played an important role in the initial divergence of the Hamamelidae and Rosidae. Early, primitive representatives of these subclasses were major elements in the primary radiation of the higher dicotyledons.

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Cloning of a cDNA for a T Cell–Specific Serine Protease from a Cytotoxic T Lymphocyte

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A new serine protease was encoded by a clone isolated from a murine cytotoxic T-lymphocyte complementary DNA library by an RNA-hybridization competition protocol. Complementary transcripts were detected in cytotoxic T lymphocytes, spleen cells from nude mice, a rat natural killer cell leukemia, and in two of eight T-helper clones (both cytotoxic), but not in normal mouse kidney, liver, spleen, or thymus, nor in several tested T- and B-cell tumors. T-cell activation with concanavalin A plus interleukin-2 induced spleen cells to express this gene with kinetics correlating with the acquisition of cytolytic capacity. The nucleotide sequence of this gene encoded an amino acid sequence of approximately 25,700 daltons, with 25 to 35 percent identity to members of the serine protease family. The active site "charge-relay" residues (His^{57}, Asp^{102}, and Ser^{195} of the chymotrypsin numbering system) are conserved, as well as the trypsin-specific Asp (position 189 in trypsin). A Southern blot analysis indicated that this gene is conserved in humans, mouse, and chicken. This serine protease may have a role in lymphocyte lysis and a "lytic cascade."

CYTOTOXIC T LYMPHOCYTES (CTL's) are a subset of antigen-induced T lymphocytes with the capacity to recognize, bind, and lyse target cells bearing the inducing antigen (*1*). Usually, the CTL "recognizes" a target cell bearing a nonself major histocompatibility (MHC) class I gene product (K or D in the mouse) or an ill-defined assemblage of antigens (often viral) plus a self-MHC class I gene product. Natural killer (NK) cells are constitutive cytotoxic cells that recognize, bind, and lyse a restricted set of target cells in an apparently MHC-unrestricted fashion. Target recognition by both active CTL's and NK cells is followed by a lysis mechanism that includes, in turn, a binding step, a lag interval, and unidirectional lysis events. Although many properties of CTL's have been characterized (*2*), the mechanisms mediating the cytolytic events are unclear. Several polypeptides released from killer cells and their cytoplasmic granules are thought to participate in the lytic event; these polypeptides include serine proteases, toxic lymphokines, and pore-forming "polyperforins" (*3, 4*).

To examine the activation and cytolytic mechanisms of CTL's, we used a natural history approach, searching for genes expressed preferentially in CTL's but absent in noncytolytic T cells. We report the isolation of a complementary DNA (cDNA) clone transcribed at high levels in CTL's but not in resting T lymphocytes. This cDNA clone encodes a previously unknown serine protease that probably has trypsin-like specificity and may play a role in cytolytic effector functions.

In order to isolate genes preferentially expressed in CTL's, we used an RNA-hybridization competition protocol (*5*) to identify sequences expressed in the CTL clone 1E4 but absent in the tumor cell line VL3. Briefly, the 1E4 cDNA library was screened with labeled 1E4 messenger RNA in the presence of excess VL3 RNA. The 1E4 cell line is a cloned cytotoxic, Lyt-2^+ T-lymphocyte line from the C57L mouse strain, lysing only Abelson virus–infected cells bearing syngeneic H-2^b class I molecules (*6*). The VL3 cell is a noncytolytic radiation virus (RadLV)–induced thymic lymphoma cell line from the C57BL/Ka (H-2^b) mouse with the surface phenotype of an immature intrathymic cell (Lyt-1^+, Lyt-2^+, GK 1.5^+) (*7*). To minimize the possible isolation of endogenous retroviral sequences, we used a CTL line from the relatively ecotropic retrovirus–deficient C57L mouse strain (*8*), for competition with RNA from the virus-producing VL3 line. We prepared a cDNA phage library from the CTL clone 1E4 (*9, 10*). This 1E4 cDNA phage library was screened with a mixture of an alkali-treated, ^{32}P-kinase–labeled 1E4 poly(A)–RNA probe and a several hundredfold excess of cold total VL3 RNA. The labeled sequences shared by VL3 and 1E4 cells would thus be diluted by competition for the probe, whereas sequences occurring only in 1E4 would not be diluted and would produce hybridization signals.

Using this RNA hybridization competition scheme, we screened 4.8×10^4 recombinant phage plaques of the 1E4 cDNA library (*11*). Initially, 70 positive phage plugs were picked and rescreened through two more rounds of RNA hybridization competition; this yielded 13 plaque-purified clones with reproducible signals. As an assay for the specificity of these clones, these putatively CTL-preferential clones were placed into an array, and replicates of the

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array were probed with ^{32}P-labeled single-stranded cDNA (*12*) from C57L liver, L691 (a Lyt-1$^+$, Lyt-2$^-$ C57L tumor), VL3, and 1E4 poly(A)–RNA's. Although some clones annealed strongly with all probes, two clones hybridized only to the 1E4 cDNA.

By Northern analysis, each of the two clones hybridizing only to the 1E4 cDNA gave the same size and distribution of transcripts (Fig. 2A). Both clones annealed to a 1-kilobase polyadenylated RNA species detectable only in the two CTL cloned lines AR1 and 1E4. These transcripts were not detectable in normal C57L liver, spleen, or kidney. Similarly, the transcript was not detected in the T-cell tumors L691, VL3, RL12, or the B-cell hybridoma M2. Subsequently, these two clones were shown to be overlapping cDNA fragments, as demonstrated by their reciprocal cross-hybridization, Northern analysis, patterns of hybridization to CTL cDNA libraries, and ultimately cDNA sequence analysis. These 560- and 450-bp 3′ cDNA fragments were used to screen again λgt10 1E4 and AR1 (another C57L CTL clone) libraries, generating a more nearly full-length cDNA clone from the AR1 library of 0.95 kb (hereafter referred to as the HF gene). In an S1 endonuclease assay with this longer cDNA, RNA from the two CTL lines 1E4 and AR1, but not several other lines, formed hybrids protected from digestion (Fig. 2B). In addition to the protected full-length cDNA, a smaller, less abundant, S1-resistant hybrid band of approximately 550 bp was detected in both AR1 and 1E4.

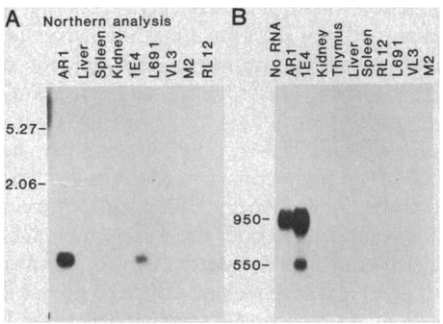

Fig. 1. Size and distribution of poly(A)-containing RNA from sequences similar to the cloned DNA fragments. (A) Northern analysis: glyoxal-treated poly(A)-containing RNA was electrophoresed through a 1.1% agarose gel and transferred to diazotized adenosine triphosphate paper as described (*31*). The RNA paper was annealed with nick-translated ^{32}P-labeled DNA of clone 5.1, a 560-bp 3′ cDNA fragment. After the RNA paper was washed, autoradiograms were made on Kodak XR-5 x-ray film (*32*). Ribosomal RNA's were molecular size standards. (B) Distribution of endonuclease S1–resistant RNA-DNA hybrids: 1 μg of poly(A)-containing RNA's from the indicated cell or tissue was annealed to single-stranded M13 phage containing the full-length HF cDNA insert and incubated with endonuclease S1 (*33*). The resistant RNA-DNA hybrids were electrophoresed on a 1.8% neutral agarose gel. The resistant hybrids were transferred to nitrocellulose and hybridized with a ^{32}P-labeled insert as described (*32*). Size markers consisted of pBR322 plasmids digested with Alu.

We investigated the relation between HF transcription and cellular function, in collaboration with Lobe and Bleackley (*13*), by using RNA dot blots to examine the kinetics of expression of HF homologous RNA in CBA/J spleen cells stimulated with concanavalin A (Con A) and interleukin-2 (IL-2) (Fig. 3A). Although no hybridizing RNA was detected in unstimulated spleen, the cells stimulated with Con A plus IL-2 showed peak expression of HF transcripts on days 3 and 4, just preceding the appearance of cytotoxicity for allogeneic targets in this culture. The temporal and quantitative association of HF gene expression with cytotoxicity implies that its translated product might be involved with cytotoxicity. Furthermore, the HF gene is detected in spleen cells from athymic C57BL nude mice but not in spleen cells from congenic normal mice. Nude mouse spleens, unlike normal mouse spleens, contain a high percentage of NK cells (*14*), suggesting again a link with cytotoxicity. In fact, the HF gene hybridized strongly to RNA dot blots of the rat NK leukemia RNK-16, but not to the noncytolytic RC31A T-cell leukemia from the same rat strain (*15*).

An examination of eight cytotoxic and eight helper T-lymphocyte cloned cell lines by RNA dot blots (Fig. 2B) tested the correlation between function and HF expression. All the CTL clones contained detectable transcripts, and at least two of the eight helper T-lymphocyte clones also contained transcripts. These two helper T-lymphocyte clones (MB2-1 and LB2-1) had measurable cytolytic activity in a 5-hour lectin-dependent ^{51}Cr-release cytotoxicity assay, whereas helper clone H39-34 had both cytotoxicity and measurable HF transcripts; one other tested helper clone (MD13-5.1) expressed cytotoxicity but lacked measurable transcripts (*16*). In summary, the HF RNA transcripts were detected in 11 of 11 CTL clones, in nude mouse spleen cells, in three of three sublines of a rat NK-cell leukemia, in two of eight T-helper cell lines, and in CTL-containing spleen cells stimulated with Con A plus IL-2. These transcripts were not detectable in normal mouse kidney, liver, spleen, or thymus, nor in the tumor T-cell lines L691, VL3, RL12, and BW5147.

The nucleotide sequence was completely determined on both strands (*17*), yielding a single open reading frame (Fig. 3). A computer homology search with the nucleotide sequence of this cDNA revealed the closest similarity (62 percent identity over 123 nucleotides) with the Christmas factor (clotting factor IX), a blood coagulation serine protease. As a result of this similarity, we referred to the gene as Hanukah factor (HF or H factor). By protein sequence homology, the DNA sequence encodes a predicted active serine protease of 232 ± 1 amino acids (see legend to Fig. 3.) with a polypeptide molecular weight of approximately 25,700. Two unusual features of this protein are 9 cysteine and 27 lysine residues. A potential *N*-linked glycosylation site is at Asn^{141}, based on the sequence Asn-X-Thr. The predicted active enzyme is probably preceded by a zymogen peptide, as in other serine proteases, cleaving either on the COOH-terminal side of Arg^{-1} or at Lys^{-2}. This zymogen peptide contains at least four lysines. The amino acid residues of the serine protease charge-relay catalytic mechanism are conserved—namely, His^{41}, Asp^{85}, and Ser^{183}, equivalent to His^{57}, Asp^{102}, and Ser^{195} of chymotrypsin. The HF serine protease contains an Asp^{177} residue equivalent to the Asp^{189} of trypsin, suggesting trypsin-like substrate specificity (*18*).

Figure 4 illustrates the homology of HF

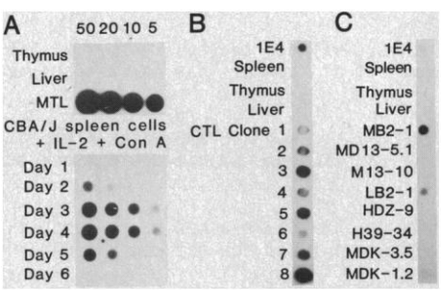

Fig. 2. RNA dot blots showing the distribution of HF in RNA of (A) thymus, liver, a cytotoxic T-cell line MTL, and spleen cells stimulated with Con A plus IL-2 (days 1 to 6); (B) several cytotoxic T-cell clones; and (C) T-helper cell lines. CBA/J spleen cells were stimulated with Con A (2 μg/ml) plus purified IL-2. The cells were cultured on the indicated day as described (*34*). Formaldehyde-denatured cytoplasmic RNA from the indicated number of cells (in units of 10^4 cells) in (A) or 5×10^5 cells in (B) and (C) was applied to the nitrocellulose (*32*). The filters were probed with a ^{32}P-labeled full-length cDNA insert. After the filters were washed, they were exposed to Cronex film for 4 days (*32*). CTL clones were derived from C57BL/6J mice by culturing single isolated T cells for 7 days in supernatants from Con A–stimulated rat spleen cells in the presence of Con A (3 μg/ml) and 5×10^5 irradiated (1500 rads) syngeneic splenocytes per well. Initially, clones were screened for CTL activity by a 5-hour lectin-dependent chromium-release cytotoxicity assay. Positive clones were expanded by weekly stimulation with Con A, irradiated splenocytes, and supernatants from Con A–stimulated rat spleen cells. Cytotoxic activity was confirmed at the time of RNA extraction. T-helper cells were derived as described (*35*).

with other eukaryotic serine proteases. With this alignment, the similarity between the predicted active HF and these other serine proteases is as follows: rat mast cell group-specific protease, 35 percent; bovine trypsin, 33 percent; human plasminogen, 33 percent; human tissue plasminogen activator, 29 percent; bovine chymotrypsin, 29 percent; human factor IX, 28 percent; and prothrombin, 25 percent.

To investigate the genomic organization and phylogenetic distribution of the HF gene, we used the HF cDNA probe for a Southern blot analysis with DNA from various organisms. The results indicate conservation of this putative serine protease as a small gene family in human, mouse, and chicken. No bands were detected in trout, drosophila, corn, or *Neisseria* DNA under conditions that reveal sequences with homology greater than 80 percent.

To summarize, we isolated from a cytotoxic T lymphocyte a T cell–specific cDNA clone encoding a putative new serine protease, HF. The distribution of HF transcripts is specific to a restricted subset of T lymphocytes. Transcripts were detected in 11 of 11 cytotoxic T lymphocytes, in two of eight T-helper cells, in a rat NK-cell leukemia, and in nude mouse spleen cells; yet no transcripts were detected in normal mouse kidney, liver, spleen, or thymus cells, nor in several tumor T-cell lines. HF transcripts are induced by activation of spleen cells with Con A plus IL-2, and expression peaked on days 3 to 4.

The HF cDNA could encode a precursor serine protease which, by analogy with other serine proteases, cleaves an activation peptide to produce the active enzyme form. This serine protease is distinct from other serine proteases, conserving active site residues His, Asp, and Ser as well as 25 of the 29 invariant residues in the serine proteases (*19*).

The Southern blot analysis of the HF gene indicates a relatively small gene family similar to those of chymotrypsin B and elastase, but unlike putatively more primitive serine proteases such as trypsin, with more than 10 related genes, and kallikrein, with 25 to 30 related genes (*20*). Highly homologous HF-like genes were detected in human and chicken genomic DNA by Southern blots, providing sequences for phylogenetic studies.

Serine proteases have many physiologic functions (*21*). The HF gene product is expressed in CTL's in a rat NK leukemia, and in nude mouse spleen cells, but not in resting cells. This suggests an "activation" gene related to a lysis mechanism. We propose that the HF-encoded protease may be one of a few serine proteases in a "lytic pathway" common to CTL's and NK cells and analogous to both the complement (*22*) and blood-clotting cascades (*23*). A number of investigators have demonstrated the inhibition of CTL- or NK-mediated target cell lysis by low and high molecular weight serine protease inhibitors (*24*). Hatcher *et al.* (*25*) isolated a cytotoxic serine protease from unstimulated human peripheral blood lymphocytes with an approximate molecular weight of 30,000. More recently, Pasternack and Eisen (*26*) have defined a trypsin-like serine protease, of molecular weight ~28,000, specific for CTL's. A potential problem is the presence of HF transcripts in two of eight helper-cell clones. The two HF-positive T-helper clones had lectin-dependent cytotoxic activity, whereas the HF-negative T-helper clones lacked cytotoxic activity. A single possible discrepancy is shown by a helper clone with lectin-dependent cytotoxicity but no measurable HF transcripts, a finding that must be examined in more detail quantitatively and kinetically.

```
                                                    ▼
-14                                              -1 +1
Trp Glu Ile Asn Leu Ser Ser Lys Arg Gly Gly Cys Glu Arg Ile Ile Gly Gly Asp Thr
TGG GAG ATC AAC CTG TCT TCC AAG AGA GGA GGC TGT GAA AGA ATC ATT GGA GGA GAC ACG

                                                                            26
Val Val Pro His Ser Arg Pro Tyr Met Ala Leu Leu Lys Leu Ser Ser Asn Thr Ile Cys
GTT GTT CCT CAC TCA AGA CCG TAT ATG GCT CTA CTT AAA CTT AGT TCA AAT ACC ATC TGT

                                                        •                   46
Ala Gly Ala Leu Ile Glu Lys Asn Trp Val Leu Thr Ala Ala His Cys Asn Val Gly Lys
GCT GGC GCT TTG ATT GAA AAG AAC TGG GTG TTG ACT GCT GCC CAC TGT AAC GTG GGA AAG

                                                                            66
Arg Ser Lys Phe Ile Leu Gly Ala His Ser Ile Asn Lys Glu Pro Glu Gln Gln Ile Leu
AGA TCT AAG TTC ATT CTT GGG GCT CAC TCA ATC AAT AAG GAG CCA GAA CAA CAG ATA TTG

                                                                        *   86
Thr Val Lys Lys Ala Phe Pro Tyr Pro Cys Tyr Asp Glu Tyr Thr Arg Glu Gly Asp Leu
ACT GTT AAG AAA GCA TTT CCC TAT CCA TGC TAT GAT GAA TAT ACA CGT GAG GGG GAT CTA

                                                                           106
Gln Leu Val Arg Leu Lys Lys Lys Ala Thr Val Asn Arg Asn Val Ala Ile Leu His Leu
CAA CTT GTA CGG CTA AAG AAA AAA GCA ACA GTT AAC AGA AAT GTG GCT ATC CTT CAC CTA

                                                                           126
Pro Lys Lys Gly Asp Asp Val Lys Pro Gly Thr Arg Cys Arg Val Ala Gly Trp Gly Arg
CCT AAA AAG GGA GAT GAT GTG AAA CCA GGA ACC AGA TGC CGA GTA GCA GGA TGG GGG AGA

                                                        +                   146
Phe Gly Asn Lys Ser Ala Pro Ser Glu Thr Leu Arg Glu Val Asn Ile Thr Val Ile Asp
TTT GGC AAT AAG TCA GCT CCC TCT GAA ACT CTG AGA GAA GTC AAC ATC ACT GTC ATA GAC

                                                                           166
Arg Lys Ile Cys Asn Asp Glu Lys His Tyr Asn Phe His Pro Val Ile Gly Leu Asn Met
AGA AAA ATC TGC AAT GAT GAA AAA CAC TAT AAT TTT CAT CCT GTA ATT GGA CTA AAC ATG

                                                $                       •   186
Ile Cys Ala Gly Asp Leu Arg Gly Gly Lys Asp Ser Cys Asn Gly Asp Ser Gly Ser Pro
ATT TGT GCA GGG GAC CTC CGT GGT GGA AAG GAC TCC TGC AAT GGG GAT TCT GGC AGC CCT

                                                                           206
Leu Leu Cys Asp Gly Ile Leu Arg Gly Ile Thr Ser Phe Gly Gly Glu Lys Cys Gly Asp
CTG CTA TGT GAT GGT ATT TTG CGA GGC ATC ACC TCT TTT GGT GGA GAG AAG TGT GGA GAT

                                                                           226
Arg Arg Trp Pro Gly Val Tyr Thr Phe Leu Ser Asp Lys His Leu Asn Trp Ile Lys Lys
CGC CGA TGG CCT GGT GTC TAT ACT TTC CTC TCA GAT AAA CAC CTC AAT TGG ATA AAG AAG

                     232
Ile Met Lys Gly Ser Val
ATT ATG AAG GGT TCT GTG TAAATGTATGTC TTTCACTCCA TCCCTGTCAC TTCTGTGTCT GATCACAAAT

      800
AAAATCAACT TGAATGGC (A)n
```

Fig. 3. Nucleotide sequence and amino acid translation of the HF cDNA cloned from a cytotoxic T cell. The amino acid sequence is numbered sequentially from the predicted amino terminus of the putative active enzyme. An arrow indicates a putative site of cleavage, generating the active enzyme predicted solely from homology alignments. The amino acids of the charge-relay system, His^{41}, Asp^{85}, and Ser^{183}, are each marked with a star. The acidic residue Asp^{177}, marked with a \$, is thought to determine substrate specificity for Lys or Arg. The AATAAA polyadenylation consensus sequence is underlined in the 3′ noncoding region. A potential Asn-linked carbohydrate site occurs at Asn^{141}, marked by +.

Fig. 4. Alignment of the HF protein sequence with some related eukaryotic serine proteases. The numbering scheme begins at the amino terminus of the predicted active enzyme; a dot was inserted at every ten residues to facilitate comparisons. Gaps were introduced to optimize the alignment. The residues of the charge-relay system are marked by a black diamond. All sequences were taken from the National Biomedical Research Foundation Protein Bank Library. The alignment makes use of the following sequences: mouse H factor(1–232), rat group–specific protease, bovine chymotrypsinogen A(16–245), bovine trypsinogen(7–229), human tissue plasminogen activator(311–562), human plasminogen(561–790), human factor IX(227–462), and human prothrombin(323–581).

In general, however, these findings confirm previous demonstrations of Lyt-1$^+$, Lyt-2$^-$ cells and helper cells with cytotoxic activity (*27*). Interestingly, Lobe and Bleackley (*13*) cloned two different, novel serine proteases with a related T-lymphocyte distribution and putative chymotrypsin-like activities.

Is there any evidence for a lytic mechanism, similar to that of complement-mediated lysis, which could involve killer-specific proteases? Morphologically recognized granules have been purified by density gradients from murine CTL's and rat NK-like leukemia cells. These cytolytic granules are capable of an efficient, rapid, Ca^{2+}-dependent lysis of tumor cells without MHC restriction or the presence of target antigen. An electron microscopic analysis of the membrane lesion caused by these lytic granules revealed a tubular ring structure, composed of proteins termed "polyperforins," reminiscent of the membrane-attack structure of complement (*3*). These polyperforins are thought to polymerize from monomers stored within the granules. Serine proteases may be responsible for controlling this cytolytic reaction by a cascade of events within the granules or at the site of their release at the CTL–target cell interface. If a protease cascade results in the lytic assembly, by analogy with other proteolytic systems, there may exist regulating protease inhibitors and protease inactivators that limit and control these reactions. A deficiency or absence in one of these hypothesized components or the inappropriate activation of this proposed lytic pathway may have some significant medical implications (*28*). Finally, just as the complement system can be activated by either the specific antigen-antibody–mediated "classical" pathway or the less specific alternative pathway, it is conceivable that by analogy the CTL represents a classical antigen receptor–mediated pathway, whereas NK and lymphokine-activated killer cells represent an alternative (non[α, β]-T-cell receptor–mediated) pathway for activation of a common lytic mechanism.

This new serine protease and, in general, the remarkable diversity of the serine protease family (kallikreins, complement proteolytic enzymes, and blood clotting enzymes, among others) pose the enigmatic questions of (i) where these enzymes come from and (ii) what selective pressures give rise to the new enzymes (*22, 29*). In considering these issues for a cytotoxic serine protease, we think host-defense interactions among unicellular phagocytic cells, and between these cells and bacteria provide a primitive, worthwhile model system. This idea is supported by the finding of an extracellular cytotoxic mechanism involving both serine proteases and polyperforin-like structures in a virulent strain of *Entamoeba histolytica* (*30*). This model suggests obvious adaptive advantages for organisms capable of synthesizing protease-specific inhibitors and protease-type inactivators. During evolution, unicellular organisms like *E. histolytica* could have usurped bacterial protease cytotoxin genes, giving the phagocytic cell a potent selective advantage in both inter- and intraspecies competition. This scheme would lead to evolution of divergent proteases in bacteria with independent acquisition of proteases by unicellular organisms.

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10. Complementary DNA libraries were made in λgt10 by modifications of a procedure initially developed by D. Kemp and improved by Huynh *et al.* (*9*). The two modifications were the replacement of all phenol-chloroform extractions with spermine precipitation as described [B. C. Hoopes, and W. R. Mc-

Clure, *Nucleic Acids Res.* **9**, 5493 (1981)], and the replacement of the BioGel A-50 m column with 1% to 2% agarose horizontal gel electrophoresis, both to remove the excess Eco RI linkers and to size-fractionate double-stranded cDNA. Spermine precipitation simplified the protocol and increased the yield, allowing all reactions to be performed in one siliconized tube. The agarose horizontal gel electrophoresis gave simple, reproducible separation, permitting visualization of the DNA with ethidium bromide. The double-stranded cDNA's were selected initially for lengths greater than 0.5 kb and subsequently for lengths greater than 0.95 kb. The selected agarose slices were electroeluted in dialysis bags [H. O. Smith *Methods Enzymol.* **65**, 371 (1980)] and precipitated with spermine. All RNA's for the cDNA libraries, Northern blots, and S1 analysis were prepared by guanidium thiocyanate extraction [J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, W. M. Rutter, *Biochemistry* **18**, 5294 (1979)] and poly(A) selected with oligo(dT)-cellulose. The 1E4 and AR1 killer cell lines were harvested and extracted 4 days after stimulation, at which time their cytotoxicity was confirmed.

11. The probe was prepared as described (*32*) and the cDNA libraries were plated at a density of ~3000 plaque-forming units per 150-cm plate as described by Huynh *et al.* (*9*).
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Novel Serine Proteases Encoded by Two Cytotoxic T Lymphocyte–Specific Genes

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Genes that are expressed exclusively in cytotoxic T cells should encode proteins that are essential for target cell lysis in cell-mediated immune responses. The sequences of two cytotoxic T lymphocyte–specific complementary DNA's (cDNA's) suggest that the two genes encode serine proteases. A full-length cDNA corresponding to one of the genes was isolated and sequenced. The predicted protein resembles serine proteases in that it includes all the residues that form the catalytic triad of the active site of serine proteases. Moreover, it has sequence characteristics thought to occur only in rat mast cell protease type II. These results are in accord with the view that a protease cascade plays a key role in cytotoxic T-cell activation.

CYTOTOXIC T LYMPHOCYTES (CTL's), also referred to as T killer cells, are effector cells in cell-mediated immune reactions. They specifically recognize foreign antigens on the surface of target cells, bind to them, and cause the target cells to lyse. Although the various steps in this process have been analyzed in considerable detail (*1, 2*), most studies have not provided insight into the mechanism by which the killer cell effects the lysis of a target cell. A means of identifying relevant molecules, based on cloning CTL-specific genes, has been described (*3*). The transcripts corresponding to two of these genes (B10 and C11) were detected exclusively in activated CTL's (*4*). Moreover, the kinetics of messenger RNA (mRNA) expression, as detected by these two cloned probes, closely paralleled but preceded cytotoxicity throughout cytotoxic responses in vitro (*3*).

Sequence analysis (*5*) of B10 and C11 (Fig. 1A) revealed that they were related to each other and that the hypothetical proteins they encode contain a short region characteristic of serine proteases, Asp-Ser-Gly-Gly (a sequence homologous to that surrounding Ser^{195} of chymotrypsin). With B10 and C11 as probes, another CTL complementary DNA (cDNA) library was screened, in which inserts greater than 1000 base pairs were cloned in λgt10. Forty thousand recombinants were screened and 39 plaques corresponding to C11 were isolated, but no evidence for a B10 recombinant could be found.

A cDNA insert of 1400 base pairs, which hybridized with C11, was selected for sequence analysis (*5*). The predicted protein sequence encoded, of molecular weight 25,319, is shown in Fig. 1B. The putative start codon is preceded by a potential ribosome binding site CCUUCCG (*6*), and a polyadenylation signal sequence AAUAAA (*7*) occurs just upstream from the poly(A) tract. Of the first 12 amino acids predicted, ten are hydrophobic, and the amino acid in position 2 (Lys) is basic, suggesting that this sequence may act as a signal to direct secretion or intracellular organelle location (*8*).

A search of the National Biomedical Research Foundation (NBRF) protein sequence data bank revealed that the protein encoded by C11 resembles a number of serine proteases (Table 1). When the se-

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