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Immunology

Cloning and chromosomal assignment of a human cDNA encoding a T cell- and natural killer cell-specific trypsin-like serine protease

(cytotoxic T lymphocyte/cytotoxicity/human chromosome 5)

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ABSTRACT **A cDNA clone encoding a human T cell- and natural killer cell-specific serine protease was obtained by screening a phage λgt10 cDNA library from phytohemagglutinin-stimulated human peripheral blood lymphocytes with the mouse Hanukah factor cDNA clone. In an RNA blot-hybridization analysis, this human Hanukah factor cDNA hybridized with a 1.3-kilobase band in allogeneic-stimulated cytotoxic T cells and the Jurkat cell line, but this transcript was not detectable in normal muscle, liver, tonsil, or thymus. By dot-blot hybridization, this cDNA hybridized with RNA from three cytolytic T-cell clones and three noncytolytic T-cell clones grown *in vitro* as well as with purified CD16$^+$ natural killer cells and CD3$^+$, CD16$^-$ T-cell large granular lymphocytes from peripheral blood lymphocytes (CD = cluster designation). The nucleotide sequence of this cDNA clone encodes a predicted serine protease of 262 amino acids. The predicted protein has a 22-amino acid presegment, a 6-amino acid prosegment, and an active enzyme of 234 amino acids with a calculated unglycosylated molecular weight of 25,820. The active enzyme is 71% and 77% similar to the mouse sequence at the amino acid and DNA level, respectively. The human and mouse sequences conserve the active site residues of serine proteases—the trypsin-specific Asp-189 and all 10 cysteine residues. The gene for the human Hanukah factor serine protease is located on human chromosome 5. We propose that this trypsin-like serine protease may function as a common component necessary for lysis of target cells by cytotoxic T lymphocytes and natural killer cells.**

Cytolytic T lymphocytes (CTL) and natural killer (NK) cells, subsets of peripheral blood lymphocytes (PBL), share the remarkable ability to recognize, bind, and lyse specific target cells. Teleologically, these cells are thought to protect their host by lysing cells bearing on their surface "nonself" antigens, usually peptides or proteins resulting from infection by intracellular pathogens—e.g., viruses (1, 2). Antigen-responsive CTL are induced by presentation of their target's nonself antigen in the context of "self" major histocompatibility (MHC) molecules. When these CTL are induced, they (*i*) recognize and bind target cells bearing the immunizing antigen in the context of self-MHC [via a number of T-cell receptor molecules (3, 4), including the T-cell receptor $\alpha\beta$ heterodimer] and (*ii*) activate the machinery that lyses these bound cells (5–7). In contrast, NK cells express an innate cytotoxic immunity (i.e., "natural") in which they recognize target cells by an ill-defined, MHC-unrestricted mechanism and then lyse them (8, 9). While many features of CTL recognition and binding have been delineated (6), the cytotoxic mechanism(s) of CTL and NK cells is not fully understood (6). From morphologic analysis of CTL–target conjugates, Zagury (10) and the Henkarts (11) proposed that the lytic mechanism of CTL cells resided in their cytoplasmic "lysosome-like" granules, which move toward the particular bound target cell to be lysed following conjugation (12, 13). Subsequently, several investigators (14–19) have focused on analyzing the contents of these granules from CTL and NK cells. Several proteins have been isolated from the granules, including one that can reconstitute cytolytic activity, a protein termed cytolysin or perforin (20–22). Perforin, a 60- to 66-kDa monomer protein, polymerizes in the target cell membranes in the presence of Ca^{2+} to create ≈160-Å tubular pores, lysing the target cells (20–22). In addition, several groups (23–29) have identified serine proteases in CTL and NK cells as defined by the binding of diisopropylfluorophosphate and have purified at least two specific granule proteases (25–29).

In an independent approach to understand lymphocyte cytotoxicity, we and others have used recombinant DNA techniques to select genes expressed preferentially in mouse CTL cells. Two of us (H.K.G. and I.L.W.; ref. 30) cloned a mouse CTL cDNA encoding a trypsin-like serine protease, Hanukah factor (HF; so-named for its nucleotide sequence similarity to blood coagulation factor IX, which was named "Christmas factor"), and Lobe *et al.* (31) and Brunet *et al.* (32) cloned two cDNAs encoding distinct CTL serine proteases. Masson, Tschopp, and co-workers have (28, 29) demonstrated that one of the granule serine proteases, granzyme A, has an N-terminal sequence identical to HF. Granzyme A is a dimer of 35-kDa disulfide-linked subunits that possesses a trypsin-like substrate specificity. To begin investigating the role of this putative enzyme in humans, we here describe the cloning, sequence analysis,[§] RNA expression, and chromosome location of the human HF (HuHF) gene homologous to the mouse HF cDNA.

MATERIALS AND METHODS

From oligo(dT)-selected RNA of human PBL stimulated with phytohemagglutinin (PHA) for 72 hr, a cDNA phage library was constructed by using the λgt10 vector with *Eco*RI cDNA inserts as described (33). The phage library was screened by plaque hybridization with nick-translated mouse HF cDNA (30, 34). All filter hybridizations were carried out at 42°C in the presence of 5× SSPE (34) containing 50% formamide; the filters were washed with 1× SSPE at 50°C. The recombinant phages were plaque-purified, isolated from overnight confluent plates, and puri-

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Abbreviations: PBL, peripheral blood lymphocytes; HF, Hanukah factor; HuHF, human HF; CTL, cytotoxic T lymphocyte; NK, natural killer; CD, cluster designation; LGL, large granular lymphocytes; FACS, fluorescence-activated cell sorter; CCP, cytotoxic T cell-specific protein; PHA, phytohemagglutinin.

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§This sequence is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03608).

fied by two CsCl step gradients (35). The phage DNA was extracted by the formamide extraction method (35). Restriction endonucleases were obtained and utilized as directed from New England Biolabs. Horizontal gel electrophoresis of RNA and DNA, transfer to nylon membranes, blot hybridization, and RNA dot blots were done as described (34). RNA was prepared by the guanidinium thiocyanate method (36). Cloned HuHF cDNA fragments were subcloned in phage M13 vectors mp18 and mp19; the M13 phage were grown and isolated, and the nucleotide sequence was determined as described (37, 38).

RESULTS

Isolation and Characterization of a HuHF cDNA Clone. Using the mouse HF cDNA, we screened 2.0×10^5 λgt10 recombinant phage plaques from a cDNA library prepared from human PBL stimulated with PHA for 3 days and found a single positive phage. This *Eco*RI-digested recombinant phage DNA revealed four *Eco*RI inserts, with only one fragment of 1.36 kilobases (kb) hybridizing with the mouse HF cDNA; this 1.36-kb fragment we designated HuHF cDNA.

On an RNA blot, the HuHF cDNA hybridized to a 1.1- to 1.3-kb polyadenylylated RNA species in human CTL cells generated in a 4-day alloreactive mixed lymphocyte culture and in the Jurkat cell line (Fig. 1 *Upper*). This transcript was not detectable in normal human muscle, liver, tonsil, or thymus, nor was it detected in the KB cell (a nasopharyngeal carcinoma), RPMI 4265 cell (a B-cell tumor), or NA and SSII cells (T-cell leukemias). We confirmed this distribution by dot-blot hybridization, except that we were now able to detect a low level of transcripts in the original PBL blast RNA. We investigated the relation between HuHF transcription (in RNA dot blots) and cytotoxic function by examining three human CTL alloreactive cloned lines (AI5.1, AMSB.3, and AMW.6), three noncytolytic long-term *in vitro* interleukin 2-grown T-cell lines (AMSH.2, AMW.8, and APL2.1) (42, 43), and two purified subsets of NK cells ($CD3^-$, $CD16^+$ and $CD3^+$, $CD16^-$; CD = cluster designation) (9) (Fig. 1 *Lower*). HuHF transcripts were detected in all of these cells, even in the long-term *in vitro* T-cell lines that lacked cytotoxicity in a lectin-linked ^{51}Cr-release cytotoxicity assay. By dot blots, the $CD16^+$ NK cell fraction had significantly more detectable transcripts than did the $CD3^+$, $CD16^-$ large granular lymphocyte (LGL) fraction.

The HuHF cDNA Clone Encodes a Trypsin-Like Serine Protease. The nucleotide sequence of this cDNA was determined on both strands yielding a single open reading frame (Fig. 2). The predicted mature prepro-HuHF protein contains 262 amino acids with a calculated molecular weight of 28,972 for the unglycosylated protein. Application of the algorithm developed by G. Von Heijne (44) shows that a signal sequence cleavage site probably occurs between Pro-7 and Glu-6. Thus, the prepiece and propiece would be 22 and 6 amino acids, respectively. Protein sequence homology suggests that the active enzyme results from cleavage on the COOH-terminal side of Lys-1. The predicted active enzyme contains 234 amino acids with a calculated unglycosylated molecular weight of 25,820. A potential N-linked glycosylation site occurs at Asn-142, based on the sequence Asn-Xaa-Thr. The amino acids of the serine protease "charge-relay"—His-57, Asp-102, and Ser-195 in chymotrypsin—are His-41, Asp-86, and Ser-184 in the HuHF serine protease. HuHF also contains the acidic residue Asp-178 equivalent to the Asp-189 of trypsin (45), suggesting that, like trypsin, the HuHF serine protease specifies cleavage C-terminal to lysine or arginine residues.

Sequence Homologies Between the Human and Mouse HF Coding Sequences. Table 1 presents the percentage similarities of the active HuHF protein to other serine proteases after optimal alignment. The HuHF and the mouse HF coding sequences are 71% and 77% similar at the amino acid and DNA level, respectively. In addition to conservation of the serine protease charge relay residues and the trypsin-like

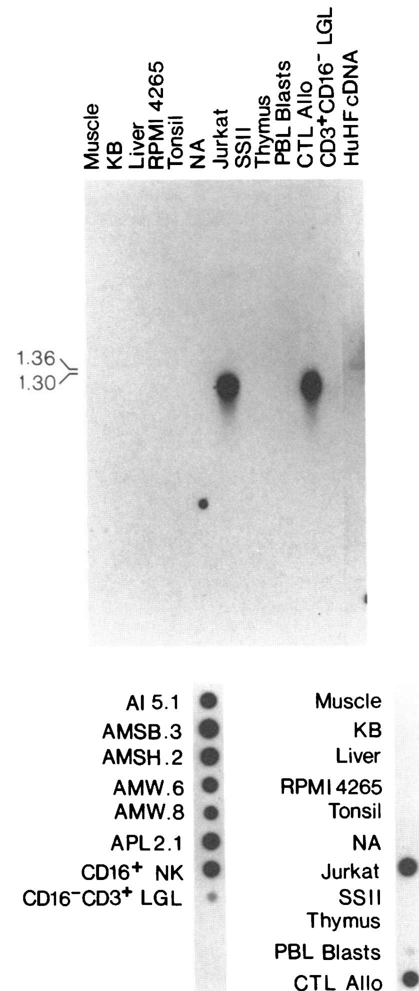

FIG. 1. Size and distribution of poly(A)-containing RNA from sequences similar to the cloned cDNA. (*Upper*) RNA blot-hybridization analysis. Glyoxal-treated total and poly(A)-containing RNA was electrophoresed through a 1.1% agarose gel and transferred to nitrocellulose as described (39). The RNA paper was hybridized with nick-translated ^{32}P-labeled DNA of a 1.3-kb HuHF cDNA fragment (34). After the RNA paper was washed with 0.1× SSPE at 50°C, autoradiograms were made on Cronex film for 6 days. Ribosomal RNA, *Hin*fI-digested pUC9, and the HuHF cDNA served as molecular size markers (shown in kb). Each lane contained 2 μg of poly(A)-containing RNA, except for muscle (20 μg of total RNA) and PBL day 3 blasts (90 μg of total RNA). KB is a human nasopharyngeal carcinoma; RPMI 4265 is a B-cell tumor, NA/SUP-T1 is a T-cell lymphoblastic leukemia ($CD1^+$, $CD4^+$, $CD5^+$, $CD7^+$, $CD8^+$, $CD2^-$, $CD3^-$), SSII/KT-1 is a T-cell acute lymphoblastic leukemia ($CD5^+$, $CD1^-$, $CD4^-$, $CD8^-$) (40), and Jurkat is a human T-cell leukemia line (41). (*Lower*) RNA dot blots showing the distribution of HuHF in RNA of several normal tissues, three cytotoxic T-cell lines (AI5, AMSB.3, and AMW.6), three noncytotlytic T cell *in vitro* lines (AMSH.2, AMW.8, and APL2.1), day 4 PHA-stimulated alloreactive CTL PBL cells (42, 43), fluorescence activated cell sorter (FACS)-purified $CD16^+$ $CD3^-$ NK cells, and FACS-purified $CD16^-$ $CD3^+$ T-cell LGL cells (9). Formaldehyde-denatured RNA [0.5 μg of poly(A) RNA from normal tissues except for total RNA from muscle (5 μg) and PBL blasts (22 μg); and cytoplasmic RNA from 5×10^5 cells of the remaining cell lines] was applied to nitrocellulose and hybridized with ^{32}P-labeled 1.3-kb HuHF cDNA insert DNA (34). After the filters were washed with 0.1× SSPE at 50°C, they were exposed to Cronex film for 6 days. Control hybridization with vector sequences gave no signal.

FIG. 2. Nucleotide sequence, complete amino acid sequence, and predicted amino acid cleavage sites of the HuHF cDNA cloned from a day 3 PHA-stimulated PBL cDNA library. The amino acid sequence of the prepro-HuHF is numbered sequentially. A thin arrow indicates the predicted signal sequence cleavage site between Pro-7 and Glu-6, generating the pro-HuHF, while the thick arrow indicates the putative cleavage site, generating the active HuHF enzyme. The amino acid residues of the charge relay system, His-41, Asp-86, and Ser-184, are each marked with a star. The acidic residue Asp-178, marked with a "$," is thought to determine substrate specificity for lysine or arginine. The AATAA polyadenylylation consensus sequence is underlined in the 3′ noncoding region. A potential asparagine-linked carbohydrate site occurs at Asn-142 marked by a "+." The nucleotide sequence of the HuHF 1.36-kb cDNA contained approximately 420 base pairs at the 5′ end, representing an inverted repeat of 3′ sequences. Only the unique sequence of the complete coding region is shown.

substrate specificity of an aspartic residue, all 10 cysteine residues (9 in the active enzyme) are conserved between the mouse and human sequences. The preprosequences have diverged, however; they have only a 21% similarity at the amino acid sequence level, reflected in a 50% DNA sequence similarity.

The HuHF Gene Maps to Chromosome 5. Using the HuHF cDNA as a probe, we have confirmed the hybridization pattern previously reported with the mouse HF probe on a Southern blot of human DNA (30). As with the mouse HF gene, Southern analysis with a variety of enzymes suggested only a single HuHF genomic gene. To ascertain the chromosomal location of the HuHF gene, we defined a species-specific restriction fragment length polymorphism (RFLP) (in kb) on Southern blots of genomic human or mouse DNA digested with the following enzymes: *Eco*RI (human 5.0-, 1.45-, and 1.1-kb fragments/mouse 5.75- and 3.5-kb fragments); *Bam*HI (9.5/10.2 and 7.9), and *Pvu* II (9.4 and 6.3/2.3 and 1.0). The *Eco*RI restriction fragment length polymorphism was used to analyze human–mouse hybrids with known human chromosomal compositions (data not shown). Table 2 demonstrates that discordance percentage, based on the pattern of hybridization, assigns the human HF gene to chromosome number 5.

DISCUSSION

We have cloned a cDNA from PBL blasts encoding a trypsin-like serine protease preferentially expressed in activated CTL, some *in vitro* activated T cells, and NK cells, but not in a variety of normal tissues (muscle, liver, thymus, and tonsil). The sequence of this cDNA is 77% similar to the mouse HF cDNA over the region known to encode the active, mouse HF serine protease. From recent work, the mouse HF protein is localized to the intracytoplasmic, cytolytic granules of CTL cells (27–29), and the enzyme activity is secreted into the medium after antigen receptor triggering (26, 51). The cloning of the HuHF gene should facilitate the extension of the mouse serine protease studies to the human system.

HF transcripts are present in a variety of cytotoxic cells: three CTL cell lines; $CD3^+$, $CD16^-$ LGL cells; and $CD3^-$, $CD16^+$ NK cells. Surprisingly, four apparently noncytolytic cell lines—namely, the Jurkat cell line and three long-term *in vitro* T-cell lines—contain HuHF transcripts. The Jurkat tumor cell line was initially $CD3^+$, $CD4^+$, $CD8^+$ and may have lost cytolytic potential during its transformation or its many years of passage. We propose that the three noncytolytic T-cell lines' expression may be nonphysiologic: the activation of HF sequences may be secondary to the persistent stimulation with antigen and/or the Con A supernatant factors required to maintain these lines. This assertion extrapolates from the experiments in the mouse by Simon *et al.* (52), which demonstrated expression of a trypsin-like enzymatic activity induced by antigen/lectin and interleukin-2 in both $Lyt\text{-}2^+$, $L3T4^-$ and $Lyt\text{-}2^-$, $L3T4^+$ T cells *in vitro* but found only in cytolytic $Lyt\text{-}2^+$, $L3T4^-$ cells *in vivo*. The expression of HuHF sequences in $CD16^+$ NK cells and $CD3^+$, $CD16^-$ T-cell LGL cells is notable for two reasons. First, these cells were isolated directly from human peripheral blood, thereby representing *in vivo* expression. Second, the expression implies that this enzyme may be involved in a common cytolytic pathway, triggered either by the classical T-cell receptor combination with antigen in T cells or by the self/nonself alternative recognition system for NK cells.

The sequence encoded by the HuHF cDNA conserves the features of a trypsin-like serine protease and demonstrates 71% homology to the mouse cDNA over the active enzyme region. The next highest similarities are to the cytotoxic T cell-specific protein-encoding C11 (CCP-C11) cDNA/CTLA-1 cDNA sequences, which encode distinct serine proteases with a remarkably similar T cell-specific expression (31, 32). The HuHF active enzyme conserves the nine cysteine residues present in the mouse sequence. By homology with other serine proteases and with the isolation of HF, establishing the mouse HF protein/granzyme A as a homodimer of 35-kDa subunits (28, 29), we suggest four intrachain disulfide bridges between Cys-26 and Cys-42, Cys-120 and Cys-190, Cys-151 and Cys-169, and Cys-180 and Cys-206 and an interchain disulfide bond between Cys-77 residues, analogous to the mouse protein (28). The intriguing divergence between the mouse and human preprosequences suggests that these sequences may be encoded on separate genomic exons, as occurs in the genomic sequences of several other serine proteases (53). A comparison of these HF, CCP-C11/CTLA-1, and perforin sequences may eventually reveal a "sorting domain"—i.e., a stretch of amino acids ensuring the appropriate sorting and transport of these proteins from the Golgi complex to the intracytoplasmic granules (54). The

Table 1. Similarity of the active HuHF protein sequence with some related eukaryotic serine proteases

Protein	EC No.	Species	Sequence position limits	% homology
HF		Human	1–234	
HF		Mouse	1–232	70.6
CCP-C11/CTLA-1		Mouse	1–227	43.1
Plasminogen	3.4.21.7	Human	561–790	38.8
Tonin	3.4.21.-	Rat	1–239	36.6
Group-specific protease	3.4.21.-	Rat	1–224	36.0
Kallikrein, pancreatic	3.4.21.8	Rat	29–265	35.6
7S nerve growth factor	3.4.21.-	Mouse	25–261	34.7
Trypsin	3.4.21.4	Bovine	7–229	33.6
Factor IX (Christmas factor)	3.4.21.22	Human	227–462	32.1
Chymotrypsinogen A	3.4.21.1	Bovine	16–245	31.5
Chymotrypsinogen B	3.4.21.1	Bovine	16–245	30.7
Elastase	3.4.21.11	Pig	1–240	30.6
Trypsin	3.4.21.4	*Streptococcus griseus*	1–221	28.4
t-Plasminogen activator	3.4.21.31	Human	311–562	27.6
Factor X (Stuart factor)	3.4.21.6	Human	195–424	26.1
Urokinase	3.4.21.31	Human	159–404	26.1
Complement C1r,b chain	3.4.21.41	Human	1–242	24.7

All sequences were taken from the National Biomedical Research Foundation Protein Bank Library and the alignment was optimized by the Dayhoff algorithm (46). C1r,b chain, b chain of the complement protease C1; t-plasminogen, the subcomponent C1r tissue plasminogen.

localization of this gene to chromosome 5 emphasizes the complex evolution of serine proteases, as no other serine protease has been linked to this chromosome (55).

We have proposed that mouse HF is one of several serine proteases involved in cytolysis. In addition, we have speculated that killer cell serine proteases might be part of a cytolytic cascade. What is the evidence for this? First, a variety of experiments replicated by several groups has shown inhibition of CTL and NK cytolysis by inhibitors of chymotrypsin or trypsin-type serine proteases (56–64). Thus, serine esterases of either specificity appear to be necessary for cytolysis. Second, Petty *et al.* (23) and Pasternack and Eisen (24) have defined murine serine proteases specific to cytolytic cells by using radioactive diisopropyl fluorophosphate, a serine-specific active-site reagent. Third, Masson and Tschopp (65) have identified by amino acid N-terminal sequencing a family of eight distinct killer cell granule serine proteases in murine CTLs, one of which is murine HF. Fourth, the pore-forming protein at the end of the complement cytolytic cascade, C9, is antigenically cross-reactive and similar by primary sequence to the killer cell pore-forming protein, perforin (66). All of these serine esterases and the perforin molecule are found in killer cell cytoplasmic granules. We interpret these data as being consistent with the model of exocytosis of intracytoplasmic granules after cell–cell antigen receptor triggering. In this scheme, the granule zymogen serine proteases and perforin could be linked in a cytolytic cascade or act together to effect cytolysis in some other fashion.

While these studies strongly implicate serine proteases in target-cell lysis, they do not formally demonstrate the existence of a serine protease cytolytic cascade. Targets of NK and CTL action (and cortisone-treated lymphocytes) undergo an early fragmentation of chromosomal DNA, presumably via activation of a target cell endonucleolytic system (67–70). This second cytolytic-associated event might be independent of a putative serine protease pathway or might be induced by it; redundant cytolytic mechanisms might be required to ensure target-cell death. It has also been proposed that killer-cell granule-mediated cytolysis (and therefore the proposed functions described above) is an *in vitro* phenomenon not normally induced *in vivo* (71, 72). Here we show that HuHF is present in human NK cell subsets (Fig. 1 *Lower*), and elsewhere some of us demonstrate that murine HF transcripts are found at high levels in cells infiltrating cardiac allografts (73). The development of probes and inhibitors specific for each of these proposed elements in cytolysis (HF, CCP-C11/CTLA-1, perforin/cytolysin, putative endonucleases activated in target cells by killer cells, etc.) that can be used to assess immune reactions *in vivo* should aid in defining the function of these proteins and the function of cells that express them *in vivo* and also in demonstrating whether killer-cell functional heterogeneity might explain the diversity of killer pathways shown to date.

Should a serine protease cascade necessary for cytotoxic lymphocyte lysis be demonstrated, a disordered homeostasis in this cascade [such as a deficiency in an enzyme or hyperactivity of a protease inactivator (74)] might play a role in syndromes with a deficiency in the postbinding steps of

Table 2. Segregation of the HuHF gene

% discordancy at human chromosome																						
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
29	30	31	37	0	27	38	43	32	33	31	23	43	43	43	33	41	33	33	33	40	38	46

Segregation of the HuHF gene, encoding a T cell-specific serine protease, with human chromosomes in human–mouse cell hybrid DNA, determining its assignment to human chromosome number 5. The HuHF cDNA was hybridized to Southern blots containing *Eco*RI-digested DNA from human–mouse hybrids. The human HF serine protease was scored (+) by the presence of the human-specific HF hybridization bands at 5.0, 1.45, and 1.1 kb and (−) by their absence (data not shown). Data were gathered on 30 cell hybrids involving 13 unrelated human cell lines and 4 mouse cell lines (47–50). Concordant hybrids have either retained or lost the HuHF serine protease gene together with a specific human chromosome. Discordant hybrids either retained the gene but not the specific chromosome or lost the gene but retained the specific chromosome. A 0% discordancy is the basis for the chromosome assignment to chromosome number 5.

lymphocyte lysis (75–79). Finally, these serine proteases could provide targets for rational drug design of inhibitors (78–80) that will block CTL and NK function, and they could be used to produce monoclonal antibodies capable of defining cytolytic cells.

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1. Townsend, A. R. M., Rothbard, J., Gotch, F. M., Bahadur, G., Wraith, D. & McMichael, A. J. (1986) *Cell* **44**, 959–968.
2. Welsh, R. M. (1986) *Nat. Immun. Cell Growth Reg.* **5**, 169–199.
3. Kronenberg, M., Siu, G., Hood, L. E. & Shastri, N. (1986) *Annu. Rev. Immunol.* **4**, 529–591.
4. Reinherz, E. L. (1987) *Nature (London)* **325**, 660–663.
5. Cohn, M. (1985) in *Neural Modulation of Immunity*, eds. Guillemin, R., Cohn, M. & Melnechuk, T. (Raven, New York), pp. 3–23.
6. Cerottini, J. & MacDonald, H. R., ed. (1987) *Ann. Immunol. (Paris)* **138**, 289–342.
7. Henkart, P. A. (1985) *Annu. Rev. Immunol.* **3**, 31–58.
8. Herberman, R. B., Reynolds, C. W. & Ortaldo, J. R. (1986) *Annu. Rev. Immunol.* **4**, 651–680.
9. Lanier, L. L., Phillips, J. H., Hackett, J., Tutt, M. & Kumar, V. (1986) *J. Immunol.* **137**, 2735–2739.
10. Zagury, D. (1982) *Adv. Exp. Med. Biol.* **146**, 149–169.
11. Henkart, M. P. & Henkart, P. A. (1982) *Adv. Exp. Med. Biol.* **146**, 227–247.
12. Yannelli, J. R., Sullivan, J. A., Mandell, G. L. & Engelhard, V. H. (1986) *J. Immunol.* **136**, 377–382.
13. Kupfer, A., Singer, S. J. & Dennert, G. (1986) *J. Exp. Med.* **163**, 489–498.
14. Podack, E. R. (1985) *Immunol. Today* **6**, 21–27.
15. Young, J. D.-E. & Cohn, Z. (1986) *J. Cell. Biochem.* **32**, 151–167.
16. Criado, M., Lindstrom, J. M., Anderson, C. G. & Dennert, G. (1985) *J. Immunol.* **135**, 4245–4251.
17. Henkart, P., Henkart, M., Millard, P., Fredriske, P., Bluestone, J., Yue, C. & Reynolds, C. W. (1985) *Adv. Exp. Med. Biol.* **184**, 121–138.
18. Henkart, P. A., Yue, C. C., Yang, J. & Rosenberg, S. A. (1986) *J. Immunol.* **137**, 2611–2617.
19. MacDermott, R. P., Schmidt, R. E., Caulfield, J. P., Hein, A., Bartley, G. T., Ritz, J., Schlossman, S., Austen, K. & Stevens, R. (1985) *J. Exp. Med.* **162**, 1771–1787.
20. Masson, D. & Tschopp, J. (1985) *J. Biol. Chem.* **260**, 9069–9072.
21. Podack, E., Young, J. D.-E. & Cohn, Z. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8629–8633.
22. Young, J. D.-E., Podack, E. R. & Cohn, Z. A. (1986) *J. Exp. Med.* **164**, 144–155.
23. Petty, H. R., Hermann, W., Dereski, W., Frey, T. & McConnell, H. M. (1984) *J. Cell Sci.* **72**, 1–13.
24. Pasternack, M. S. & Eisen, H. N. (1985) *Nature (London)* **314**, 743–745.
25. Kramer, M. D., Binninger, L., Schirrmacher, V., Moll, H., Prester, M., Nerz, G. & Simon, M. A. (1986) *J. Immunol.* **136**, 4644–4651.
26. Pasternack, M. S., Verret, C. R., Liu, M. A. & Eisen, H. N. (1986) *Nature (London)* **322**, 740–743.
27. Young, J. D.-E., Leong, L. G., Liu, C., Damiano, A., Wall, D. A. & Cohn, Z. A. (1986) *Cell* **47**, 183–194.
28. Masson, D., Zamai, M. & Tschopp, J. (1986) *FEBS Lett.* **208**, 84–88.
29. Masson, D., Nabholz, M., Estrade, C. & Tschopp, J. (1986) *EMBO J.* **5**, 1595–1600.
30. Gershenfeld, H. K. & Weissman, I. L. (1986) *Science* **232**, 854–858.
31. Lobe, C. G., Finlay, B. B., Paranchych, W., Paetkau, V. H. & Bleackley, R. C. (1986) *Science* **232**, 858–861.
32. Brunet, J., Dosseto, M., Denizot, F., Mattei, M., Clark, W., Haggi, T., Ferrier, P., Nabholtz, M., Schmitt-Verhalst, A., Luciani, M. & Golstein, P. (1986) *Nature (London)* **322**, 268–271.
33. Concannon, P., Pickering, L. A., Kung, P. & Hood, L. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6598–6602.
34. Meinkoth, J. & Wahl, G. (1984) *Anal. Biochem.* **138**, 267–284.
35. Huynh, T. V., Young, R. A. & Davis, R. W. (1984) in *DNA Cloning: A Practical Approach*, ed. Glover, D. (IRL, Oxford), pp. 49–78.
36. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. (1979) *Biochemistry* **18**, 5294–5299.
37. Messing, J. (1983) *Methods Enzymol.* **101**, 20–78.
38. Henikoff, S. (1984) *Gene* **28**, 351–359.
39. McMaster, G. K. & Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4835–4838.
40. Smith, S. D., Morgan, R., Link, M. P., McFall, P. & Hecht, F. (1986) *Blood* **67**, 650–656.
41. Weiss, A., Wiskocil, R. & Stobo, J. (1984) *J. Immunol.* **133**, 123–128.
42. Krensky, A. M., Reiss, C. S., Mier, J. W., Strominger, J. L. & Burakoff, S. J. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2365–2369.
43. Clayberger, C., Holmes, N., Wang, P. L., Koller, T. D., Parham, P. & Krensky, A. M. (1985) *J. Exp. Med.* **162**, 1709–1714.
44. Von Heijne, G. (1986) *Nucleic Acids Res.* **14**, 4683–4690.
45. Hartley, B. S. (1970) *Philos. Trans. R. Soc. London Ser. B* **257**, 77–87.
46. Dayhoff, M. O. (1976) in *Atlas of Protein Sequence and Structure*, ed. Dayhoff, M. O. (Natl. Biomed. Res. Found., Washington, DC), Vol. 5, Suppl. 2, pp. 1–8.
47. Shows, T. B., Sakaguchi, A. Y. & Naylor, S. L. (1982) *Adv. Human Genet.* **12**, 341–452.
48. Sakaguchi, A. Y. & Shows, T. B. (1982) *Somatic Cell Genet.* **8**, 83–94.
49. Shows, T. B., Eddy, R., Haley, L., Byers, M., Henry, M., Fujita, T., Matsui, H. & Taniguchi, T. (1984) *Somatic Cell Mol. Genet.* **10**, 315–318.
50. Shows, T. B. (1983) in *Current Topics in Biological and Medical Research: Isozymes*, eds. Rattazzi, M., Scandalios, J. & Shitt, G. (Liss, New York), Vol. 10, pp. 323–329.
51. Takayama, H., Trenn, G., Humphrey, W., Bluestone, J. A., Henkart, P. A. & Sitkovsky, M. V. (1986) *J. Immunol.* **138**, 566–569.
52. Simon, M. M., Fruth, U., Simon, H. G. & Kramer, M. D. (1986) *Eur. J. Immunol.* **16**, 1559–1568.
53. Rogers, J. (1985) *Nature (London)* **315**, 458–459.
54. Kelly, R. B. (1987) *Nature (London)* **326**, 14–15.
55. Eighth International Workshop on Human Gene Mapping (1985) *Cytogenet. Cell Genet.* **40**, (1–4).
56. Redelman, D. & Hudig, D. (1980) *J. Immunol.* **124**, 870–878.
57. Chang, T. & Eisen, H. N. (1980) *J. Immunol.* **124**, 1028–1033.
58. Hudig, D., Haverty, T., Fulcher, C., Redelman, D. & Mendelsohn, J. (1981) *J. Immunol.* **126**, 1569–1574.
59. Quan, P. C., Ishizaka, T. & Bloom, B. R. (1982) *J. Immunol.* **128**, 1786–1791.
60. Pasternack, M. S., Sitkovsky, M. V. & Eisen, H. N. (1983) *J. Immunol.* **131**, 2477–2483.
61. Ristow, S. S., Starkey, J. R. & Hass, G. M. (1983) *Immunology* **48**, 1–8.
62. Ades, E. W., Hinson, A., Chapuis-Cellier, C. & Arnaud, P. (1982) *Scand. J. Immunol.* **15**, 109–113.
63. Simon, M. M., Fruth, U., Simon, H. G. & Kramer, M. D. (1987) *Ann. Immunol. (Paris)* **138**, 309–314.
64. Lavie, G., Leib, Z. & Servadio, C. (1985) *J. Immunol.* **135**, 1470–1476.
65. Masson, D. & Tschopp, J. (1987) *Cell* **49**, 679–685.
66. Lowrey, D. M., Rupp, F., Aebischer, T., Grey, P., Hengartner, H. & Podack, E. (1987) *Ann. Immunol. (Paris)* **138**, 297–300.
67. Russell, J. H., Masakowski, V. R. & Dobos, C. B. (1980) *J. Immunol.* **124**, 1100–1105.
68. Duke, R. C., Chervenak, R. & Cohen, J. J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6361–6365.
69. Cohen, J. J. & Duke, R. C. (1984) *J. Immunol.* **132**, 38–42.
70. Ucker, D. S. (1987) *Nature (London)* **327**, 62–64.
71. Berke, G. (1987) *Ann. Immunol. (Paris)* **138**, 305–308.
72. Berke, G. & Rosen, D. (1987) *Transplant. Proc.* **19**, 412–416.
73. Mueller, C., Gershenfeld, H. K., Lobe, C. G., Okada, C. Y., Bleackley, R. C. & Weissman, I. L. (1987) *J. Exp. Med.*, in press.
74. Ganea, D., Teodorescu, M. & Dray, S. (1986) *Immunology* **57**, 85–92.
75. Pedersen, B. K. (1985) *Allergy* **40**, 547–557.
76. Sullivan, J. L., Byron, K. S., Brewster, F. E. & Purtilo, D. T. (1980) *Science* **210**, 543–545.
77. Katz, P., Zaytoun, A. M., Lee, J. H., Panush, R. S. & Longley, S. (1982) *J. Immunol.* **129**, 1966–1971.
78. Katzman, M. & Lederman, M. M. (1986) *J. Clin. Invest.* **77**, 1057–1062.
79. Klimpel, G. R., Herndon, D. N., Fons, M., Albrecht, T., Asuncion, M. T., Chin, R. & Stein, M. D. (1986) *Clin. Exp. Immunol.* **66**, 384–392.
80. Baugh, R. J. & Schnebli, H. P. (1980) in *Proteinases and Tumor Invasion*, eds. Strauli, P., Barrett, A. J. & Baici, A. (Raven, New York), pp. 157–179.