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Immunology

Sequential expression of new gene programs in inducer T-cell clones

(cell-specific genes/differentiation)

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ABSTRACT **We have prepared a cDNA probe that detects genes that are rapidly and abundantly expressed after exposure of inducer T-lymphocyte clones to antigen or mitogen. All inducer cells tested express a characteristic set of new mRNA, and these mRNAs are not expressed after activation of other lymphocytes. This initial burst of mRNA synthesis is paralleled by synthesis and secretion of a family of polypeptides that mediate inducer cell activity, including T- and B-cell growth factors, interferon, and molecules that bind to antigen. Expression of this initial genetic program precedes mitosis and is replaced within 72 hr by a different genetic program, which may control further cell division. The action of these sequential sets of genetic programs defines two stages of the cell's differentiation and accounts for altered expression of the cell's immunological functions.**

One result of thymus-dependent differentiation is the generation of several morphologically identical sublines of mature lymphocytes. Each set coordinately expresses genes that encode a unique surface protein "marker" and a particular immunologic function (1). Analysis of cells separated according to these molecular labels has shown that the level of immune reactions is determined by a set of T cells called inducer or "helper" cells, which synthesize and secrete a family of polypeptides that stimulate different target cells to divide or differentiate (2).

After activation by antigen, clones of inducer cells rapidly synthesize and secrete biologically active inducer polypeptides and undergo a round of cell division (3, 4). The genes coding for these inducer cell polypeptides have not been defined. We show here that, within 15 hr of exposure to antigen or mitogen, in the absence of cell division, inducer cells synthesize large amounts of several mRNA species that are not detected in other types of T lymphocytes or other lymphocytes after activation. Expression of this new set of genes is (*i*) accompanied by a burst of synthesis and secretion of new proteins and (*ii*) replaced by expression of another set of genes within 78 hr after activation.

MATERIALS AND METHODS

Cells. Cl.Ly1-T1 is a 2,4,6-trinitrophenyl (TNP)-specific $Thy1^+$ $Ly1^+2^-3^-$ inducer T-cell clone isolated from a BALB/c mouse. Cl.Ly1-N5 is a (4-hydroxy-3-nitrophenyl)acetyl (NP)-specific $Thy1^+$ $Ly1^+2^-3^-$ inducer T cell clone isolated from a C57BL/6 mouse (5). Cl.Ly23.4, a sheep glycophorin-specific suppressor T-cell clone of C57BL/6 origin, and NK-11 have been described in detail (6, 7). The biological properties of these clones (and others used for comparison) are summarized in Table 1.

Activation of T-Cell Clones. Inducer T-cell clones were grown in the absence of other cell types, unless otherwise indicated, using a modification of the method of Nabel *et al.* (3). Fifteen hours before harvesting for RNA extraction, concanavalin A (Con A) was added to cultures at 5 μg/ml. In some cases 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (10 ng/ml) was added as well. It had little or no effect on the pattern or amount of protein synthesis.

Table 1. Properties of clones and tumor cells used in this analysis

Cell	Surface phenotype	Function	Strain	Specificity*
Cl.Ly1-T1	$Thy1^+$ $Ly1^+2^-$	Inducer	BALB/c	TNP
Cl.Ly1-N5	$Thy1^+$ $Ly1^+2^-$	Inducer	C57BL/6	NP
Cl.Ly1-A	$Thy1^+$ $Ly1^+2^-$	Inducer	C57BL/6	Unknown
Cl.Ly23.4	$Thy1^+$ $Ly1^-2^+$	Suppressor	C57BL/6	Sheep glycophorin
Cl.NK-11	$Thy1^+$ $Ly1^-2^-$	Cytolysis	C57BL/6	Unknown
EL4	$Thy1^+$ $Ly1^+$	Interleukin 2 (thymoma)	C57BL/6	(Thymoma)
2PK3	sIg^+ Ia^+	(B-cell lymphoma)	BALB/c	—
MOPC 315	Ig^+	Ig secretion (myeloma)	BALB/c	TNP
101.6	$Thy1^-$ $Ly1^-2^-$ Ig^-	—	C57BL/6	—
L929	—	Fibroblast tumor		

* All inducer T cells listed here react to the indicated antigens in association with products of major histocompatibility genes.

RNA Isolation. RNA was extracted by using the guanidinium thiocyanate procedure of Chirgwin *et al.* (8). After sedimentation of the RNA through cesium chloride, the supernatant material was aspirated and the bottom 0.5 cm of the tube, containing the clear RNA pellet, was excised with a razor blade. Pellets were dissolved thoroughly in 5 ml of 10 mM Tris·HCl, pH 7.5/1 mM EDTA/5% Sarkosyl/5% phenol, made up to 0.1 M in NaCl, and vigorously shaken with 10 ml of phenol/chloroform/isoamyl alcohol, 50:49:1 (vol/vol). RNA was precipitated from the aqueous phase with 2.5 vol of ethanol in the presence of 0.2 M sodium acetate, pH 5.5. $Poly(A)^+$ RNA was purified by binding to and elution from oligo(dT)-cellulose (9).

cDNA Synthesis. ^{32}P-Labeled cDNA was synthesized in a reaction mixture containing 50 mM Tris·HCl at pH 8.2, 50 mM KCl, 6 mM $MgCl_2$, 10 mM dithiothreitol, $(dT)_{12\text{-}18}$ at 45 μg/ml, placental ribonuclease inhibitor at 350 units/ml, dATP, dCTP, dGTP, and dTTP at 60 μM each, each ^{32}P-labeled nu-

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Abbreviations: BGG, bovine gamma globulin; Con A, concanavalin A; NP, (4-hydroxy-3-nitrophenyl)acetyl; TNP, 2,4,6-trinitrophenyl; TPA, 12-*O*-tetradecanoylphorbol 13-acetate.

cleoside triphosphate at 1 mCi/ml (1 Ci = 3.7×10^{10} Bq), poly(A)$^+$ RNA at 100 μg/ml, and avian myeloblastosis virus reverse transcriptase at 800 units/ml. Reaction mixtures were incubated for 1 hr at 42°C. The RNA template was removed by alkaline hydrolysis (10) and freed of unincorporated nucleoside triphosphates by three cycles of precipitation with ethanol in the presence of 2 M ammonium acetate.

RNA·cDNA Hybridizations. Hybridizations were performed according to Alt *et al.* (11) and Mather *et al.* (12). Briefly, L-cell poly(A)$^+$ RNA (6-fold excess over the amount of mRNA used to make labeled cDNA transcript) was hybridized to the ^{32}P-labeled cDNA until a R_0t of 5,200 mol·sec/liter was attained. The solution was then brought to a concentration of 0.1 M sodium phosphate at pH 6.8, applied to a 1.5-ml hydroxyapatite column equilibrated with the same buffer, and maintained at 60°C. Single-stranded cDNA was eluted with 0.1 or 0.12 M sodium phosphate, pH 6.8, and RNA·cDNA hybrids were eluted with 0.2 M sodium phosphate, pH 6.8.

RNA Blotting Experiments. Poly(A)$^+$ RNA was denatured in 2.2 M formaldehyde and electrophoresed in a 1.3% agarose gel, according to Seed (13). Transfer to nitrocellulose and hybridizations were performed as described by Seed (13) and Wahl *et al.* (14). Filters were stringently washed with three changes of 15 mM NaCl/0.1 mM EDTA/2 mM Tris·HCl, pH 8/0.1% sodium pyrophosphate/0.1% $NaDodSO_4$/10 mM sodium phosphate, pH 7, for 20 min at 50°C and exposed to x-ray film for 1–14 days.

Synthesis and Secretion of Polypeptides. Antigen-pulsed adherent cells were prepared by incubating 5×10^6 irradiated [1,500 roentgens (1 roentgen = 2.6×10^{-4} coulombs/kg)] C57BL/6 spleen cells plus 100 μg of NP-ovalbumin in 1 ml of RPMI 1640 medium/5% fetal calf serum for 2 hr. Nonadherent cells and soluble antigen were removed by vigorous pipetting; the adherent cells (approximately 10^5 per well) were washed twice with phosphate-buffered saline; and 10^6 Cl.Lyl-N5 cells were added. Every 6 hr the culture supernatant was removed from one well and 1 ml of labeling medium was added (RPMI 1640 medium minus methionine, containing 2 mM glutamine, penicillin at 50 units/ml, streptomycin at 50 μg/ml, methionine at 0.3 μg/ml, soybean trypsin inhibitor at 100 μg/ml, and [^{35}S]methionine at 100 μCi/ml). Supernatants were harvested 6 hr later and centrifuged at 1,000 × *g* for 10 min, and the resulting supernatants were frozen at −20°C.

Both the cell pellet after centrifugation and the cells remaining in the culture well were resuspended in 1 ml of phosphate-buffered saline with 0.7 mM EDTA. Total intracellular protein synthesis was determined by measuring the radioactivity in a 0.01-ml aliquot in duplicate by liquid scintillation spectrophotometry.

Determination of NP-Specific Antigen-Binding Proteins. A 0.4-ml sample of each radiolabeled supernatant was added to 100 μl of NP-BGG-Sepharose (BGG, bovine gamma globulin) and to 100 μl of BGG-Sepharose (BGG at 1 mg/ml) in 12 × 75 mm tubes. Samples were incubated on a rotator at 4°C for 2 hr and washed three times with phosphate-buffered saline/5% fetal calf serum/0.1% Nonidet P-40, and the pellet was dried overnight. Then 0.25 ml of $NaDodSO_4$/polyacrylamide gel electrophoresis sample buffer was added and the samples were boiled for 3 min and centrifuged. Radioactivities of 0.025-ml samples of each supernatant were determined in duplicate by liquid scintillation spectrophotometry. Nondenatured molecules eluted from NP-BGG-Sepharose induce *in vitro* antibody production by primed B cells in an antigen and *H-2* locus-restricted manner (unpublished data).

NP-specific binding was calculated as cpm bound to NP-BGG-Sepharose minus cpm bound to BGG-Sepharose. Total trichloroacetic acid-precipitable radiolabeled protein was determined by adding acid-precipitable cpm in the unfractionated supernatant and acid-precipitable cpm in the cell pellet.

Polypeptides Synthesized and Secreted by Cl.Lyl-N5. Cl.Lyl-N5 cells (10^6) were incubated in media supplemented with Con A at 5 μg/ml or Con A at 5 μg/ml and TPA at 10 ng/ml. After 9 hr the culture supernatant was removed and 1 ml of labeling medium with [^{35}S]methionine was added. Culture supernatants were harvested 6 hr later and centrifuged at 1,000 × *g* for 10 min. The supernatant was made 0.15 mM in phenylmethylsulfonyl fluoride and centrifuged at 100,000 × *g* at 4°C for 1 hr. Proteins in the supernatant were concentrated by precipitation with 10% trichloroacetic acid, washed three times with acetone, dried, and dissolved in $NaDodSO_4$/polyacrylamide gel electrophoresis sample buffer. Electrophoresis was carried out in 0.7-mm-thick slab gels as described (4, 6). Fluorographic treatment was used to enhance sensitivity.

RESULTS

The inducer T-cell clones, Cl.Lyl-T1 and Cl.Lyl-N5, carry surface recognition structures for the haptens TNP and NP, respectively, in association with class II major histocompatibility gene products (see Table 1; ref. 5). Incubation with these antigens is rapidly followed (within 24 hr) by synthesis and secretion of a family of polypeptides, including growth factors for other lymphocytes and mast cells (4, 15), interferon, and antigen-binding molecules that stimulate hapten-specific B cells to secrete antibody (unpublished data).

Our initial experiments were designed to define optimal conditions for synthesis and secretion of polypeptides by inducer cells. The tempo of polypeptide synthesis and secretion by Cl.Lyl-N5 as well as [^{3}H]thymidine incorporation 5 days after activation are shown in Table 2 and Fig. 1. The cells continue to synthesize new polypeptides over these 5 days, but the proliferative rate markedly decreases by the 5th day. Secretion of antigen-binding molecules, which consist of several discrete polypeptides showing sizes of 40–50 kilodaltons on polyacrylamide gel electrophoresis (unpublished data), rises quickly after activation and peaks between 18 and 24 hr. The tempo of secretion of interleukin 2 (T-cell growth factor) is virtually identical (Fig. 1). Analysis 9–15 hr after activation shows that Cl.Lyl-N5 secretes a family of polypeptides characteristic of most inducer clones (4, 16) (Fig. 2).

Stimulation by either Con A or antigen is followed by similar kinetics of polypeptide secretion, peaking at 18 hr (refs. 3 and 4; unpublished results). We assumed that maximal mRNA

Table 2. Kinetics of cellular activation: Protein synthesis and secretion

Time after activation, hr	Trichloroacetic acid-precipitable cpm		
	Total intracellular and extracellular	NP-specific	Total extracellular
18	548,060	38,045	55,760
24	1,452,960	39,900	72,960
30	2,269,480	16,765	177,800
54	3,857,080	9,065	102,480
72	ND	ND	57,800
90	ND	ND	122,100
Not activated	377,400	ND	10,570

Cl.Lyl-N5 cells were incubated in culture wells with antigen-pulsed adherent cells. Aliquots of culture supernatants were obtained at the indicated times after addition of [^{35}S]methionine 6 hr before harvesting. The cells were centrifuged at 1,000 × *g* for 10 min and their supernatants were centrifuged at 100,000 × *g* for 1 hr to obtain supernatant extracellular material. ND, not determined.

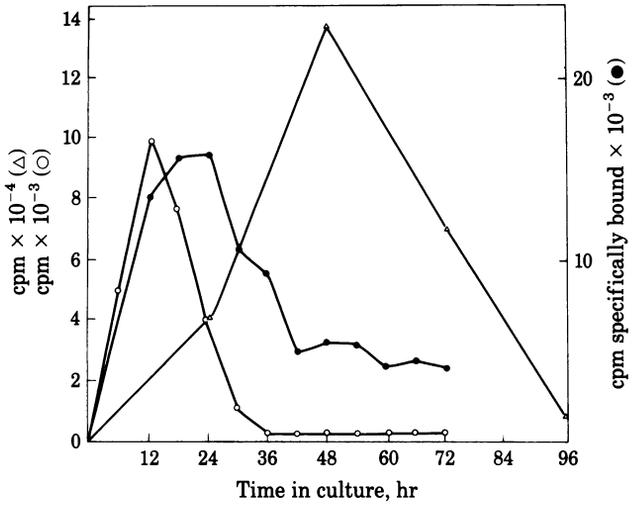

FIG. 1. Kinetics of proliferation and production of interleukin 2 and antigen-binding proteins. Cl.Ly1-N5 cells were incubated with antigen-pulsed adherent cells for the indicated times. Interleukin 2 production was measured by determining the [^{3}H]thymidine incorporation of Cl.Ly23.4 [an interleukin 2-dependent T cell (6)] in response to a 1:1 dilution of culture supernatant taken at the indicated times (○). Proliferation of Cl.Ly1-N5 was measured by adding 1 μCi of [^{3}H]thymidine to the culture and determining the ^{3}H incorporated 12 hr later (△). Secretion of antigen-binding proteins was measured by radiolabeling the cells with [^{35}S]methionine for 6 hr. Culture supernatants were harvested at the indicated times and NP-specific binding was calculated as cpm bound to NP-BGG-Sepharose minus the cpm bound to BGG-Sepharose (●). Eluate from NP-BGG columns, but not fluorescein isothiocyanate-conjugated BGG columns, activated B lymphocytes in cultures containing these cells and the hapten NP coupled to various proteins.

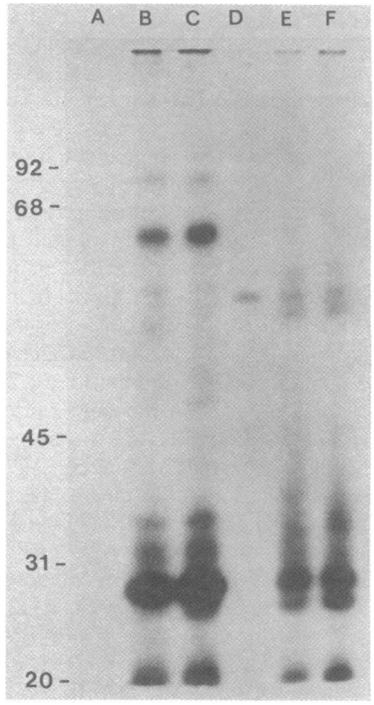

FIG. 2. Polypeptides synthesized and secreted by inducer T-cell clones before and after activation. Inducer T cells were labeled with [^{35}S]methionine from hr 9–15 after the addition of mitogen or media (see below). Proteins secreted into the extracellular medium were prepared as described in the text. A fluorogram of a NaDodSO$_4$/12.5% polyacrylamide gel is shown. Molecular masses in kilodaltons are given on the left for marker proteins. The secreted polypeptides are from: lane A, unactivated Cl.Ly1-N5; lane B, Cl.Ly1-N5 activated with Con A at 5 μg/ml; lane C, Cl.Ly1-N5 activated with Con A at 5 μg/ml and TPA at 10 ng/ml; lane D, unactivated Cl.Ly1-T1; lane E, Cl.Ly1-T1 activated with Con A at 5 μg/ml; lane F, Cl.Ly1-T1 activated with Con A at 5 μg/ml and TPA at 10 ng/ml.

expression slightly precedes maximal secretion. We extracted RNA from cells at 15 hr after Con A activation for production of a cDNA probe. We activated the inducer clone with Con A rather than with antigen and splenic adherent cells to ensure that all mRNA extracted from cell cultures was synthesized solely by the clone.

Definition of Genes Expressed After Activation of Inducer T Cells. A cDNA probe corresponding to mRNA sequences newly expressed in activated T inducer cells was prepared by subtractive hybridization (see *Materials and Methods*). ^{32}P-Labeled cDNA was made from the mRNA of activated Cl.Ly1-T1 by reverse transcription (see Table 1 for properties of this and other cells used in these experiments). The labeled cDNA was depleted of mRNA sequences not specific to lymphocytes by hybridization to a 6-fold excess of mRNA from L929 cells, a mouse fibroblast cell line. After a single cycle of hybridization, the remaining single-stranded cDNA, representing 14–16% of the input cDNA, was isolated by hydroxyapatite chromatography.

The specificity of the single-stranded cDNA probe was initially tested by hybridization to a nitrocellulose blot of mRNA from five different cell types (Fig. 3). Stringent hybridization and wash conditions were employed. There was little or no hybridization to the mRNA of unactivated Cl.Ly1-T1. The probe did not hybridize with mRNA sequences expressed in 2PK3, a B cell lymphoma, or to 101.6, a null cell lymphoid tumor. By contrast, there was strong hybridization to mRNA sequences present in the activated inducer Cl.Ly1-T1 clone: dominant species of approximately 1,700, 1,400, and 900 nucleotides as well as diffuse areas of hybridization spanning 600–1,000 nucleotides were apparent. The cDNA probe also showed an almost identical pattern of hybridization to mRNA from a second inducer cell clone, Cl.Ly1-N5. Four prominent areas of hybridization were detected, corresponding to mRNAs of approximately 1,400, 1,100, 900, and 700 nucleotides. In contrast,

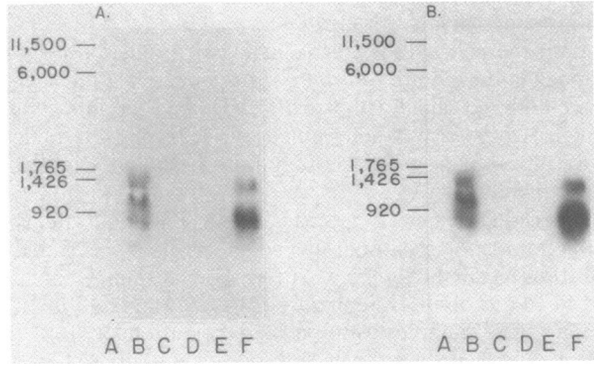

FIG. 3. Specificity of the cDNA probe for mRNAs newly expressed in activated inducer T cells. RNA was extracted from cells, and 5 μg of poly(A)$^+$ RNA was applied to each lane, electrophoresed on a 1.3% agarose gel, and transferred to nitrocellulose paper. The RNA was hybridized to a cDNA probe prepared by subtractive hybridization of cDNA synthesized from mRNA of activated Cl.Ly1-T1. *A* and *B* are different exposures (2 hr and 5 days) of the same gel. Lane A, 2PK3; lane B, activated Cl.Ly1-N5; lane C, Cl.Ly23.4; lane D, 101.6; lane E, unactivated Cl.Ly1-T1; lane F, activated Cl.Ly1-T1. See Table 1 for the properties of these cells. The virion and mRNAs of vesicular stomatitis virus were used as size markers and their lengths in nucleotides are indicated on the left (17).

there was little hybridization to mRNA from a T suppressor cell clone, Cl.Ly23.4, although a faint band (approximately 1,800 nucleotides) was detected here and in subsequent blots (see below).

The gel was exposed an additional 5 days to detect hybridization to minor mRNA species. None was found (Fig. 3*B*). Finally, hybridization of the unsubtracted cDNA probe to mRNA from both unactivated and activated inducer clones resulted in hybridization to diffuse mRNA ranging in size between approximately 0.5 and 4.5 kilobases (not shown).

Expression of Unique Species of mRNA After Activation of Inducer Cells. A second [^{32}P]cDNA probe was made according to the previous protocol, except that 8-fold more cpm were used for the blot hybridization to ensure greater sensitivity. Virtually identical results were obtained: 85% of cDNA hybridized to L-cell mRNA and the single-stranded cDNA showed the same pattern of hybridization to mRNA from the cells used in the previous blot (Fig. 4). This experiment also showed that mRNA from the myeloma MOPC 315, a cell containing large amounts of mRNA coding for immunoglobulin and activated EL4 (a T-cell lymphoma that is stimulated by Con A to produce interleukin 2, a growth factor normally synthesized by activated inducer clones), did not hybridize with the inducer cDNA probe.

The inducer cDNA probe did not hybridize to mRNA from activated natural killer lymphocytes or Con A-activated T suppressor clones, although again a faint area of hybridization to mRNA was detected in an area corresponding to about 1,800 nucleotides, similar to the result shown in Fig. 3.

Identification of Less Abundant Inducer mRNA. When the probe used above (containing 8-fold more cpm) was hybridized to mRNA from inducer clones, the pattern of hybridization was very similar to that seen in Fig. 3 except that two additional minor bands were detected. These mRNAs, corresponding to 3,900 and 3,400 nucleotides, were detected only in activated Cl.Lyl-N5 and Cl.Lyl-A and, less prominently, in activated Cl.Lyl-T1 mRNA. A very faint hybridization was detected to a mRNA of approximately 1,400 nucleotides from unactivated Cl.Lyl-T1 or Cl.Lyl-N5. We tested this probe for hybridization to mRNA of an inducer T-lymphocyte clone (Cl.Lyl-A) that does not specifically react to hapten but synthesizes interleukin 2 and B-cell growth factors after mitogen activation. Hybridization to the mRNA species of about 900, 3,400, and 3,900 nucleotides was observed. However, the mRNA species of about 1,400 nucleotides, prominent in the hapten-specific inducers, was absent from this non-hapten-specific cell. This is intriguing because this sized mRNA is appropriate for encoding polypeptides of 40–50 kilodaltons, the size of partially purified antigen-binding molecules (unpublished data).

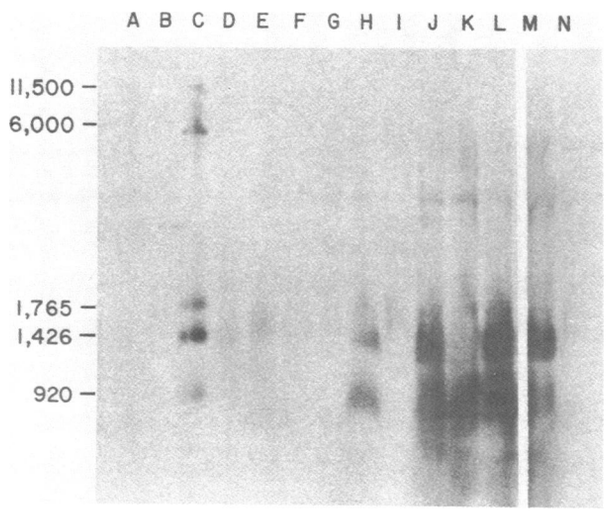

FIG. 4. Blot analysis of hybridization of a cDNA probe to RNAs from functionally distinct lymphocyte clones and lymphoid tumors. RNA was extracted from cells, electrophoresed on a 1.5% agarose gel, and transferred to nitrocellulose paper. The blotted RNA was hybridized to a cDNA probe prepared by subtractive hybridization of cDNA synthesized from mRNA of activated Cl.Lyl-T1. Lanes A–L contain 5 μg of poly(A)$^+$ RNA. Lanes M and N contain 12.5 μg of total cellular RNA. Lanes: A, activated EL-4; B, MOPC 315; C, ^{32}P-labeled vesicular stomatitis virus virion and mRNAs (the length in nucleotides of these marker RNAs is indicated on the left); D, activated Cl.NK-11; E, activated Cl.Ly23.4; F, unactivated Cl.Ly23.4; G, unactivated Cl.Lyl-T1; H, activated Cl.Lyl-T1; I, unactivated Cl.Lyl-N5; J, activated Cl.Lyl-N5; K, activated Cl.Lyl-A; L, activated Cl.Lyl-T1 (a different mRNA preparation from that in lane H); M, Cl.Lyl-N5 at 24 hr after activation with antigen; N, Cl.Lyl-N5 at 78 hr after activation with antigen.

Inducer Cells Express More Than One Genetic Program After Activation. The mRNA species detected by the cDNA probe in cells early after activation by antigen (24 hr; Fig. 4, lane M) corresponded closely to those expressed after mitogen activation (Fig. 4, lane J). Although the mRNA species detected in cells early after activation (24 hr) was fully apparent 3 hr after exposure of the gel, no hybridization was detected with RNA from cells 78 hr after activation despite 5 days of exposure. This cannot be accounted for by termination of protein synthesis: the cell synthesizes high levels of new extracellular polypeptides at this time and for at least 12–24 hr afterwards (Table 2). More recent analysis of the life span of the early mRNA species shows that it is shut off by 48–60 hr after activation.

In sum, these data indicate that (*i*) activation of inducer clones results in a rapid burst of mRNA synthesis and secretion of large amounts of several polypeptides, including growth factors and antigen-binding molecules, which mediate inducer cell activity, and (*ii*) this early mRNA species is relatively short lived. It is not detectable at 78 hr after activation, as shown here, and more extensive analysis of its life span shows that it is not detectable by 48–60 hr, despite the fact that the cell continues to synthesize extracellular proteins for at least 90 hr after activation.

DISCUSSION

Perhaps the most important point that emerges from these data is that a panel of nontransformed lymphocyte clones that maintain specialized physiologic function allows detection of sequential gene expression associated with acquisition of new cellular function. The rapid but transient synthesis of the initial mRNA species after activation of T cells by antigen is unlikely to be detected in T-cell tumors or continuously dividing hybrids between tumors and T cells ("hybridomas"). These cells, in our hands, do not retain the chromosomal information for complete and stable expression of the gene sets that control sequential stages of a normal cell's response to antigen.

Although cloned T cells have been used to provide homogeneous proteins for biochemical analysis, they have not provided insight into the genetic basis of T-cell function. The approach taken here defines inducer T cells according to the set of gene products that are strongly and uniquely expressed after activation of these cells. It is implicit that the collective activities of these gene products account for the functions associated with this lymphocyte set and that this approach should reveal immune functions that only are dimly understood at present.

Kinetic hybridization studies have yielded estimates that mRNA species expressed in heterogenous lymphocytes and uncloned fibroblasts differ by about 25% (18). We have found that 15% of the mRNA species expressed in an activated inducer T-cell clone do not hybridize with fibroblast cell mRNA. Because only a single cycle of hybridization was performed, this is an

upper estimate of the difference between the cells. As measured by blot hybridization analysis, a cDNA probe prepared in this way detects genes expressed strongly and uniquely by activated inducer T cells. This is most clearly demonstrated by the absence of hybridization to mRNA from unactivated inducer T-cell clones as well as to B- and T-cell tumors. This probe does not detect sequences common to all lymphocytes, even though they have not been removed by hybridization with fibroblast mRNA. Sequences individually in low abundance, both in the specific radioactive cDNA probe and in the blotted mRNA, are not detectable by blot analysis because hybridization is concentration dependent.

The strategy for detecting mRNA after activation also deserves comment. We first defined the optimal conditions and time course of polypeptide secretion by the clone (Table 2 and Fig. 1). These experiments showed that maximal levels of protein secretion and at least some inducer-specific polypeptides were reached rapidly after exposure to antigen (18–24 hr): we therefore extracted RNA 3 hr before appearance of maximal levels of antigen-binding molecules (Fig. 1) and other inducer polypeptides (3) secreted into the extracellular medium. This cDNA probe revealed a set of mRNAs rapidly synthesized by cloned inducer cells after activation. Because this cDNA probe detects similar mRNA species in uncloned Con A-activated Lyl cells but does not hybridize to mRNA from lipopolysaccharide-activated B cells, the cDNA probe described here defines genes that are normally expressed by activated inducer cells, rather than genes that may be expressed after long-term cell culture.

Repeated analyses of the kinetics of the cell cycle after activation of nondividing inducer clones show that DNA synthesis does not occur during the initial 15 hr after stimulation. The lack of a requirement for cell division before expression of the first set of new mRNA species is notable because mitosis is sometimes thought of as a necessary prelude to cellular differentiation. Induction of prothymocytes to express surface molecules characteristic of thymocytes represents a second example of division-independent transcription during T-cell differentiation (19). Although gene products that are secreted by inducer cells are of special interest (4), some gene products that are uniquely and strongly expressed by activated inducer cells may not be secreted. Both define the specialized physiology of this differentiated cell. We therefore have not attempted to correlate discrete species of mRNA with inducer proteins secreted at high levels, such as antigen-binding molecules (Table 2, Fig. 1). However, the absence of a mRNA species of about 1,400 nucleotides in clones that lack 40- to 50-kilodalton receptors for hapten (unpublished data) is intriguing (Fig. 4).

These results also bear directly on the developmental relationship among T lymphocytes and other lymphocytes sets. T inducer and suppressor cell types are secretory cells (4, 18, 20, 21) and differentiate in the thymus. The two types of T lymphocyte are distinguishable only by the Ly surface markers (1–4). These data show that, after activation by Con A, the two morphologically indistinguishable lymphocytes express different major mRNA species and, like prothymocytes (20), are already committed to express their respective genetic programs after nonspecific activation.

The cell continues to synthesize and secrete polypeptides at about the same rate over 5 days (Table 2). However, 78 hr after activation, synthesis of polypeptides is controlled by a new set of mRNAs expressed during or after mitosis; i.e., the cell expresses a new "genetic program" (20–22). The function of late-appearing genes is not clear. An important clue comes from the observation that, although inducer cells continue to synthesize polypeptides for 5 days, proliferation of these cells is markedly decreased during the last 2 days (Fig. 1), and these cells are refractory to restimulation by antigen or mitogen. Moreover, 3 days after activation, inducer cells no longer activate B cells to divide and secrete Ig; instead, they inhibit activation of resting B cells by antigen and induce partially activated B cells to secrete Ig, but not to divide (unpublished data). Possibly the early genetic program is supplanted by a set of genes that in part suppresses further cellular division of T and B cells to avoid uncontrolled lymphocyte growth.

Translation of the individual mRNA species expressed as part of the early genetic program remains to be characterized. Analysis of the biologic activity of these proteins should define the major inducer proteins synthesized and possibly secreted shortly after activation.

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