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Complexity of the Primary Genetic Response to Mitogenic Activation of Human T Cells

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We describe the isolation and characterization of more than 60 novel cDNA clones that constitute part of the immediate genetic response to resting human peripheral blood T cells after mitogen activation. This primary response was highly complex, both in the absolute number of inducible genes and in the diversity of regulation. Although most of the genes expressed in activated T cells were shared with the activation response of normal human fibroblasts, a significant number were more restricted in tissue specificity and thus likely encode or effect the differentiated functions of activated T cells. The activatable genes could be further differentiated on the basis of kinetics of induction, response to cycloheximide, and sensitivity to the immunosuppressive drug cyclosporin A. It is of note that cyclosporin A inhibited the expression of more than 10 inducible genes, which suggests that this drug has a broad genetic mechanism of action.

As a rule, lymphocyte proliferation and effector functions are regulated by antigen or mitogen and lymphokine binding to cell surface receptors. The binding of such extracellular ligands results in a cascade of intracellular biochemical events (45, 46) that ultimately set in motion a genetic program for activation-related growth and expression of differentiated functions. Thus, quiescent T cells undergo a series of sequentially dependent, ordered transcriptional events after activation of quiescent cells that culminates in the initiation of DNA synthesis after about 24 h (5, 17). Primary gene transcriptional events, defined by their early kinetics and lack of dependence on prior protein synthesis, including proto-oncogenes (e.g., c-*myc* and c-*fos*, lymphokines (e.g., interleukin-2 [IL-2], gamma interferon [IFN-γ], granulocyte-macrophage colony-stimulating factor [GM-CSF]), and a cell surface lymphokine receptor (IL-2 receptor) (6, 9, 12, 17, 27, 38, 46), all of which are thought to have significant effects on T-cell proliferation or regulation of an immune response. It is expected that some of the immediately induced genes play a role in initiating and thus regulating the cascade of molecular events that follow mitogenic activation. Such genes are also likely targets of tumorigenic events. For example, a variety of data support the notion that uncontrolled growth occurs when the mitogen-inducible c-*myc* gene is expressed inappropriately (11, 28).

At present, a limited number of primary induced genes in T cells have been described. In the past, genes induced in activated T cells, such as those encoding IL-2 and IL-2 receptor, often have been identified on the basis of their encoded proteins. However, the transcriptional response of T cells is likely more complex than has been reported to date (2, 4, 15, 24, 29, 33, 42); therefore, we sought to characterize thoroughly the initial response of T cells to activation by cloning a maximal number of induced genes. Thus, we have used an unbiased approach to clone novel, mitogen-induced genes on the basis of the property of differential expression.

Human peripheral blood (PB) T cells were used as the model system in which to study activation for several reasons. First, PB T cells are primary quiescent cells that have not been selected for growth in culture, and they can be polyclonally stimulated in vitro. Second, upon activation, T cells express a variety of differentiated functions that play critical roles in the progression of an immune response. For example, novel lymphokines-cytokines would be expected to be included within the family of inducible genes, since a primary function of PB T cells after activation is to secrete soluble factors that affect ultimately a number of physiological functions (5, 6, 9, 13). Third, an important aspect in investigating the genetic response to activation is identification of mechanisms of gene regulation. Use of the immunosuppressive drug cyclosporin A (CSA), which acts on T cells, provides a tool for initially dissecting regulatory categories of induced genes. CSA appears to inhibit one or a few early T-cell activation pathways while leaving others intact (16, 25, 30). Finally, the T-cell system allows us to dissect and contrast the activation requirements mediated through various distinct surface molecules that are thought to play roles in T-cell development and function (23).

MATERIALS AND METHODS

Cell culture. Human PB T cells were obtained from healthy volunteers and were isolated over a Ficoll-Hypaque gradient and nylon wool columns. The resulting cell preparations were consistently more than 90% T cells as judged by anti-CD3 staining. PB T cells were cultured at a concentration of 2×10^6/ml in RPMI 1640 containing 10% fetal calf serum (FCS). PB T cells were stimulated for various periods of time with phytohemagglutinin (PHA)-P (1 μg/ml; Burroughs Welcome Co., Research Triangle Park, N.C.) and phorbol 12-myristate 13-acetate (PMA) (20 ng/ml) either with or without cycloheximide (10 μg/ml). The Jurkat cell line was provided by K. Hardy. Jurkat cells were maintained in RPMI 1640 supplemented with 10% FCS and 25 μg of gentamicin per ml. Jurkat cells were stimulated for various periods of time at a concentration of 5×10^5 with PHA-P (1 μg/ml) and PMA (20 ng/ml) with or without CSA (1 μg/ml; Sandoz). The human fibroblast lines CCD-11LU and WI38 were obtained from the American Type Culture Collection, Rockville, Md. Fibroblasts were grown to confluence in minimal essential medium containing 10% FCS and then maintained in minimal essential medium with 0.25% FCS for 3 to 5 days. To reinitiate growth, the spent medium was

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replaced by minimal essential medium supplemented with 20% FCS either with or without cycloheximide (10 μg/ml).

Subtraction cloning and hybridization. PB T cells were isolated and cultured as described above. RNA was isolated as described previously (8) from unstimulated cells or after stimulation for 4.5 h with PHA-P and PMA in the presence of cycloheximide. Poly(A)$^+$ RNA was purified by one passage over an oligo(dT) column (3). cDNA was synthesized by using oligo(dT) priming (18) from 20 μg of poly(A)$^+$ RNA from activated T cells. After hydrolysis of the RNA, this cDNA was hybridized to a C_0t value to 2,000 mol · s/liter with a 10-fold excess of poly(A)$^+$ RNA from unstimulated cells. The single-stranded molecules then were separated from the double-stranded cDNA-mRNA hybrids by chromatography, using a hydroxyapatite column (14, 20). After the first round of subtraction, 15% of the molecules appeared in the single-stranded fraction as judged by the distribution of counts. This fraction was again hybridized to a 10-fold excess of mRNA from unstimulated cells, and this second round of subtraction yielded about 90% single-stranded material. After size fractionation on a Sepharose CL-6B column, cDNA molecules that were larger than 400 nucleotides in size were used as templates by DNA polymerase I for second-strand synthesis. Double-stranded DNA was subsequently cloned into lambda gt10 according to standard procedures (22). A library with a base of 45,000 individual clones was obtained. About 40% of the subtracted cDNA library was screened with a subtracted cDNA probe synthesized as described above except that the probe was labeled to a high specific activity (5×10^8 cpm/μg) and was subtracted only once. After tertiary screening with subtracted probes and plaque purification, differential screening was performed. Duplicate filters were hybridized to a cDNA probe prepared from activated cell mRNA and a cDNA probe derived from unstimulated-cell mRNA. A total of 528 differentially hybridizing clones were obtained.

Northern (RNA) analyses. Total cellular RNA was extracted with guanidine isothiocyanate and purified by centrifugation through 5.7 M CsCl (8), separated by electrophoresis in formaldehyde-agarose gels, blotted onto GeneScreen membrane filters (Dupont, NEN Research Products, Boston, Mass.), and hybridized to ^{32}P-labeled cDNA inserts. Quantitative loading of RNA was determined by hybridization to a beta-2 microglobulin probe (data not shown).

Probes. Probes were kindly supplied by the following individuals or institutions: GM-CSF, Genetics Institute; IFN-γ, Meloy Laboratories; c-*fos*, T. Curran; IL-2 receptor, W. Greene; IL-3, S. Clark (Genetics Institute); Met- and Leu-preproenkephalin, S. Sabol; Human IL-4, American Type Culture Collection; p53, D. Givol; lymphotoxin, S. Gray (Genentech); IL-5, K. Arai (DNAX); ornithine decarboxylase, D. Nathans; *bcl-2*, A. Bakhshi; IL-6, H. Goldstein; c-*myb*, F. Mushinski; the heat shock protein 70 gene; and unwinding ATPase, R. Morimoto. M. Sporn provided a nick-translated transforming growth factor beta probe.

RESULTS

Subtractive cloning of inducible genes. To clone immediately inducible genes in mitogen-activated T cells, we used the method of subtractive cDNA cloning followed by subtractive probe hybridizations (summarized in Fig. 1), as these techniques afford the greatest sensitivity in detecting even those genes whose mature mRNAs appear at low levels upon activation (14, 20). Human PB T cells were polyclonally activated for 4.5 h in the presence of the mitogens PHA and PMA as well as the protein synthesis inhibitor cycloheximide. The latter agent is known to superinduce a number of growth-related genes (1, 27, 31, 35). In addition, cycloheximide prevents mRNA induction that follows IL-2 and IL-2 receptor synthesis and interaction. This focuses our analysis on the primary response of activated cells, defined by those genes which are inducible independent of new protein synthesis. To date, we have screened approximately 40% of the subtracted lambda gt10 cDNA library with subtracted probes. Purified phage that hybridized to subtracted probes were subjected further to a differential screen in which cDNA probes synthesized from activated and resting T-cell mRNA were used on duplicate filters of the phage. Finally, 528 phage clones were selected which harbored induced cDNAs as judged by both the subtractive and the differential screening methodologies.

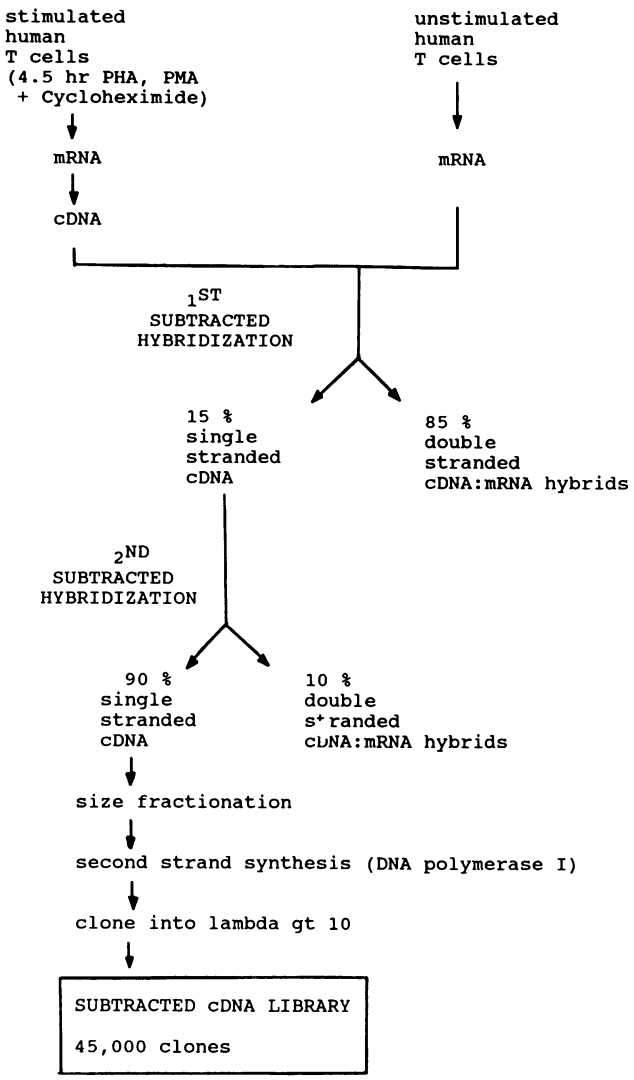

FIG. 1. Outline of the procedure used to isolate genes that are induced immediately upon mitogenic stimulation of human PB T lymphocytes (see Materials and Methods).

To determine the number of distinct genes among these 528 phage clones, we cross-hybridized subcloned cDNA inserts with the 528 phages. In this way, we have identified 66 unique cDNA clones, the majority of which appear to represent distinct genes. A limited number of groups may

derive from different segments of the same mRNA, thus leading to an overestimation of individual genes. However, the number of unique inducible genes will exceed 66, since 120 of the 528 phages have not hybridized to the selected cDNA inserts tested to date. The number of isolated phage clones belonging to a given group via cross-hybridization varied considerably and ranged from 1 to as many as 86 (see footnote *a* of Table 1). Forty-four of the novel inducible gene clones were studied further (see below). All hybridized to an inducible and, with few exceptions, single-sized message by Northern blot analyses (Table 1). Furthermore, none of the cDNA inserts contained repetitive sequences, but a few appeared to be members of small multigene families, as determined by Southern blot analyses (Table 1).

To assess the extent to which our subtracted library and the 528 selected phage clones might represent previously described induced genes, we subjected both to cross-hybridization analyses with many of the genes known to be inducible in T cells. The composition of the subtracted library was shown to represent a typical activated T-cell phenotype as determined by enrichment for clones encoding IL-2, GM-CSF, IFN-γ, c-*myc*, and c-*fos*, the latter of which is induced in T cells at 4.5 h in the presence of cycloheximide but to a much lesser extent than is c-*myc* (38). Among the selected 528 phages, we detected several isolates of c-*myc*. In addition, the 528 phages include cDNAs homologous to the IL-2 receptor, the IL-3 and IL-4 growth factors, and Met- and Leu-preproenkephalin (47, 49, 50) (Table 1). Among the 528 isolated phages, we could rule out the presence of IL-2, GM-CSF, IFN-γ, c-*fos*, p53, ornithine decarboxylase, *bcl-2*, lymphotoxin, transformation growth factor beta, c-*myb*, IL-5 and -6, the heat shock protein 70 gene, and the unwinding ATPase (6, 9, 13, 21, 26, 36, 38, 39, 48), confirming that many novel genes had been isolated. The detection of known genes (e.g., IL-2) in the library that were not present in the 528 phages selected with subtracted cDNA probes resulted in part from the much stronger signal generated by nick-translated versus heterogenous cDNA probes. In addition, although the length of the induction period (4.5 h) was optimal for a great number of genes, it was not optimal for those that are expressed with relatively delayed kinetics. Nonetheless, the hybridization data indicate that the 528 selected phages encompass many but not all of the genes expected and that the subtracted library contains the known induced genes that have been assayed.

Regulation of induced genes and sensitivity to CSA. To assess the heterogeneity of expression for the isolated inducible genes, kinetic analyses of mRNA levels for many of the genes were performed. Figure 2 displays typical Northern analyses for four novel inducible genes. One pattern of expression, exemplified by pAT 249, displayed a very rapid appearance after activation of T cells by PHA and PMA, i.e., by 30 min or less; another common pattern shown for pAT 464 is characterized by the appearance of mRNA only after 2 to 4 h. Additional mRNA species (e.g., pAT 129 and pAT 139; Fig. 2) were induced at intermediate times. The observed kinetic differences with regard to both the onset and duration of expression suggest a highly diverse regulation of the primary response. Approximately 70% of the genes (Table 1) were superinduced by cycloheximide, and the others were affected only minimally or not at all. The use of cycloheximide was critical for isolation of the rapidly induced genes, as many (e.g., pAT 129 and pAT 249) were expressed transiently and were detectable at 4.5 h only in the presence of the protein synthesis inhibitor. Cycloheximide alone did not cause expression of these genes (Fig. 2), which suggested that many may be transcriptionally regulated, as has been confirmed in several cases by nuclear run-on data (K. C. Gunter, S. G. Irving, P. F. Zipfel, U. Siebenlist, and K. Kelly, submitted for publication). The detailed requirements for expression of nine newly isolated genes are discussed in the accompanying paper (23).

The human $CD4^+$ helper cell line Jurkat is known to have retained the inducibility of several genes, including the lymphokines IL-2 and IFN-γ as well as the IL-2 receptor (19, 37, 45). We tested a number of the isolated genes for expression and regulation in this tumor line as well as for sensitivity to the immunosuppressive drug CSA. Many lymphokines elaborated by activated T cells are known to be suppressed by this drug, which appears to inhibit transcriptional induction (16, 37, 40). Four patterns of expression could be distinguished (Fig. 3 and Table 2). Of the 35 newly cloned genes tested, the expression of 22 was induced in Jurkat cells after treatment with PHA and PMA. In 11 of these (e.g., pAT 602; Fig. 3), induction was essentially unaffected by CSA. However, the induction of a surprisingly large number of genes, 11 of 22 (e.g., pAT 464; Fig. 3), was suppressed by CSA. This finding implies that CSA affects a step during activation which is common to a fairly large group of genes (possibly including many lymphokines-cytokines), which further suggests that a distinct activation path is required for this set of genes.

The remaining 13 genes tested were not induced in Jurkat T cells. Ten of these (e.g., pAT 133; Fig. 3) failed to hybridize to any message in Jurkat cells, as determined at a number of time points after induction (data not shown). Possibly, these messages exist in a cell type distinct from Jurkat, such as $CD8^+$ T cells. Alternatively, Jurkat cells are transformed and may have lost or modified these genes or the signaling machinery necessary to induce their mRNAs. Members of the last group of genes (3 of 35) were constitutively expressed in Jurkat cells (e.g., pAT 129; Fig. 3). These genes may contribute to the uncontrolled growth of this tumor line (see Discussion).

Tissue specificity of genes induced in T cells. An important question in the initial characterization of mitogen-induced genetic responsiveness is the tissue specificity of the transcriptional activation process. Do cells derived from different tissues exhibit a largely shared activation response or are they very dissimilar, given that the receptors for mitogenic agents are generally tissue specific? We studied activation of the normal human lung fibroblast cells WI38 and CCD-11LU (12). These cells enter quiescence after growth to confluence and subsequent incubation in 0.25% serum, and they can be stimulated to reenter the cell cycle in the presence of high concentrations of FCS, which contains multiple growth-promoting activities. An analysis of three representative induced genes is shown in Fig. 4, in which quiescent fibroblasts were stimulated with 20% serum for various amounts of time in the presence or absence of cycloheximide. The kinetics of expression for individual mitogen-induced genes were relatively similar in T cells and fibroblasts (compare Fig. 4 and Table 1). Interestingly, induction of mRNA in the presence of cycloheximide alone occurred frequently for a variety of genes in fibroblasts but not in T cells (compare Fig. 2 and 4). Such a result suggests that (i) human lung fibroblasts such as those used here contain a background of activated cells or (ii) the mechanisms for suppressing gene expression during quiescence differ in the two cell types. Approximately 80% of the novel induced T-cell genes analyzed here could be detected in fibroblasts by Northern analyses (Table 2). Thus, although

TABLE 1. Characterization of mitogen-induced genes

Clone	Frequency grouping[a]	Insert size (base pairs)	mRNA size (nucleotides)[b]	Kinetics of mRNA induction (min)[c]	Cycloheximide effect[d]	Genomic organization[e]
pAT 120	V	1,600	2,000	60/60	S	F
pAT 125	IV	400	3,500	60/60	S	S
pAT 127	V	900	2,000	30/60	S	S
pAT 129	II	600	2,000	60/60	S	M
pAT 133	II	600	2,000	120/240	S	S
pAT 139	III	800	2,500	60/120	S	–
pAT 140S	I	200	2,600	270/270	S	S
pAT 140L	II	300	2,600	240/270	S	S
pAT 154	V	800	3,400	30/120	S	S
pAT 158	III	1,000	3,400	+	–	S
pAT 189	I	1,100	3,000	+	S	M
pAT 201	III	350	3,500	+	–	–
pAT 204	III	360	2,700	+	–	S
pAT 225	V	600	3,900	30/60	S	S
pAT 229	III	600	6,700	240/240[f]	S	S
pAT 232S	I	400	2,600	120/120	S	S
pAT 232L	IV	900	1,900	60/270[f]	S	M
pAT 237	V	1,800	2,000	30/120	S	S
pAT 239	I	400	8,100	+	–	S
pAT 243	IV	1,300	4,500	60/420	S	S
pAT 270	III	1,200	2,100	+	–	–
pAT 276	III	800	3,300	+	–	–
pAT 281	I	900	900	30/420	NE	S
pAT 383	IV	300	2,000[g]	120/270[f]	S	M
pAT 402	II	500	2,700	+	S	F
pAT 407	I	800	2,300	60/600	NE	S
pAT 416	III	600	2,400	60/120	S	S
pAT 428	III	500	5,800	+	–	S
pAT 464	III	900	900	120/420	NE	F
pAT 466	I	900	1,450	+	–	S
pAT 478	II	400	2,200	+	NE	S
pAT 483	I	300	1,800	+	–	S
pAT 485	II	700	6,800	240/240	S	S
pAT 496	I	400	2,000	+	–	S
pAT 516	II	450	1,300[h]	+	–	–
pAT 542	III	500	6,800[i]	+	S	–
pAT 563	III	1,800	2,400[j]	270/420	S	F
pAT 591	II	500	3,500	60/420	S	–
pAT 594	II	500	2,000	120/270	NE	S
pAT 603	I	300	4,800	+	NE	S
pAT 607	I	1,100	3,100	+	–	–
pAT 620	III	300	1,250	240/420	NE	–
pAT 730	II	550	1,900	+	NE	–
pAT 744	IV	800	800	120/420	NE	S
c-*myc*	II		2,400	60/120	S	S
IL-3	I		800	+		S
IL-4	II		700	+		S
IL-2R	I		3,500	+		S
PPE	II		1,300	+		S

[a] Number of cross-hybridizing phage clones among the 528 clones isolated that were detected by the subcloned insert listed: I, 1; II, 2 to 5; III, 6 to 10; IV, 11 to 25; V, more than 25. Among the total of 66 different clones that have been isolated to date, the frequency distribution is as follows: I, 22 clones; II, 17 clones; III, 16 clones; IV, 6 clones; and V, 5 clones.

[b] Determined relative to the migration of the 28S and 18S rRNA on formaldehyde-agarose gels.

[c] Time at which the induced mRNA species was first detected after PHA (1 μg/ml) and PMA (20 ng/ml) stimulation of PB T cells; the second number denotes the time at which maximal steady-state mRNA levels were noted. +, Gene induction in response to the combination of PHA, PMA, and cycloheximide, although extensive kinetic studies were not performed.

[d] Effect on PHA-PMA-induced steady-state mRNA levels in PB T cells relative to levels obtained with PHA or PMA alone. S, superinduced; NE, no or marginal effect of cycloheximide; –, effect not determined.

[e] Number of bands detected per digest on a Southern blot of human thymus DNA digested with *Bam*HI, *Eco*RI, or *Sst*I. S, Fewer than four bands per lane in all three digests; F, four or more bands per lane in at least two digests; M, five or more bands per lane in all three digests; –, genomic organization not determined.

[f] mRNA could be detected only when cycloheximide (10 μg/ml) was included in the PHA-PMA stimulation.

[g] pAT 383 also detected a constitutively expressed mRNA species of 900 nucleotides that was unaffected by cycloheximide.

[h] pAT 516 also detected a constitutively expressed mRNA of 1,700 nucleotides.

[i] pAT 542 also detected an mRNA species of 4,600 nucleotides that was coordinately expressed with the predominant species of 6,800 nucleotides.

[j] pAT 563 also detected mRNA species of 3,600, 4,100, and 8,500 nucleotides that were coordinately expressed with the predominant species of 2,400 nucleotides.

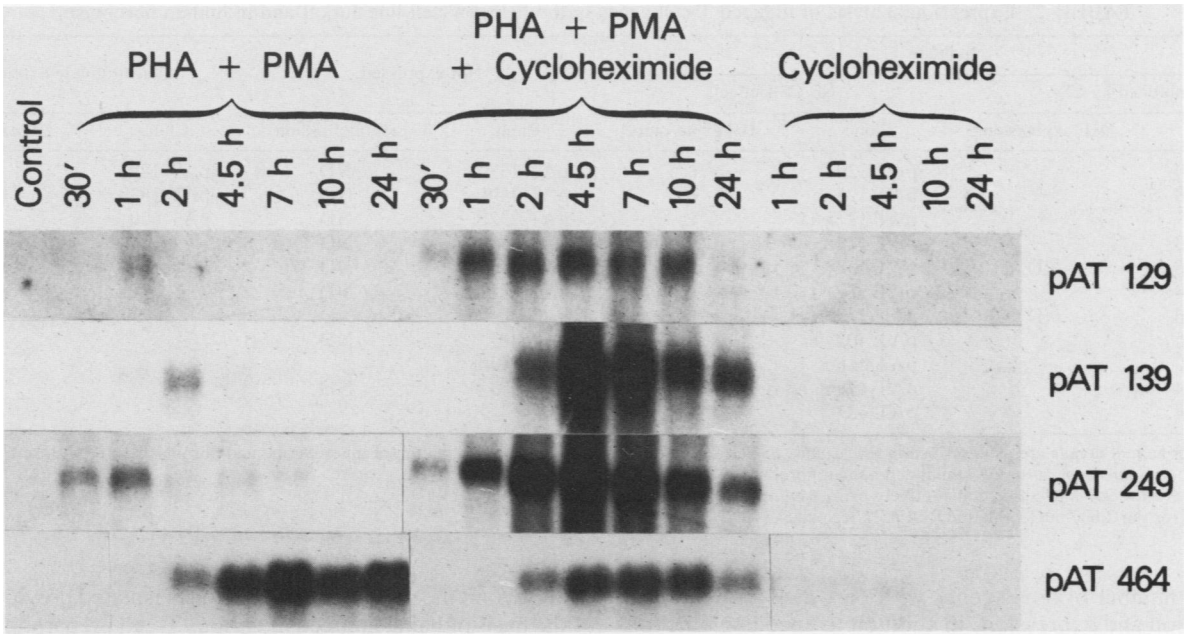

FIG. 2. Kinetics of mRNA induction of selected cDNA clones upon stimulation of resting human PB T cells. Human PB T cells were stimulated with PHA and PMA. Total cellular RNA was extracted at the times indicated. Equal amounts of RNA were loaded in each lane, as shown by hybridization of the filters to a beta-2 microglobulin probe (data not shown). pAT 249 is a member of the pAT 225 family (see Table 1). In multiple experiments, the induction of clones pAT 464 and pAT 744 was sometimes observed to be partially inhibited in the presence of cycloheximide, as seen here, but most often this drug had no noticeable effect on their steady-state message levels.

initial responses appeared to be largely similar in completely distinct cell types, some or the genes were more restricted in tissue specificity and thus likely to encode or effect differentiated functions of lymphocytes or T cells. To date, all four genes which display a restriction in tissue specificity have been inhibited in their induction by CSA. Interestingly, some of the induced genes expressed by both tissues are suppressed by CSA in T cells (Table 2), providing model systems for studying the tissue specificity of the action of CSA.

DISCUSSION

As demonstrated in this report, the primary genetic response to the activation of T cells is very complex, involving

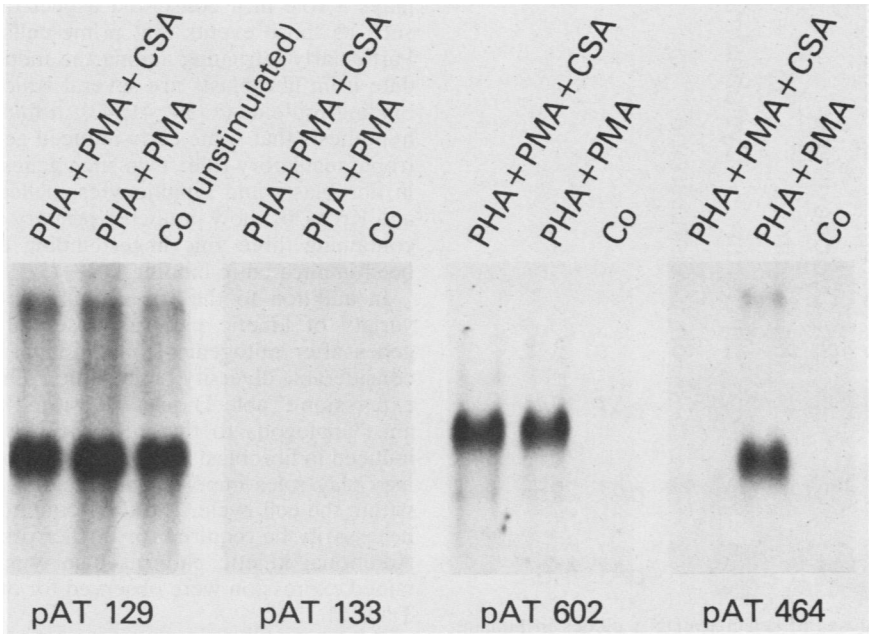

FIG. 3. Diverse expression and regulation of selected cDNA clones in the human helper T-cell line Jurkat. Total cellular RNA was isolated from constitutively growing Jurkat cells (control [Co]), from cells stimulated with PHA and PMA for 4 h, or from cells stimulated with PHA and PMA in the presence of the immunosuppressive drug CSA for 4 h and subjected to Northern analyses. pAT 602 is a member of the pAT 127 family (see Table 1).

TABLE 2. Expression analysis of induced T-cell genes in the helper T-cell line Jurkat and in human fibroblasts[a]

Induced				Not expressed		Constitutively expressed	
Inhibited by CSA		No CSA effect					
Clone	HF expression	Clone	HF expression	Clone	HF expression	Clone	HF expression
pAT 154	+	pAT 120	+	pAT 133	ND	pAT 129	+
pAT 229	+	pAT 125	+	pAT 140L	ND	pAT 383[b]	ND
pAT 237	−	pAT 127	+	pAT 232S	ND	pAT 620	+
pAT 243	+	pAT 140S	ND	pAT 239	ND		
pAT 428	ND	pAT 158	+	pAT 276	ND		
pAT 464	−	pAT 189	+	pAT 281	ND		
pAT 466	ND	pAT 225	+	pAT 383[c]	ND		
pAT 485	+	pAT 402	+	pAT 407	ND		
pAT 542	+	pAT 416	+	pAT 563	+		
pAT 594	−	pAT 478	ND	pAT 603	ND		
pAT 744	−	pAT 483	ND				

[a] Experimental details are as described in the legends to Fig. 3 and 4. +, Induction upon stimulation of quiescent human fibroblasts (HF) with serum; −, no expression in quiescent or serum-stimulated human fibroblasts; ND, not determined.
[b] Data for the 900-nucleotide constitutively expressed mRNA species (see Table 1, footnote *g*).
[c] Data for the 2,000-nucleotide induced mRNA species (see Table 1, footnote *g*).

a large number of novel genes with very distinct patterns of regulation and expression. In addition to encoding functions involving progression through the cell cycle and commitment to proliferation, these genes may encode other functions, such as modulation of the immune system. Specifically, those genes that exhibit limited tissue distribution of expression (Table 2) may be expected to play such roles in the differentiated function of activated T cells. Indeed, two of these genes, pAT 464 and 744, have been sequenced and display predicted hydrophobic leader peptides and structural homology with known secreted proteins, which suggests that they may be lymphokines (P. F. Zipfel, J. Balke, S. G. Irving, K. Kelly, and U. Siebenlist, J. Immunol., in press).

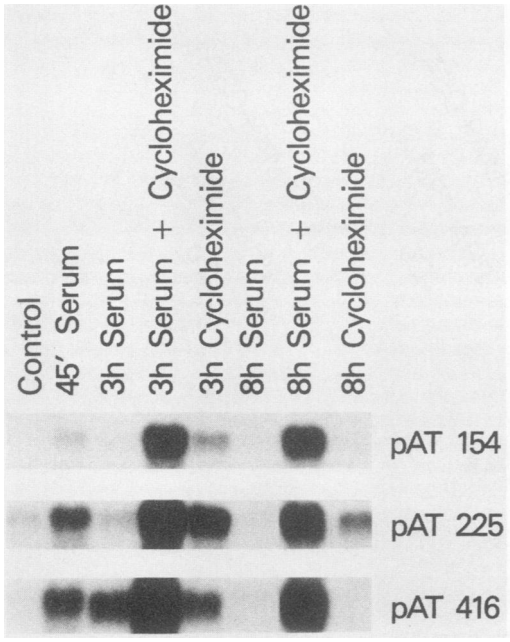

FIG. 4. Northern analyses of selected cDNA clones in human fibroblasts. Quiescent CCD-11LU cells were stimulated with minimal essential medium containing 20% FCS in the presence of absence of cycloheximide. Total cellular RNA was isolated at the indicated times and analyzed by Northern blotting.

Beyond the limited number of genes isolated previously on the basis of being induced in various T-cell preparations, cell lines, or clones (2, 4, 15, 23, 29, 33, 42), more extensive research has been conducted on the serum-induced activation process in mouse 3T3 fibroblasts. Previous studies in which immediately activatable genes were cloned yielded a number much smaller than discovered here (10, 31). However, a recent study using the mouse 3T3 fibroblast system concludes that more than 70 genes are induced (1), in good agreement with our studies on human PB T cells. As shown here, a majority of induced genes are expressed similarly in both human fibroblasts and lymphocytes. Such an observation suggests that the complexity of the immediate-early transcriptional response to mitogens does not result primarily from the induction of differentiated functions. Rather, it is more likely that a ubiquitously expressed activation gene plays a role in a conserved aspect of cellular metabolism such as those events that prime cells for DNA synthesis. Particularly intriguing among the induced genes isolated to date from fibroblasts are several which may encode DNA-binding proteins (7, 34, 41, 43), a finding that supports the hypothesis that some early-induced genes may play a pleiotropic regulatory role. Two such genes which are expressed in fibroblasts and lymphocytes, called Egr-1 (or NGFI-A) and Krox-20, show predicted primary amino acid structures containing three zinc finger-binding domains, which have been implicated in binding DNA (7, 34, 43, 44).

In addition to the large number of genes induced, the variety of kinetic patterns observed among the induced genes after mitogenic stimulation of PB T cells suggests considerable diversity in regulation. One common pattern of expression (Table 1) displays a rapid and transient appearance analogous to that of c-*fos* (34) and of many genes induced in fibroblasts (1, 32). As one possibility, such genes may play roles important in initiating a G_0-to-G_1 transition within the cell cycle, and their continued presence may not necessarily be required for further progression through G_1. Additional kinetic patterns displaying later onset or sustained expression were observed for other genes (Fig. 2 and Table 1).

Further evidence pointing to diverse regulatory groups among the genes studied here is the variable effects of CSA on gene induction. CSA appears to interfere with induced gene expression before or at the initiation of transcription

(16; Gunter et al., submitted). Therefore, we anticipate that CSA-inhibitable genes share a common activation component required for induction which may be reflected in shared regulatory motifs for these genes. In addition, the broad effect of this drug on an unexpectedly large proportion of genes leads us to speculate that its immunosuppressive action may be due to more than the suppression of only a small number of genes, such as the gene encoding IL-2.

A common characteristic observed among 70% of the induced genes studied was the superinduction of message levels by cycloheximide. In addition, in the presence of cycloheximide many transiently expressed genes showed increasingly sustained expression kinetics. Thus, most induced genes appear to be subject to regulatory mechanisms that are mediated by labile proteins, a system that provides maximum flexibility for the modulation of message levels within the cell. Evidence has been presented in fibroblasts for cycloheximide both inhibiting transcriptional shut-off and increasing mRNA stability, which suggests the involvement of labile transcriptional repressors and degrading enzymes in the regulation of induced gene expression (1, 32).

It seems likely that a subset of the genes described here plays a role in programmed cell growth. It is particularly interesting to note the constitutive expression in Jurkat tumor cells of three genes that are normally expressed only after mitogenic activation (Fig. 3 and Table 2). As one possibility, the expression of such genes may contribute to the uncontrolled growth of this tumor-derived cell line. We speculate that an analysis of many different types of tumors may yield characteristic patterns of constitutive expression for normally inducible genes, thus providing diagnostic tools. Furthermore, molecular analyses of the normal and abnormal expression of such genes should render insights into the etiology of certain tumors. A detailed analysis of the structure, regulation, and function of this large collection of inducible genes promises to advance our understanding of programmed proliferation and activation-associated differentiation of T cells.

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