin pair. Clones ME10 (blue; IL-4-null) and GW4 (red; IL-4+) were treated with control IgG (designated R for Resting) or anti-CD3 (designated A for Activated) for 4 h, after which RNA was isolated and analyzed on Genechips monitoring the expression of 6,800 human genes from the Unigene collection. Genes whose expression was modulated at least 2-fold in either clone were chosen for clustering analysis with the Self-Organizing Map algorithm (15). This method was used to cluster genes into six distinct groups, based on differential expression patterns between ME10 and GW4, independent of expression magnitude. The first group displays the six patterns represented when all genes meeting the 2-fold change criterion are used. The other groupings reveal the differential expression patterns of selected gene functional classes. Individual genes of each functional class falling into the different clusters are identified in Table 1.

panel of Fig. 2 displays the results for all genes meeting the 2-fold criterion, and the other 11 panels show the results for specific functional classes. The dominant pattern that emerged is represented in row 1, column 2, and contains genes that were up-regulated upon activation in the IL-4-secreting clone but that were nonresponsive to stimulation in the IL-4-null clone. This finding was true for all functional classes examined, indicating a profound defect in transcriptional induction for a large number of genes in the IL-4-null clone. However, examination of the other five clusters revealed that the transcriptional dysregulation in the IL-4-null clone is more complex than merely a global nonresponsiveness, as evidenced by a group of genes that were induced in this clone but not in the IL-4-secreting clone (row 1, column 1) and by a group that contained genes that were down-regulated in the IL-4-null clone but up-regulated in the IL-4-secreting clone (row 2, column 2). Clearly, the IL-4-null clone is able to respond to stimulation through the T cell receptor. The identity of the genes in each cluster for the 11 functional classes is listed in Table 1.

An identification of the pattern of genes activated in a particular cell type may provide information predictive of the function of that cell. The suggested primary effector function of invariant CD161+ T cells, direct regulation of T cell Th2 bias, is thought to be mediated in part by burst secretion of IL-4 in response to CD1d without prior IL4 priming (1). This concept as the *in vivo* function for these cells has been controversial for several reasons, among which are the observations that CD1d knockout mice retain the ability to mount antigen-specific Th2 responses and that natural killer T cells have a demonstrated role in tumor surveillance (16–18). When examined on the DNA microarrays, activation of Vα24JαQ T cell clones by anti-CD3 resulted in significant changes in transcripts of the cytokine/chemokine family. Changes in gene expression patterns in this category are particularly relevant given the association of cytokine secretion and the *in vivo* function for these cells. Marked differences in the expression of genes in this category were found when comparing the IL-4+ with the IL-4-null clone (Fig. 3). The transcriptional changes noted in macrophage inflammatory protein-1α, macrophage inflammatory protein-1β, tumor necrosis factor-α, tumor necrosis factor-β, IL-5, IL-13, and granulocyte–macrophage colony-stimulating factor each have been verified at the protein level by ELISA (our unpublished data). When comparing clones from the disease-discordant twins, robust changes were detected in several transcripts in the IL-4-null clone, including those in common with the IL-4-secreting clone, and a total of 1,523 transcripts were present at significant levels, 535 of which were unique to the IL-4-null clone. In addition, the clone pairs secreted equivalent amounts of IFN-γ in response to anti-CD3. Importantly, if a significant portion of the effector function of CD161+ Vα24JαQ T cells occurs through cytokine secretion, then the IL-4-null clone has failed to engage the complete spectrum of differentiated function. Recently, defects in the ability to both respond to activation and subsequently

Table 1. Genes differentially expressed between natural killer T cell clones ME10 and GW4

Functional category	Accession no.	Common name	Cluster (row, column)
Surface receptor			
	U38276	Semaphorin III	(1,1)
	U82169	Frizzled	(1,1)
	M32315	TNF-R	(1,2)
	U03397	4-1BB	(1,2)
	S77812	VEGF-R	(1,2)
	X01057	IL-2Rα	(1,2)
	Y00285	IGF-R II	(1,2)
	L08096	CD27	(1,2)
	Z30426	CD69	(1,2)
	U76764	CD97	(1,2)
	U60800	CD100	(1,2)
	M24283	Rhinovirus-R	(1,2)
	U19906	Arginine vasopressin-R	(1,2)
	Z48042	p137	(1,2)
	D79206	Ryudocan	(1,3)
	HT3125	CD44	(1,3)
	L39064	IL-9R	(2,1)
	X14046	CD37	(2,1)
	L31584	EBI-1	(2,1)
	X97267	LPAP	(2,1)
	M33680	TAPA-1	(2,2)
	M63175	AMFR	(2,2)
	U60975	gp250	(2,2)
	Z50022	C21orf3	(2,2)
	U90546	Butyrophilin BT4	(2,3)
	U90552	Butyrophilin BT5	(2,3)
	X96719	AICL	(2,3)
Cytoskeleton			
	U80184	Flightless I homolog	(1,1)
	X00351	β-Actin	(1,2)
	U20582	Actin-like peptide	(1,2)
	X82207	β-Centractin	(1,2)
	X98534	VASP	(1,2)
	D83735	Calponin	(2,1)
	J00314	β-Tubulin	(2,3)
	M21812	Myosin LC	(2,3)
	X98411	Myosin-IE	(2,3)
Kinase/phosphatase			
	X79510	PTP D1	(1,1)
	L10717	ITK	(1,2)
	X60673	AK3	(1,2)
	X85545	PKX-1	(1,2)
	D13720	LYK	(1,2)
	HT1153	Nm23-H2S	(1,2)
	M30448	CK II β	(1,2)
	M90299	Glucokinase	(1,2)
	U08316	ISPK-1	(1,2)
	X80910	PPP1CB	(1,2)
	X93920	DUSP-6	(1,2)
	U24152	PAK-1	(1,3)
	D11327	PTPN7	(1,3)
	U15932	DUSP-5	(1,3)
	L16862	GRK-6	(2,1)
	L27071	TXK	(2,1)
	J03805	PPP2CB	(2,2)
	HT3678	CLK-1	(2,3)
	U66464	HPK-1	(2,3)
	X62535	DAG kinase	(2,3)
	M31724	PTP-1B	(2,3)
Cytokine			
	U89922	LT-β	(1,1)
	J00219	IFN-γ	(1,2)
	V00536	IFN-γ	(1,2)
	M13207	GM-CSF	(1,2)
	M16441	TNF-α	(1,2)
	X02910	TNF-α	(1,2)
	X04688	IL-5	(1,2)
	U31120	IL-13	(1,2)
	M37435	M-CSF	(1,2)
	U02020	PBEF	(1,2)
	U37518	TRAIL	(1,2)
	U46461	Dishevelled homolog	(1,2)
	M90391	IL-16	(2,3)
Nuclear protein			
	U73477	Nuclear pp32	(1,1)
	U62962	Int-6	(1,2)
	L25931	Lamin B receptor	(1,3)
	M17733	Thymosin-β4	(2,3)
Transcription factor			
	M69043	IκBα	(1,2)
	X58072	GATA-3	(1,2)
	U43185	STAT-5A	(1,2)
	X51345	Jun-B	(1,2)
	X56681	Jun-D	(1,2)
	U15460	B-ATF	(1,2)
	HT4899	C-myc	(1,2)
	L00058	C-myc	(1,2)
	M13929	C-myc	(1,2)
	U26173	NF-IL3A	(1,2)
	M97796	Id-2	(1,2)
	M96843	Id-2B	(1,2)
	D14826	CREM	(1,2)
	S68271	CREM	(1,2)
	J03827	Y box BP	(1,2)
	U09412	ZNF134	(1,2)
	U13044	NRF-2α	(1,2)
	U22431	HIF-1α	(1,2)
	X78925	HZF-2	(1,2)
	Z47727	RNA POL2K	(1,2)
	J04076	EGR-2	(1,3)
	D61380	DJ-1	(1,3)
	HT4567	PC4	(1,3)
	HT4921	BTF-3 homolog	(2,1)
	L41067	NFAT-4C	(2,3)
	L78440	STAT-4	(2,3)
	M82882	ELF-1	(2,3)
	M83667	NF-IL6	(2,3)
Signal transduction			
	HT5108	TRAP-3	(1,1)
	X80200	MLN62	(1,1)
	U20158	SLP-76	(1,2)
	U26710	Cbl-b	(1,2)
	D78132	RHEB	(1,2)
	M63573	SCYLP	(1,2)
	M75099	FK506 BP	(1,2)
	Z35227	TTF	(1,2)
	U19261	EBV-independent	(1,3)
	M28209	RAB-1	(2,2)
	D78577	14-3-3-Eta	(2,3)
	X89399	Ins(1345)P4 BP	(2,3)
RNA Metabolism			
	D38251	RNP B5	(1,1)
	U90547	RNP homolog	(1,1)
	X17567	RNP B	(1,2)
	M29064	RNP B1	(1,2)

Table 1. (*continued*)

Functional category	Accession no.	Common name	Cluster (row, column)
	HT110	RNP A/B	(1,2)
	Z23064	RNP G	(1,2)
	HT3238	RNP K	(1,2)
	X52979	RNP SmB	(1,2)
	U15009	RNP SmD3	(1,2)
	X85372	RNP Sm F	(1,2)
	U30827	SF SRp40	(1,2)
	X70944	SF (PTP-associated)	(1,2)
	M60858	Nucleolin	(1,2)
	U10323	NF45	(1,2)
	U38846	Stimulator of TAR	(1,2)
	X59417	PROS-27	(1,2)
	X59892	IFN-independent γ2	(1,2)
	X66899	EWS	(1,2)
	X71428	fus	(1,2)
	X72727	Tunp	(1,2)
	X75755	PR264	(1,2)
	Z24724	Poly A site	(1,2)
	L28010	RNP F	(1,3)
	HT4788	RNP I	(1,3)
	L03532	M4	(1,3)
	U69546	RNA BP	(2,3)
Apoptosis			
	Z23115	Bcl-X_L	(1,2)
	U45878	IAP-1	(1,2)
	U11821	Fas ligand	(1,2)
	S81914	IEX-1	(1,2)
	U37546	MIHC	(1,2)
Chemokine			
	M23178	MIP-1α	(1,2)
	J04130	MIP-1β	(1,2)
	M69203	MCP-1	(1,2)
	L19686	MIF	(1,3)
Protein metabolism			
	D28473	ILE-tRNA synthase	(1,2)
	U09510	GLY-tRNA synthase	(1,2)
	L25085	Sec61-β	(1,2)
	X74801	Chaperonin cctg	(1,2)
	X77584	Thioredoxin	(1,2)
	Y00281	Ribophorin I	(1,2)
	Y10807	ARG-methyltransferase	(1,3)
	D13748	EIF-4AI	(1,3)
	X55733	EIF-4B	(2,1)
	X76648	Glutaredoxin	(2,3)

Genes populating the six expression clusters for the 11 gene functional categories shown in Fig. 2 are listed. Each gene is identified by GenBank accession no. [or The Institute for Genomic Research (TIGR) identifier for HT designations], followed by a common name and the specific cluster into which it fell (row, column).

secrete cytokines also were noted in the natural killer T cells of nonobese diabetic mice (19). In addition, this combination of cytokines/chemokines suggests that CD161+ T cells also may recruit and regulate immature dendritic cells and monocytes (our unpublished data).

When comparing the IL-4+ and IL-4-null clones, significant differences in expression were noted in other genes important for cell survival, cytokine secretion, and calcium flux that in part are activated through PI 3-kinase signaling, such as BCLxL, IAP, PLCγ1, and the tec family kinase, Itk (20–23). These transcripts were found at significantly greater abundance in the IL-4+ clone. Differences also were noted in the

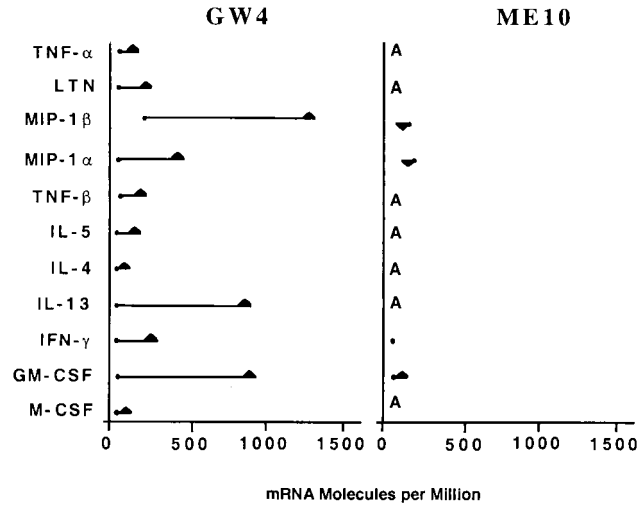

Fig. 3. Changes of expression on genes for cytokines and chemokines. Vα24JαQ T cell clone GW4 (IL-4+) is compared with ME10 (IL-4-null). For each individual transcript, the anti-CD3-induced hybridization intensity is shown. RNA was isolated, amplified, and hybridized to Genechips, displaying probes for 250 genes of immunological interest. This chip is custom designed for quantitative analysis by increasing the number of probes for the detection of each specific transcript. In the graph, the position of the black dot represents hybridization intensity for each gene before anti-CD3 stimulation, and a line is drawn to the position of hybridization intensity after stimulation. Genes that were called significantly different by a gene expression algorithm, and that changed by at least 2-fold, are indicated by a bold line. Upward- or downward-pointing arrowheads indicate increases or decreases in gene expression in the stimulated cells relative to the unstimulated cells. A, the transcript was not detected; TNF, tumor necrosis factor; LTN, lymphotactin; MIP, macrophage inflammatory protein; GM-CSF, granulocyte–macrophage colony-stimulating factor; M-CSF, macrophage colony-stimulating factor.

expression of mRNAs encoding transcription factors and signaling modulators important for cytokine secretion and Th phenotype. These included GATA3, STAT1, STAT4, JunB, JunD, and NFAT4. Notably, JunB and GATA3 recently were reported to be preferentially expressed in Th2 T cells (24, 25). Transcriptional activation of GATA3, JunB, as well as JunD, was found selectively in the IL-4+ clone. The transcripts for STAT1 (IFN-γ signaling), STAT4 (IL-12 signaling), and CD161 (a coactivator of Vα24JαQ T cell proliferation and IFN-γ secretion) (26–28) were overexpressed in the IL-4-null clone relative to the those in the IL-4+ clone. Importantly, the transcription factor NFAT4, thought to act in part as a suppressor of IL-4 transcription (29, 30), was overexpressed in the IL-4-null clone relative to the IL-4+ clone. Based on this data, the discordant regulation of other genes such as transcription factors might be predicted to be important for controlling Th phenotype. A model for regulated genes whose expression concurs with multiple independent biological observations is presented in Fig. 4.

In summary, the transcriptional profile of activated Vα24JαQ T cell clones revealed that the defect in IL-4 secretion seen in the clone from a diabetic patient (as compared with the identical nondiabetic twin) is only one of a large number of differences in gene expression. Importantly, differences were found in the expression of gene products whose activation in part is regulated by PI3-kinase, and they seem to be necessary for the generation of a fully differentiated Vα24JαQ T cell. Finally, a variety of genes with unknown functions also were associated with T cell activation, a subset of which was not expressed in the IL-4-null clone.

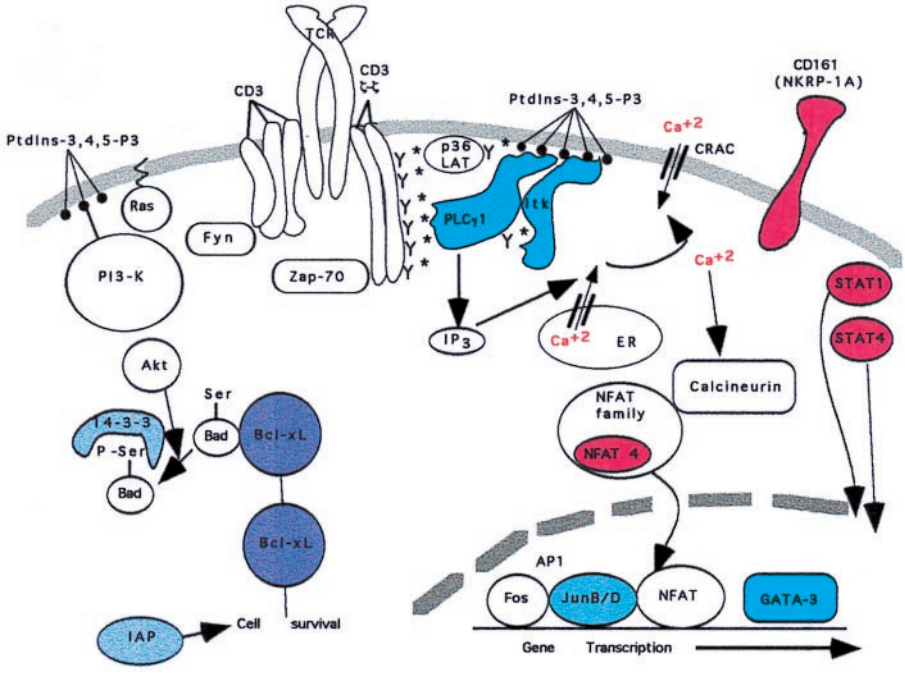

Fig. 4. A model for identified transcripts whose discordant expression is in accord with observed cell phenotypes. Pictured in cartoon format are genes whose transcripts have clearly defined cellular roles and whose discordant expression between GW4 and ME10 correlated with the established phenotypic differences. The genes are related by either being downstream of PI3-kinase, particularly several genes that regulate cell survival, or are directly required for calcium flux and calcium-regulated gene transcription. The proteins shaded various hues of blue represent genes expressed at significantly greater levels in GW4 (IL-4+) when compared with ME10 (IL-4-null). Conversely, those colored red were overexpressed in ME10 relative to GW4. Average copies of mRNA molecules per million for genes that were significantly altered by anti-CD3 treatment for GW4 (resting, activated) and ME10 (resting, activated), respectively, were: Itk (49, 115) and (25, 19); GATA3 (13, 26) and (8, 11); Jun-B (15, 118) and (14, 30); Jun-D (107, 291) and (68, 130); NFAT4 (33, 35) and (64, 26); STAT4 (36, 29) and (76, 36); 14–3-3 (24, 45) and (83, 41); Bcl-XL (11, 50) and (6, 7); and IAP (14, 211) and (10, 41). Selected genes whose expression was constitutive but discordantly expressed for GW4 vs. MW10 (GW4, ME10) were NKR-P1A (29, 459) and STAT1 (27, 79).

We thank E. L. Brown, G. Tucker-Kellogg, and K. Griffiths for assistance with data analysis, and M. Exely and M. Atkinson for critical reading of the manuscript. This work was supported by National Institutes of Health Grants RO1 AI44447 and RO1 DK52127 to S.C.K. and D.A.H., R35 CA47554 to J.L.S., and K11 DK02345 and RO1 AI45051 to S.B.W.

1. Bendelac, A., Rivera, M. N., Park, H.-S. & Roark, J. H. (1997) *Annu. Rev. Immunol.* **15,** 535–562.
2. Takeda, K. & Dennert, G. (1993) *J. Exp. Med.* **177,** 155–164.
3. Mieza, M. A., Itoh, T., Cui, J. Q., Makino, Y., Kawano, T., Tsuchida, K., Koike, T., Shirai, T., Yagita, H., Matsuzawa, A., *et al*. (1996) *J. Immunol.* **156,** 4035–4040.
4. Baxter, A. G., Kinder, S. J., Hammond, K. J. L., Scollay, R. & Godfrey, D. I. (1997) *Diabetes* **46,** 572–582.
5. Lehuen, A., Lantz, O., Beaudoin, L., Laloux, V., Carnaud, C., Bendelac, A., Bach, J. F. & Monteiro, R. C. (1998) *J. Exp. Med.* **188,** 1831–1839.
6. Hammond, K. I. L., Poulton, L. D., Palmisano, L. J., Silveira, P. A., Godfrey, D. I. & Baxter, A. G. (1998) *J. Exp. Med.* **187,** 1047–1056.
7. Exley, M., Garcia, J., Balk, S. P. & Porcelli, S. (1997) *J. Exp. Med.* **186,** 1–11.
8. Wilson, S. B., Kent, S. C., Patton, K. T., Orban, T., Jackson, R. A., Exley, M., Porcelli, S. P., Schatz, D. A., Atkinson, M. A., Balk, S. P., Strominger, J. L. & Hafler, D. A. (1998) *Nature (London)* **391,** 177–181.
9. Rincon, M. & Flavell, R. A. (1997) *Curr. Biol.* **7,** R729–R732.
10. Dumont, F. J., Staruch, M. J., Fischer, P., DaSilva, C. & Camacho, R. (1998) *J. Immunol.* **160,** 2579–2589.
11. Lockhart, D. J., Dong, H., Byrne, M. C., Follettie, M. V., Gallo, M. V., Chee, M. S., Mittmann, M., Wang, C., Kobayashi, M., Horton, H. & Brown, E. L. (1996) *Nat. Biotechnol.* **14,** 1675–1680.
12. Holstege, F. C. P., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Green, M. R., Golub, T. R., Lander, E. S. & Young, R. A. (1998) *Cell* **95,** 717–728.
13. Iyer, V. R., Eisen, M. B., Ross, D. T., Schuler, G., Moore, T., Lee, J. C. F., Trent, J. M., Staudt, L. M., Hudson, J., Jr., Boguski, M. S., *et al*. (1999) *Science* **283,** 83–87.
14. Cho, R. J., Campbell, M. J., Winzeler, E. A., Steinmetz, L., Conway, A., Wodicka, L., Wolfsberg, T. G., Gabrielian, A. E., Landsman, D., Lockhart, D. J. & Davis, R. W. (1998) *Mol. Cell* **2,** 65–73.
15. Tamayo, P., Slonim, D., Mesirov, J., Zhu, Q., Kitareewan, S., Dmitrovsky, E., Lander, E. S. & Golub, T. R. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 2907–2912.
16. Mendiratta, S. K., Martin, W. D., Hong, S., Boesteanu, A., Joyce, S. & Van Kaer, L. (1997) *Immunity* **6,** 469–477.
17. Smiley, S. T., Kaplan, M. H. & Grusby, M. J. (1997) *Science* **275,** 977–979.
18. Cui, J., Shin, T., Kawano, T., Sato, H., Kondo, E., Toura, I., Kaneko, Y., Koseki, H., Kanno, M. & Taniguchi, M. (1997) *Science* **278,** 1623–1626.
19. Falcone, M., Yeung, B., Tucker, L., Rodriguez, E. & Sarvetnick, N. (1999) *J. Exp. Med.* **190,** 963–972.
20. Cantley, L. C. & Neel, B. G. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 4240–4245.
21. Schaeffer, E. M., Debnath, J., Yap, G., McVicar, D., Liao, X. C., Littman, D. R., Sher, A., Varmus, H. E., Lenardo, M. J. & Schwartzberg, P. L. (1999) *Science* **284,** 638–641.
22. Liu, K.-Q., Bunnell, S. C., Gurniak, C. B. & Berg, L. J. (1998) *J. Exp. Med.* **187,** 1721–1727.
23. August, A., Sadra, A., Dupont, B. & Hanafusa, H. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 11227–11232.
24. Li, B., Tournier, C., Davis, R. J. & Flavell, R. A. (1999) *EMBO J.* **18,** 420–432.
25. Zheng, W. & Flavell, R. A. (1997) *Cell* **89,** 587–596.
26. Leonard, W. J. & O'Shea, J. J. (1998) *Annu. Rev. Immunol.* **16,** 293–322.
27. Arase, H., Arase, N. & Saito, T. (1996) *J. Exp. Med.* **183,** 2391–2396.
28. Exley, M., Porcelli, S., Furman, M., Garcia, J. & Balk, S. (1998) *J. Exp. Med.* **188,** 867–876.
29. Oukka, M., Ho, I. C., de la Brousse, F. C., Hoey, T., Grusby, M. J. & Glimcher, L. H. (1998) *Immunity* **9,** 295–304.
30. Ranger, A. M., Oukka, M., Rengarajan, J. & Glimcher, L. H. (1998) *Immunity* **9,** 627–635