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THE STRUCTURE OF THE MOUSE LYMPHOCYTE PORE-FORMING PROTEIN PERFORIN

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Summary Purified murine lymphocyte pore-forming protein (PFP or perforin) was partially sequenced. Oligonucleotides synthesized on the basis of this sequence information were used to screen a murine cytotoxic T lymphocyte (CTL) cDNA library. Seven clones were obtained, two of which were sequenced, providing full-length sequence information on PFP. Murine PFP (534 a.a.) is 68% identical to human PFP. Hydropathic analysis revealed a predominantly hydrophilic protein with some hydrophobic domains, including a region (a.a. 191-251) that could contain putative membrane-spanning domains. PFP is approx. 20% identical to human C7, C8 and C9 within a region encompassing 270 a.a., confirming previous immunological cross-reactivity studies. Northern blot analysis showed that expression of PFP but not of a serine esterase transcript is enhanced in a CTL line by antigen receptor-stimulation. Southern blot analysis of mouse genomic DNA indicated that PFP is encoded as a single-copy gene with the coding region contained within 10 kilobases of genomic DNA.

The lymphocyte PFP, stored in the cytoplasmic granules of CTL and natural killer (NK) cells, is a potent mediator of lymphocyte-mediated killing (1-4). PFP is exocytosed from lymphocytes and, in the presence of calcium, it lyses a variety of target cells by polymerizing into transmembrane channels on target membranes. Previous studies from this and other laboratories have already shown that PFP is related structurally and functionally to the terminal components of the complement cascade (5-8). Recently, murine PFP was purified to homogeneity and 25% of its primary sequence determined (9). This sequence information was used to derive oligonucleotide probes for cDNA cloning of PFP. The full-length sequence of murine PFP and an initial sequence analysis are presented here.

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Materials and Methods

Cloning of PFP/Perforin. PFP was purified from the murine CTL line CTLL-R8 and sequenced as described (9). From the amino terminal information (9), three degenerate oligonucleotides were synthesized (5' to 3'): AC(A/G)AA(C/T)T T(A/G)TG(C/T)TT(C/T)TG(C/T)TT (20-mer corresponding to a.a. 11 to 17, with degenerate bases given in brackets), AC(T/G/C/A)CC(T/G/C/A)GG(T/G/C/A)AC(A/G)AA (C/T)TT (17-mer corresponding to a.a. 15-20) and CAT(T/G/C/A)CC(T/C)TC(T/G/C/A) CC(T/G/C/A)GCCAT (18-mer, a.a. 22-27). A λgt11 cDNA library constructed from the murine CTL line CTLL-A11 (10) was screened initially with the 17-mer probe; plaques giving positive signals were subsequently rescreened with the other two probes. The EcoRI inserts of hybridization-positive clones were subcloned into M13 vectors and sequenced by dideoxy chain termination technique, essentially as described (11).

RNA Blot Hybridization. Total cellular RNA was prepared from mouse CTLL-R8, CTLL-L3, P815 mastocytoma and YAC-1 lymphoma cells by the guanidinium thiocyanate-CsCl method. As described in detail elsewhere (12), L3 cells were washed free of leukocyte-conditioned medium and cultured with concanavalin A (con A; 2 μg/ml) for 6 h, or with recombinant human interleukin-2 (rIL-2 from Cetus Corp., Emeryville, CA; 100 U/ml) for 6 h, or with immobilized clonotypic anti-T cell receptor (TcR) monoclonal antibody (mAb) 384.5 (13) for the indicated time periods. L3 cell preparations were kindly provided by Dr. David Lancki of the Univ. of Chicago. RNA was electrophoresed on a 1% formaldehyde denaturing agarose gel and transferred to Gene-Screen Plus (NEN, Boston, MA). Purified inserts of a PFP cDNA (PFP-6) or of a serine esterase cDNA (L3G10 #6, ref. 10) were labeled with ^{32}P, and hybridization and washing of the blots were carried out as described (10), except that the membranes were finally washed at 60° C in 0.25 x SSC/1% SDS.

Genomic DNA Blot Analysis. High molecular weight mouse DNA was prepared and digested with restriction endonucleases as described (10). After transfer to a nylon membrane, the blot was hybridized and washed essentially as described (10).

Structural Predictions. Hydropathy analysis was done by the method of Kyte and Doolittle (14) using a span setting of 9 a.a.. Amphiphilic α helices were searched using helical net diagrams and Edmunson wheel plots (15). Regions of alternating hydrophobic residues that could form amphiphilic β sheets were obtained by visual inspection. The Dayhoff and Genbank databases were employed for homology search using the program "fasta".

Results and Discussion

cDNA Cloning and Sequence Analysis of PFP/Perforin. Seven clones from the murine CTLL-A11 cDNA library gave positive hybridization signals with all 3 oligonucleotide probes. Two of these, designated PFP-6 (1.7 kb) and PFP-15 (0.64 kb), were sequenced, providing the full-length nucleotide sequence for PFP (Fig. 1). PFP-6 and PFP-15 encoded nucleotides 97-1800 and -194 to 445, respectively, as shown in Fig. 1. The sequence flanking the assigned ATG initiation codon (nucleotide residues -5 to +4) contains 7 out of 9 residues identical to the consensus sequence described by Kozak (16). The primary a.a. sequences obtained from purified PFP (9) are underlined in Fig. 1. These sequences, comprising 25% of the full length sequence of PFP, were noted to be identical to the deduced sequences shown in Fig. 1, thus further confirming the cDNA sequences reported here. The codon specifying the carboxyl terminal tryptophan is followed by the translational termination codon TAA (nucleotide residues 1663 to 1665). The long poly(A) sequence seen in clone PFP-6 may not

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                                                                    -194  TTCT GCAGTTGTCA CAGATGGAGG AGATCTTTTT GTTTGTGGCC TGAATAAAGA -151
TGGGCAGTTG GGGCTTGGTC ACACAGAGGA AGTTCTGCGT TTTACCATCT GCAAGCCTCT CCGTGGTTGT CCCATCCGAC AGTGGCGTCT TGGTGGGACT TCAGCTTTCC -31
AGAGTTTATG ACTACTGTGC CTGCAGCATC      (1) ATG GCC ACG TGC CTG TTC CTC CTG GGC CTT TTC CTG CTG CTG CCA CGA CCT GTC CCT GCT  60
                                          Met Ala Thr Cys Leu Phe Leu Leu Gly Leu Phe Leu Leu Leu Pro Arg Pro Val Pro Ala
                                         -20                                 -10                                  -1

CCC TGC TAC ACT GCC ACT CGG TCA GAA TGC AAG CAG AAG CAC AAG TTC GTG CCA GGT GTA TGG ATG GCT GGG GAA GGC ATG GAT GTG ACT  150
Pro Cys Tyr Thr Ala Thr Arg Ser Glu Cys Lys Gln Lys His Lys Phe Val Pro Gly Val Trp Met Ala Gly Glu Gly Met Asp Val Thr
 1              10                  20                  30

ACC CTC CGC CGC TCC GGC TCC TTC CCA GTG AAC ACA CAG AGG TTC CTG AGG CCT GAC CGC ACC TGC ACC CTC TGT AAA AAC TCC CTA ATG  240
Thr Leu Arg Arg Ser Gly Ser Phe Pro Val Asn Thr Gln Arg Phe Leu Arg Pro Asp Arg Thr Cys Thr Leu Cys Lys Asn Ser Leu Met
             40                  50                  60

AGA GAC GCC ACA CAG CGC CTA CCT GTG GCA ATC ACC CAC TGG CGG CCT CAC AGC TCA CAC TGC CAG CGT AAT GTG GCC GCA GCC AAG GTC  330
Arg Asp Ala Thr Gln Arg Leu Pro Val Ala Ile Thr His Trp Arg Pro His Ser Ser His Cys Gln Arg Asn Val Ala Ala Ala Lys Val
         70                  80                  90

CAC TCC ACG GAG GGT GTG GCC CGG GAG GCA GCT GCT AAT ATC AAT AAC GAC TGG CGT GTG GGG CTG GAT GTG AAC CCT AGG CCA GAG GCA  420
His Ser Thr Glu Gly Val Ala Arg Glu Ala Ala Ala Asn Ile Asn Asn Asp Trp Arg Val Gly Leu Asp Val Asn Pro Arg Pro Glu Ala
        100                 110                 120

AAC ATG CGC GCC TCC GTG GCT GGC TCC CAC TCC AAG GTA GCC AAT TTT GCA GCT GAG AAG ACC TAT CAG GAC CAG TAC AAC TTT AAT AGC  510
Asn Met Arg Ala Ser Val Ala Gly Ser His Ser Lys Val Ala Asn Phe Ala Ala Glu Lys Thr Tyr Gln Asp Gln Tyr Asn Phe Asn Ser
        130                 140                 150

GAC ACA GTA GAG TGT CGC ATG TAC AGT TTT CGC CTG GTA CAA AAA CCT CCA CTC CAC CTT GAC TTC AAA AAG GCG CTC AGA GCC CTC CCC  600
Asp Thr Val Glu Cys Arg Met Tyr Ser Phe Arg Leu Val Gln Lys Pro Pro Leu His Leu Asp Phe Lys Lys Ala Leu Arg Ala Leu Pro
        160                 170                 180

CGC AAC TTT AAC AGC TCC ACA GAG CAT GCT TAC CAC AGG CTC ATC TCC TCC TAT GGC ACG CAC TTT ATC ACG GCT GTG GAC CTC GGT GGC  690
Arg Asn Phe Asn Ser Ser Thr Glu His Ala Tyr His Arg Leu Ile Ser Ser Tyr Gly Thr His Phe Ile Thr Ala Val Asp Leu Gly Gly
        190                 200                 210

CGC ATC TCG GTC CTT ACA GCC CTG CGT ACC TGT CAG CTG ACC CTG AAT GGG CTC ACA GCT GAT GAG GTA GGA GAC TGC CTG AAC GTG GAG  780
Arg Ile Ser Val Leu Thr Ala Leu Arg Thr Cys Gln Leu Thr Leu Asn Gly Leu Thr Ala Asp Glu Val Gly Asp Cys Leu Asn Val Glu
        220                 230                 240

GCC CAG GTC AGC ATC GGT GCC CAA GCC AGC GTC TCC AGT GAA TAC AAA GCT TGT GAG GAG AAG AAG AAA CAG CAC AAA ATG GCC ACC TCT  870
Ala Gln Val Ser Ile Gly Ala Gln Ala Ser Val Ser Ser Glu Tyr Lys Ala Cys Glu Glu Lys Lys Lys Gln His Lys Met Ala Thr Ser
        250                 260                 270

TTC CAC CAG ACC TAC CGT GAG CGT CAC GTC GAA GTA CTT GGT GGC CCT CTG GAC TCC ACG CAT GAT CTG CTC TTC GGG AAC CAA GCT ACA  960
Phe His Gln Thr Tyr Arg Glu Arg His Val Glu Val Leu Gly Gly Pro Leu Asp Ser Thr His Asp Leu Leu Phe Gly Asn Gln Ala Thr
        280                 290                 300

CCA GAG CAG TTC TCA ACC TGG ACA GCC TCA CTG CCC AGC AAC CCT GGT CTG GTG GAC TAC AGC CTG GAG CCC CTG CAC ACA TTA CTG GAA 1050
Pro Glu Gln Phe Ser Thr Trp Thr Ala Ser Leu Pro Ser Asn Pro Gly Leu Val Asp Tyr Ser Leu Glu Pro Leu His Thr Leu Leu Glu
        310                 320                 330

GAA CAG AAC CCG AAG CGG GAG GCT CTG AGA CAG GCT ATC AGC CAT TAT ATA ATG AGC AGA GCC CGG TGG CAG AAC TGT AGC AGG CCC TGC 1140
Glu Gln Asn Pro Lys Arg Glu Ala Leu Arg Gln Ala Ile Ser His Tyr Ile Met Ser Arg Ala Arg Trp Gln Asn Cys Ser Arg Pro Cys
        340                 350                 360

AGG TCA GGC CAG CAT AAG AGT AGC CAT GAT TCA TGC CAG TGT GAG TGC CAG GAT TCA AAG GTC ACC AAC CAG GAC TGC TGC CCA CGA CAG 1230
Arg Ser Gly Gln His Lys Ser Ser His Asp Ser Cys Gln Cys Glu Cys Gln Asp Ser Lys Val Thr Asn Gln Asp Cys Cys Pro Arg Gln
        370                 380                 390

AGG GGC TTG GCC CAT TTG GTG GTA AGC AAT TTC CGG GCA GAA CAT CTG TGG GGA GAC TAC ACC ACA GCT ACT GAT GCC TAC CTA AAG GTC 1320
Arg Gly Leu Ala His Leu Val Val Ser Asn Phe Arg Ala Glu His Leu Trp Gly Asp Tyr Thr Thr Ala Thr Asp Ala Tyr Leu Lys Val
        400                 410                 420

TTC TTT GGT GGC CAG GAG TTC AGG ACC GGT GTC GTG TGG AAC AAT AAC AAT CCC CGG TGG ACT GAC AAG ATG GAC TTT GAG AAT GTG CTC 1410
Phe Phe Gly Gly Gln Glu Phe Arg Thr Gly Val Val Trp Asn Asn Asn Asn Pro Arg Trp Thr Asp Lys Met Asp Phe Glu Asn Val Leu
        430                 440                 450

CTG TCC ACA GGG GGA CCC CTC AGG GTG CAG GTC TGG GAT GCC GAC TAC GGC TGG GAT GAT GAC CTT CTT GGT TCT TGT GAC AGG TCT CCC 1500
Leu Ser Thr Gly Gly Pro Leu Arg Val Gln Val Trp Asp Ala Asp Tyr Gly Trp Asp Asp Asp Leu Leu Gly Ser Cys Asp Arg Ser Pro
        460                 470                 480

CAC TCT GGT TTC CAT GAG GTG ACA TGT GAG CTA AAC CAC GGC AGG GTG AAA TTC TCC TAC CAT GCC AAG TGT CTG CCC CAT CTC ACT GGA 1590
His Ser Gly Phe His Glu Val Thr Cys Glu Leu Asn His Gly Arg Val Lys Phe Ser Tyr His Ala Lys Cys Leu Pro His Leu Thr Gly
        490                 500                 510

GGG ACC TGC CTG GAG TAT GCC CCC CAG GGG CTT CTG GGA GAT CCT CCA GGA AAC CGC AGT GGG GCT GTG TGG TAA  CATAATAACA ACAAT   1680
Gly Thr Cys Leu Glu Tyr Ala Pro Gln Gly Leu Leu Gly Asp Pro Pro Gly Asn Arg Ser Gly Ala Val Trp End
        520                 530

AACATGCCGG AGAGCTGGGT GTAGTAGCAC ACGCCTTTAA TCCCAGCATT TGGGAGGCAG AGACAGGTGG ATATCTATGA GTTCGAGGCC AGCCTGGGTC TACAGGGTCT 1790
CAAAAAAAAA  1800
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Figure 1. Nucleotide and deduced amino acid sequences of mouse PFP/perforin. The nucleotides of the sense strand are numbered in the 5′ to 3′ direction, as shown on the right side of the sequence. The 5′ non-coding sequence and the putative signal sequence are indicated by negative numbers. The nucleotide sequences corresponding to the oligonucleotide probes used for library screening are underlined. The amino acid sequences determined from the purified PFP are also underlined.

represent the authentic poly(A) tail of the PFP mRNA since the consensus polyadenylation signal (AATAAA) was not found upstream of this poly(A) tract. This suggests that the complete sequence of PFP transcript may extend much further downstream. In fact, a third PFP cDNA clone, PFP-9, has an insert of 2.9 kb, which is also the size of the message for PFP (Fig. 4); this clone is presently being sequenced. While this work was being completed, sequences for murine (17) and human (18) PFP/perforin were published. Our sequence was identical to that in ref. 17, with the exception of 2 single base changes at positions 8 and 686. The nucleotide change from T (ref. 17) to C at position 8 resulted in the a.a. change from methionine to threonine at a.a. position -18 while the other nucleotide substitution did not result in any a.a. change. Unlike the sequence shown in ref. 17, our sequence extends 194 bp upstream of the initiation codon and 126 bp downstream past the stop codon, followed by the long poly(A) stretch.

Alignment of the murine PFP sequence with the published sequence for human PFP (18) revealed 68% a.a. identity between the two forms, including conservation of all 20 cysteine residues (Fig. 2). Taking into account conservative amino acid substitutions, the homology between the two forms increased to 80%. In accord with previously published studies from this and other laboratories that have suggested structural and functional homologies between PFP and the terminal components of the complement (C) cascade (5-8), about 20% amino acid identity was found between PFP and C7 (19), C8 (20-22), and C9 (23,24), in a region covering 270 a.a. The homology was more prominently observed between positions 200 and 380 of the PFP sequence, where 7 out of 8 Cys residues are conserved in C9.

A Kyte-Doolittle hydropathic plot (Fig. 3) revealed that although PFP is predominantly hydrophilic, it contains a domain (a.a. 191-251) with strong hydrophobic tendency, flanked by highly hydrophilic stretches of a.a. on each side. This candidate membrane-spanning domain is actually long enough to cross the bilayer at least twice. Several other features of this region are noteworthy. The region between a.a. positions 189 and 218 (α_1 in Figs. 2 and 3) would be amphiphilic if it were to assume α helical configuration, making it a good candidate for a membrane spanning/pore-forming domain. C7, C8, and C9 present comparable amphipathic stretches which may serve the same function (19-25). Amino acid residues 237-257, contained between Cys-236 and Cys-258, on the other hand, would be highly amphiphilic if in β sheet configuration (β_1 in Figs. 2 and 3). This characteristic is well conserved in human PFP but is only partially conserved in C9 and C8, and not at all in C7. While β_1 is long enough to span the bilayer, this region may also represent a good candidate for a membrane-binding domain, especially since a cluster of positive charges follows β_1 (5 in the mouse sequence; 6 in the human). Two other amphiphilic

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                 ..          ..                                                        ..  ..
Mouse PFP  1 PCYTATRSEC KQKHKFVPGV WMAGEGMDVT TLRRSGSFPV NTQRFLRPDR TCTLCKNSLM
Human PFP  1 --H--A---- -RS------A -L----V--- S--------- D--------G -----E-A-Q

                                   *
          61 RDATQRLPVA ITHWRPHSSH CQRNVAAAKV HSTEGVAREA AANINNDWRV GLDVNPRPEA
             EGTL----L- L-N--AQG-G ---H-TR--- S---A---D- -RS-R---K- ----T-K-TS
                                                                β2
                                                *
         121 NMRASVAGSH SKVANFAAEK TYQDQYNFNS DTVECRMYSF RLVQKPPLHL DFKKALRALP
             -VHV------ -QA-----Q- -H----S-ST ------F--- HV-HT----P ---R--GD--

                                                       *           *
         181 RNFNSSTEHA YHRLISSYGT HFITAVDLGG RISVLTALRT CQLTLNGLTA DEVGDCLNVE
             HH--A--QP- -L----N--- ---R--E--- ---A------ -E-A-E---D N--E---T--
                        ______________α1________________                  ____
                              *
         241 AQVSIGAQAS VSSEYKACEE KKKQHKMATS FHQTYRERHV EVLGGPLDST HDLLFGNQAT
             ---N--IHG- I-A-A----- ---K---TA- ---------S --V--HHT-I N-----I--G
             ______β1_________
                                                                       *    *
         301 PEQFSTWTAS LPSNPGLVDY SLEPLHTLLE EQNPKREALR QAISHYIMSR ARWQNCSRPC
             ---Y-A-VN- V-GS------ T-----V--D S-D-R----- R-L-Q-LTD- ---RD-----

                          *  *  *          **
         361 RSGQHKSSHD SCQCECQDSK VTNQDCCPRQ RGLAHLVVSN FRAEHLWGDY TTATDAYLKV
             PP-RQ--PR- P---V-HG-A --T------- ----Q-E-TF IQ-WS----W F---F--V-L
                                                                  α2
                                                                       *
         421 FFGGQEFRTG VVWNNNNPRW TDKMDFENVL LSTGGPLRVQ VWDADYGWDD DLLGSCDRSP
             ------L--S T--D----I- SVRL--GD-- -A------L- ---Q-S-R-- ----T--QA-

                     *                   *             *
         481 HSGFHEVTCE LNHGRVKFSY HAKCLPHLTG GTCLEYAPQG LLGDPPGNRS GAVW 534
             K--S---R-N ----HL--R- --R-----G- ----D-V--M ---E------ ----
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Figure 2. Alignment of amino acid sequences of mouse and human PFP. Identical a.a. in the human sequence are not displayed (dashes). Cys are indicated by a *. The region of high hydrophobicity (191-251) and the candidate amphiphilic α or β domains are underlined.

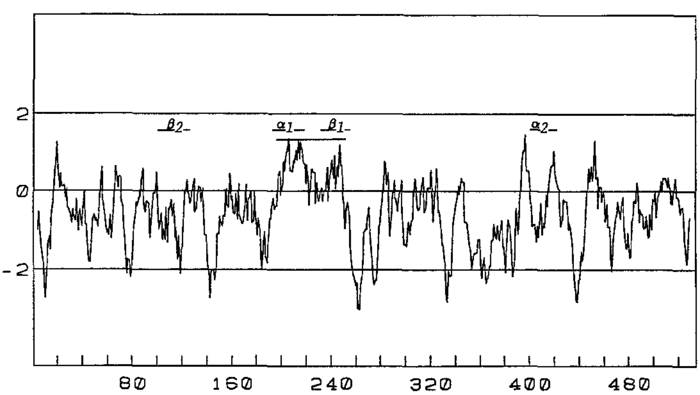

Figure 3. Kyte and Doolittle hydrophathy analysis of mouse PFP using a window of 9 a.a. Hydropathy values are plotted as a function of residue position in the sequence. Positive values are hydrophobic. The region of high hydrophobicity and the candidate α helical and β sheet domains are marked (see text for details).

stretches large enough to span the membrane have been designated α_2 (403-422) and β_2 (100-126). β_2 is conserved in C8α, C8β and C9 while α_2 is absent from C7, C8 and C9. It should be noted that amphiphilic β structures have been shown to have strong self-aggregation tendency (26), suggesting a similar role in PFP polymerization for the two long β stretches described here.

There is some accumulation of negative charges in the carboxyl terminus, as noted earlier by Stanley and Luzio (27), ranging between Asp-442 and Asp-524. Most of these negatively charged residues are shared by PFP/perforin of both species.

The cysteine residues can be distributed broadly into four regions. (1) The amino terminus (a.a. 1-81), which shows virtually no homology with the C family, contains 5 sparsely distributed Cys. (2) The second region (a.a. 356-387), also referred to as EGF precursor-like domain, contains 7 Cys that are well conserved in several other proteins, including C7, C8 and C9, von Willebrand factor, coagulation factor XII and keratin. This region may be responsible, at least in part, for the immunological cross-reactivity observed earlier between mouse PFP and human C7, C8 and C9---this cross-reactivity is manifested only when these proteins are disulfide bond-reduced (5,8). (3) The third region (a.a. 476-513) contains 4 Cys residues that are not present in the C family. (4) The remaining 4 Cys are between a.a. 155 and 258, with Cys-236 and Cys-258, but not Cys-155 and Cys-221, being conserved in the C family. Cys-236 and Cys-258 are inside the region of high hydrophobicity mentioned earlier.

Domains with putative calcium-binding function are not clearly evident. However, in analogy with zinc-binding proteins such as methallothionein (28), at least two candidate zinc-binding fingers involving Cys-rich domains are observed in murine PFP: a.a. 57-77 and 356-376. The putative zinc-binding finger 57-77 is not conserved in the human sequence since residues 73 and 76 have not conserved the key a.a. (His) seen in the murine sequence. Another candidate calcium-binding region lies around Cys-236 and Cys-258 (β_1 segment), both of which are flanked by clusters of negative charges. This characteristic is conserved in the human PFP species but not in the C family.

Northern Blot Analysis. A preliminary survey of cell types expressing PFP/perforin by Northern blot analysis revealed that a single 2.9 kb mRNA was present in some but not all CTL lines and was absent from non-CTL lines (Fig. 4a shows a representative blot). The expression of PFP mRNA can be modulated with various kinds of stimuli. As shown in Fig. 4b, unstimulated murine CTLL-L3 cells contain PFP mRNA, as expected, since these cells are maintained in leukocyte-conditioned medium (a source of T cell growth factors). Upon antigen receptor stimulation with a monoclonal antibody, PFP mRNA levels rapidly increase by 4.5 fold over a course of 24 hours (Table 1). In marked contrast, a serine esterase mRNA was expressed at high level in unstimulated L3 cells and

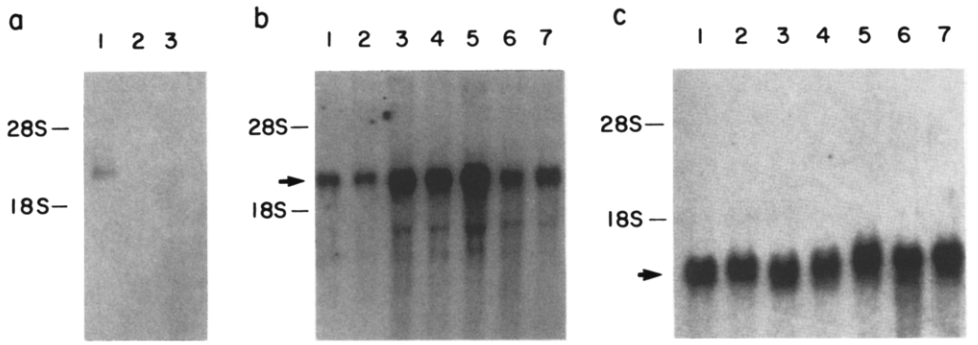

Figure 4. Northern blot analysis of murine PFP/perforin. (a) Expression of PFP RNA was assessed in various cell lines using PFP-6 as probe. Lanes contain total cellular RNA of CTLL-R8 (lane 1, 5 μg), P815 (lane 2, 10 μg) and YAC-1 (lane 3, 10 μg); the autoradiogram was exposed for 48 h. (b,c) PFP and a serine esterase (HF gene) mRNA expression in cloned CTLL-L3. L3 cells were stimulated with clonotypic anti-TcR mAb 384.5 for 0, 0.5, 6, 12 or 24 h (lanes 1-5), or with Con A (lane 6) for 6 h, or with rIL-2 for 6 h (lane 7). Ten μg of total RNA was fractionated in each lane. Same blot was hybridized against [^{32}P]-labeled PFP-6 (b) and L3G10#6 (c) successively. Positions of 28S and 18S rRNA markers are each indicated. An arrow indicates the specific hybridization signal. The autoradiograms in (b) and (c) were exposed for 48 h.

the level of expression was not affected by antigen receptor-stimulation (Fig. 4c and Table 1). On the other hand, Con A (lane 6, Fig. 4b and c) and exogenously added IL-2 (lane 7) produced little effect on the basal levels of either PFP or serine esterase mRNA (see also Table 1). Thus, although perforin and serine esterase are thought to be contained in and released from the same cytoplasmic granules, their mRNA levels appear to be regulated independently. The availability of specific probes for PFP and serine esterases should allow detailed future studies on the regulation of these important genes.

Table I. Expression of PFP and serine esterase transcripts in stimulated CTLL-L3

Stimulant	Time (h)	Relative optical density*	
		PFP-6	Serine esterase
Anti-TCR mAb 384.5	0	1	1
	0.5	0.66	0.96
	6	3.34	1.06
	12	2.92	0.87
	24	4.71	1.40
Con A	6	1.51	1.38
rIL-2	6	1.68	1.46

* The autoradiograms shown in Fig. 4b and c were scanned with a video densitometer (Bio-Rad, model 620). The optical density for 0 time was taken to equal unity.

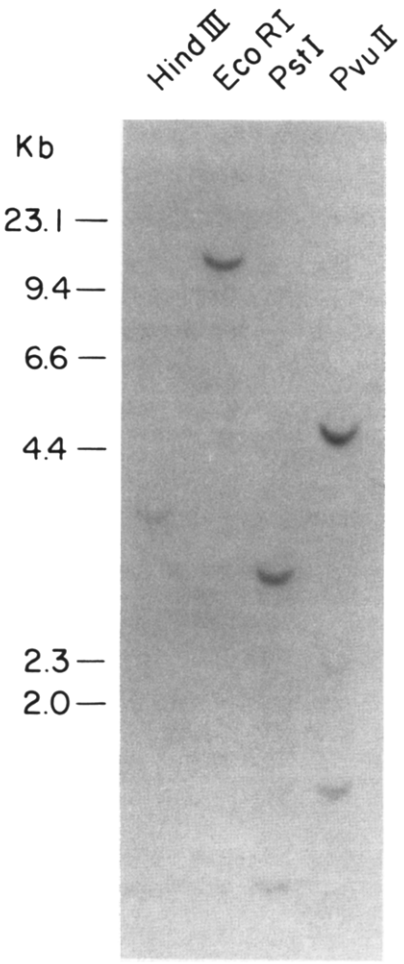

Figure 5. Southern blot analysis of murine PFP/perforin. Each lane contains 15 μg of mouse genomic DNA digested with the indicated restriction endonuclease. Hybridization was carried out with labeled PFP-6. Autoradiography was performed for 48 h.

Southern Blot Analysis. Southern blot analysis of mouse genomic DNA with PFP-6 cDNA (Fig. 5) showed that murine PFP is encoded in a single locus in the mouse haploid genome. PFP coding sequences were contained within 10 kb of genomic DNA.

Conclusions. Although the sequence of PFP/perforin suggests that it represents a member of the C family that includes C7, C8 and C9, as previously speculated (5-8), there are some important differences between PFP and the C components. PFP must undergo a conformation change in the presence of calcium that allows it to bind to, insert into, and polymerize in the target membrane. That is, PFP pores are formed by a homopolymer, in contrast to the heteropolymeric complex of C5b-6, C7, C8 and C9, which together form the C channel (reviewed in ref. 4). This important difference between the two immune

effector systems also implies that PFP should display domain-specific functions that are unique to this protein species.

A better understanding of the structure of PFP/perforin has not resolved several controversial issues related to lymphocyte-mediated killing. It is not clear, for example, whether PFP and granule exocytosis are absolutely required for all forms of lymphocyte-mediated killing. The development of probes and specific antibodies (9) should now enable a rigorous assessment of the distribution and regulation of this molecule in cell-mediated killing.

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