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Immunology

Murine plasma cell membrane antigen PC-1: Molecular cloning of cDNA and analysis of expression

(gene expression/randomly primed cDNA library/genetic polymorphism)

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ABSTRACT **PC-1 is a membrane glycoprotein expressed selectively on murine antibody-secreting plasma cells. Previously, we have obtained partial amino acid sequence data for this protein. Here, we describe the use of these data in the isolation of PC-1 cDNA clones. To avoid the "3′-bias" of conventional cDNA libraries we constructed a cDNA library in λgt10 by priming the first strand of cDNA synthesis with random hexadeoxynucleotides. The library was screened with oligonucleotide probes, 17 nucleotides in length, the design of which was based on the amino acid sequence data. Two cDNA clones of 1.0 and 0.9 kilobase pairs (λRR3 and λRR20, respectively) were isolated. Both contained a sequence encoding the 15-residue tryptic peptide that was used to derive the sequences of the oligonucleotide probes. λRR3 cDNA hybridized to an ≈3.5-kilobase mRNA that was present in plasmacytomas, spleen, and liver but not in other cell types screened. We were unable to detect PC-1 mRNA or protein in mouse brain. In the spleens of mice chronically infected with *Mesocestoides corti*, PC-1 mRNA was present at 2.5-fold higher levels than found in normal mouse spleens, whereas immunoglobulin mRNA levels were 15-fold higher. Southern blot analyses revealed the presence of only one gene copy per haploid mouse genome. Restriction fragment length polymorphisms were detected in genomic DNA from mice bearing different PC-1 alleles. A related gene is present in rat genomic DNA.**

The molecular changes that cause and accompany the development of B lymphocytes into antibody-secreting cells remain to be fully elucidated. The plasma cell antigen PC-1 is the only murine lymphocyte protein known to be expressed exclusively on the surface of terminally differentiated B lymphocytes (1, 2), but as yet its function is unknown. We are currently investigating the structure of PC-1 (2–4) in an attempt to gain some indication of its role in the final stage of B-cell maturation. At present, it is plausible that PC-1 is involved in the secretory mechanism (2), is a receptor for a lymphokine needed for B-cell growth or differentiation, or has some other recognition function. The primary structures of several receptors (5–15), recognition molecules (16, 17), and other cell surface proteins (18–20) have been determined. Comparison of the PC-1 amino acid and nucleotide sequences with sequences of known function may yield valuable information.

Biochemical analysis has demonstrated that PC-1 is an acidic disulfide-bonded dimeric membrane protein with indistinguishable subunits of relative molecular mass (M_r) 120,000 (2–4, 21). Thus PC-1 is one of only three known disulfide-bonded homodimeric proteins, the others being the transferrin receptor (22–24) and the T8 antigen (17). PC-1 homologues have been identified in the rat and hamster (4). Indirect evidence for PC-1 being found in the brain, liver, and kidney has been reported (1, 4, 25).

We have previously purified PC-1 to homogeneity and determined the amino acid sequence of seven tryptic peptides (3). The amino acid sequence data have now been employed to isolate PC-1 cDNA clones from a randomly primed cDNA library that was constructed to overcome the "3′-bias" of conventional cDNA libraries. The cDNA clones were used to investigate PC-1 mRNA expression in various cell lines and tissues and the induction of this mRNA in splenocytes induced to differentiate into antibody-forming cells *in vivo* by chronic parasite infection. Preliminary investigations into the organization of PC-1 genomic DNA were also undertaken.

MATERIALS AND METHODS

Tissue Culture. Cell lines were maintained in exponential growth in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and 50 μM 2-mercaptoethanol.

Isolation of RNA. Total cellular RNA was isolated from splenocytes by the guanidine thiocyanate/lithium chloride method (26). Cytoplasmic poly(A)$^+$ RNA was isolated from cultured cell lines as follows. Cells (10^9) were lysed in 20 ml of 10 mM Tris·HCl, pH 8.5/0.14 M NaCl/1.5 mM $MgCl_2$/1.0% Triton X-100 for 15 min on ice. Cellular debris was removed by centrifugation at 800 × *g* for 15 min and the supernatant was made up to 50 ml and 0.1 M Tris·HCl at pH 7.5, 12.5 mM EDTA, 0.4 M NaCl, 0.4% Triton X-100, 2% $NaDodSO_4$ and 200 μg of proteinase K (Merck) per ml with water and concentrated stock solutions. After incubation at 37°C for 1 hr, ≈1 ml of hydrated oligo(dT)-cellulose (Collaborative Research, Waltham, MA) was added and the mixture was agitated for 4 hr. The oligo(dT)-cellulose was washed and poly(A)$^+$ RNA was eluted as described (27). To isolate total cellular poly(A)$^+$ RNA from brain and liver, tissues were homogenized in 10 mM Tris·HCl, pH 7.5/0.1 M NaCl/1 mM EDTA/0.5% $NaDodSO_4$/containing proteinase K at 200 μg/ml. The rest of the procedure was as above.

Construction of cDNA Library. Cytoplasmic poly(A)$^+$ RNA was extracted from the plasmacytoma NS-1. Single-stranded cDNA was synthesized by avian myeloblastosis virus reverse transcriptase (Life Sciences, St. Petersburg, FL), using the reaction conditions of Huynh et al. (28) except that in place of oligo(dT), hexadeoxynucleotides generated by DNase I digestion of calf thymus DNA (Pharmacia) were used at 0.2 mg/ml as primer. The optimal concentration of hexadeoxynucleotides was determined in preliminary experiments (see *Results*). The following procedures were performed as described by Huynh *et al.* (28). After hydrolysis of the RNA template and Sephadex G-50 chromatography to separate unincorporated nucleotides and hexadeoxynucleo-

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Abbreviation: kbp, kilobase pair(s).

tides from cDNA, double-stranded cDNA was synthesized by "self-priming" using the Klenow fragment of *Escherichia coli* DNA polymerase I (New England Nuclear). The resulting hairpin loop was cleaved with mung bean nuclease (Pharmacia) and the ends were made flush by the Klenow fragment of *E. coli* DNA polymerase I. After methylation with *Eco*RI methylase (New England Biolabs), *Eco*RI linkers (New England Biolabs) were ligated to the cDNA by using T4 DNA ligase (New England Biolabs) and digested with *Eco*RI (Boehringer Mannheim). The material was size-fractionated by electrophoresis in 1% agarose and size pools of 0.6–1.0 kilobase pairs (kbp) and 1.0–6.0 kbp were collected by using DEAE-paper (NA45, Schleicher & Schull). The two size pools were ligated into the imm434 *Eco*RI insertion vector λgt10 (1 μg per pool). Ligated DNA was packaged *in vitro* by using extracts supplied by Promega Biotec (Madison, WI). Phage were plated onto *E. coli* RY1073 (29). The smaller size pool contained 10^5 clones and the larger contained 5×10^4 clones.

Oligonucleotide Probes. Oligodeoxynucleotides were synthesized by the phosphoramidite method (30), using an Applied Biosystems (Foster City, CA) model 380A automated DNA synthesizer, and were purified as described (7). The 5′-OH groups of the oligonucleotides were radiophosphorylated by using T4 polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP (Amersham). The efficiency of the phosphorylation reaction was assessed by separation of labeled oligonucleotides from unincorporated nucleotides by Sephadex G-25 chromatography. The specific activity of the labeled oligonucleotides was ≈100 μCi/μg (1 Ci = 37 GBq). Probes were used to screen the cDNA library as previously described (7). It was found to be essential to screen each plate in duplicate to eliminate artifactual spots.

Blot Hybridization of RNA. To estimate the M_r of RNA, samples of poly(A)$^+$ RNA were denatured with formamide/formaldehyde and electrophoresed in 1% agarose gels containing formaldehyde (31). RNA was transferred to nitrocellulose by the method of Thomas (32). The filter-bound RNA was prehybridized and then hybridized to cDNA radiolabeled by the "oligo labeling" method of Feinberg and Vogelstein (33) (specific activity ≈500 μCi/μg) for 16 hr at 65°C in 0.6 M NaCl/0.06 M sodium phosphate, pH 7.6/6 mM EDTA/0.02% polyvinylpyrrolidone/0.02% Ficoll 400/0.02% bovine serum albumin/0.1% NaDodSO$_4$. Filters were washed for 30 min at room temperature in 0.3 M NaCl/0.03 M sodium citrate/0.1% NaDodSO$_4$ and then for 1 hr in the same buffer at 65°C. After drying, filters were exposed to Kodak XAR-5 film or Fuji RX film, using DuPont Cronex intensifying screens at −70°C. To quantitate mRNA, dilutions of total cellular RNA were denatured in 100 μl of 1.5 M NaCl/0.15 M sodium citrate/7.5% (vol/vol) formaldehyde for 15 min at 60°C, blotted onto nitrocellulose that was equilibrated in 3 M NaCl/0.3 M sodium citrate, dried, and hybridized as above. The final wash conditions were 15 mM NaCl/1.5 mM sodium citrate/0.1% NaDodSO$_4$ at 65°C for 1 hr. Films were exposed without intensifying screens to ensure linearity of signal-to-radioactivity ratios.

Blot Hybridization of DNA. DNA was isolated, digested to completion with restriction enzymes, size-fractionated by agarose gel electrophoresis, blotted onto nitrocellulose, and analyzed as described (31).

Blot Analysis of Proteins. Glycoproteins were isolated from detergent-solubilized extracts by using concanavalin A-agarose and separated in NaDodSO$_4$/polyacrylamide gels. Proteins were electroblotted onto nitrocellulose (34) and probed with a rabbit anti-PC-1 antiserum (4) and radioiodinated staphylococcal protein A.

DNA Sequencing. DNA was digested with *Sau*3AI and subcloned in the M13 vector mp9. The dideoxy chain termination sequencing method of Sanger *et al.* (35) was then employed.

RESULTS

Construction and Screening of cDNA Library. To overcome the 3′-bias of libraries primed with oligo(dT), a cDNA library was constructed by priming the first strand of cDNA synthesis with a random mixture of hexadeoxynucleotides. The optimal concentration of hexadeoxynucleotides was determined by varying the concentration of this reagent while keeping the concentration of the other reagents in the mixture constant. The incorporation of tracer ^{32}P-labeled nucleotides into trichloroacetic acid-precipitable material and the lengths of the reverse transcripts were then analyzed. With increasing concentration of hexadeoxynucleotides, the mass synthesized increased but the length of cDNAs decreased. We chose the highest concentration of hexadeoxynucleotides that gave transcripts comparable in length to cDNAs synthesized by oligo(dT) priming. Under these conditions, the mass of cDNA synthesized was ≈25% of the amount primed by oligo(dT). It was essential to completely separate hexadeoxynucleotide from single-stranded cDNA before the synthesis of the second strand, because very low concentrations of the primer will prime the second strand synthesis reaction with an efficiency comparable to that of the "self-priming" reaction, resulting in rather short double-stranded cDNA.

The 10^5 clones of the smaller size pool (0.6–1.0 kbp) were screened with two mixtures of oligodeoxynucleotides whose sequences were derived from the amino acid sequence of a tryptic peptide of PC-1 [peptide 1 (3); see Fig. 1]. Both probes cover the same region of the peptide but differ in the leucine codons. Four plaques hybridized to the probe A mixture only, while 14 hybridized to the probe B mixture only, and 2 hybridized to both. The inserts that hybridize to both probe mixtures probably did so by G→T base pairing. The sequences GA$\begin{matrix}T\\C\end{matrix}$ would hybridize to the leucine codons of both mixtures.

To determine which of these clones was likely to encode PC-1, "oligo-labeled" (33) inserts of the phage that hybridized to the oligonucleotides were used to probe poly(A)$^+$ RNA derived from various cell lines known to express or not to express PC-1. Two phages, λRR3 and λRR20, containing inserts of 1.0 and 0.9 kbp, respectively, hybridized to an RNA with sufficient size and the expected cellular distribution for a PC-1 mRNA (see below). Both phage hybridized to probe A but not to probe B, and the inserts hybridized to each other.

To confirm that these cDNA molecules were derived from PC-1 mRNA, we sequenced a 401-bp *Sau*3AI restriction fragment of λRR3 that hybridized to probe A. This fragment contained an open reading frame encoding a peptide nearly

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PEPTIDE:          AlaGluTyrLeuHisThrTrpGlyGlyLeuLeuProValIleIleLys

PROBE A:       5′-GCNGA(A/G)TA(T/C)TT(A/G)CA(T/C)AC-3′

PROBE B:       5′-GCNGA(A/G)TA(T/C)CTNCA(T/C)AC-3′

cDNA:       5′-AGAGCTGAGTATTTGCACACCTGGGGTGGACTTCTTCCTGTCATTAGCAAG-3′

PROTEIN:    ...ArgAlaGluTyrLeuHisThrTrpGlyGlyLeuLeuProValIleSerLys...
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FIG. 1. Design of oligonucleotide probes and cDNA sequence. The top line is the amino acid sequence of a PC-1 tryptic peptide (3). The next two lines give the sequence of the two oligonucleotide probes (N designates any of the four nucleotides) and the nucleotides that differ between the probes are underlined. The fourth line is the nucleotide sequence of a portion of λRR3 and the last line is the predicted amino acid sequence. The sequences in lines 1 and 5 are identical except for the residue underlined in line 5.

identical to the tryptic peptide used to predict the oligonucleotide sequences (see Fig. 1). The only discrepancy was that the penultimate residue (residue 14) of the peptide was tentatively assigned as isoleucine, while the nucleotide sequence predicts serine. Serine residues give poor signals after Edman degradation. The preceding residue (residue 13) is isoleucine, and it is probable that residue 14 was incorrectly assigned due to "carry-over" of the previous residue. The nucleotide sequence predicts an arginine residue preceding the peptide, consistent with the specificity of trypsin.

On the basis of the sequence data and the distribution and size of the mRNA (see below), we conclude that λRR3 and λRR20 contain cDNA sequences derived from PC-1 mRNA.

Analysis of PC-1 Protein and mRNA Expression. The results of hybridizing oligo-labeled RR3 to size-fractionated poly(A)$^+$ RNA from various cell lines and tissues are shown in Fig. 2. The probe recognizes an ≈3.5-kilobase RNA present in RNA samples from the plasmacytomas NS-1 (36) and C1.18 (37) and from mouse liver. No hybridization was detectable to poly(A)$^+$ RNA from the B-lymphoma WEHI-231 (38), T-lymphomas EL-4 (39) and TIKAUT (40), mast cell line 32D (41), mouse brain, or poly(A)$^-$ RNA from NS-1.

These data agree with the known tissue distribution of PC-1 protein (1, 4), except that PC-1 has been reported to be present in the brain (1, 18). In an attempt to examine directly the presence of PC-1 protein in the brain, size-fractionated glycoproteins were blotted onto nitrocellulose and probed with a polyclonal antiserum against PC-1 (4). Results are shown in Fig. 3. PC-1 was relatively abundant in extracts of NS-1 plasmacytoma cells. The PC-1 protein was present in lower quantities in mouse spleen and liver but was not detectable in WEHI-231 or mouse brain extracts.

Increased Levels of PC-1 in *Mesocestoides corti*-Infected Mice. Mice that are chronically infected with larva of the cestode *Mesocestoides corti* have very high levels of serum IgG1 due to elevated numbers of plasma cells (42). As PC-1 is an antigenic marker for this cell type, the levels of PC-1 protein and mRNA should also be increased. To test this hypothesis, total cellular RNA was isolated from spleens of normal BALB/c mice and BALB/c mice infected 4 months previously with *M. corti* and "dot-blotted" onto nitrocellulose. The filters were probed with either oligo-labeled RR3 or an immunoglobulin γ1-chain cDNA (43), and relative levels of hybridization were determined by densitometer scanning.

Electrophoresis of serum from the infected mice confirmed that they had abnormally high levels of serum immunoglobulin (not shown). The amounts of γ1 and PC-1 mRNA per unit mass of total cellular RNA were induced 15.5-fold and 2.5-fold, respectively, over levels found in normal mice.

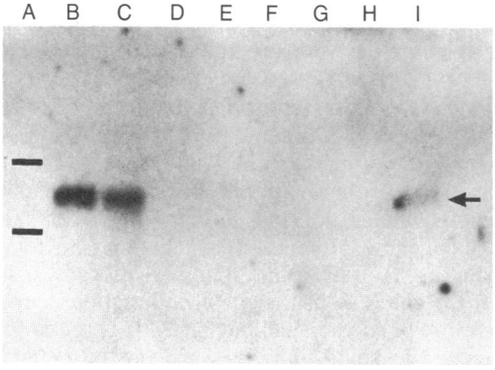

FIG. 2. Hybridization of ^{32}P-labeled RR3 to RNA. RNA (3 μg) was electrophoresed in agarose, transferred to nitrocellulose, and probed with oligo-labeled RR3 cDNA. Lane A, poly(A)$^-$ RNA from NS-1. Lanes B–I, poly(A)$^+$ RNA from NS-1 (lane B), C1.18 (lane C), WEHI-231 (lane D), TIKAUT (lane E), EL-4.1 (lane F), 32D (lane G), BALB/c mouse brain (lane H), and BALB/c mouse liver (lane I). The arrow indicates the band in lane I. The bars indicate the migration of the 28S and 18S ribosomal RNAs.

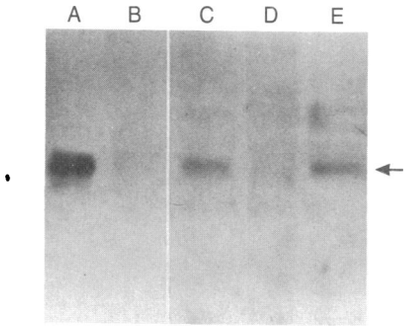

FIG. 3. Immunoblot analysis of PC-1 protein. Glycoproteins were isolated with concanavalin A-agarose from 5 × 10^6 NS-1 cells (lane A), 5 × 10^6 WEHI-231 cells (lane B), 0.1 g of BALB/c mouse spleen (lane C), 0.13 g of BALB/c mouse brain (lane D), and 0.13 g of BALB/c mouse spleen (lane E) and electrophoresed in $NaDodSO_4$/polyacrylamide gels. Proteins were transferred to nitrocellulose and the filters were probed with a rabbit anti-PC-1 antiserum (4) and ^{125}I-labeled protein A. The arrow indicates the migration of purified PC-1 electrophoresed in the same gel.

Analysis of Genomic DNA. DNAs from the BALB/c (*PC-1^a* allele) plasmacytomas NS-1 and ABPC 20, BALB/c embryo, (C57BL/6 × DBA/2)F_1 (*PC-1^b* allele) mouse liver, rat liver, and human peripheral blood lymphocytes were digested with restriction enzymes, and fragments were examined by Southern blot analyses (Fig. 4). Several fragments hybridized at high stringency to the complete RR3 cDNA in the mouse and rat DNA digests. In contrast, analysis of human DNA revealed a heterogeneous smear, indicating hybridization

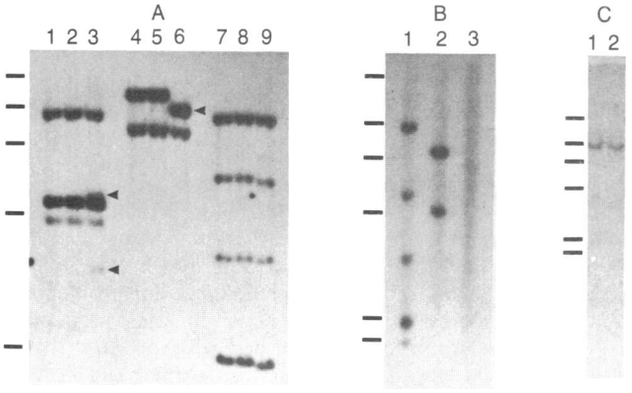

FIG. 4. Analysis of PC-1 genomic DNA. (*A*) Genomic DNA (5 μg) from NS-1 cells (lanes 1, 4, and 7), BALB/c embryo (lanes 2, 5, and 8), or (C57BL/6 × DBA/2)F_1 mouse liver (lanes 3, 6, and 9) were digested with *Bam*HI (lanes 1–3), *Pst* I (lanes 4–6), or *Eco*RI (lanes 7–9), size-fractionated in 0.7% agarose, and blotted onto nitrocellulose. The filter was probed with oligo-labeled RR3 cDNA and washed at high stringency. Arrowheads indicate fragments unique to the (C57BL/6 × DBA/2)F_1 mouse DNA. Horizontal bars on the left designate the migration of *Hin*dIII-digested phage λ DNA standards; 23.1, 9.4, 6.6, 4.4, and 2.3 kbp. (*B*) Genomic DNA (15 μg) from BALB/c embryo (lane 1), rat liver (lane 2), or human peripheral blood lymphocytes (lane 3) was digested with *Eco*RI, fractionated in 1.0% agarose, and blotted onto nitrocellulose. The filter was probed with oligo-labeled RR3 cDNA and washed at high stringency. Molecular weight markers are as in *A*, except a 2.0-kbp fragment is also shown. (*C*) Genomic DNA (5 μg) from ABPC 20 cells (lane 1) or (C57BL/6 × DBA/2)F_1 mouse liver (lane 2) was digested with *Eco*RI and fractionated in 1.0% agarose and blotted onto nitrocellulose. The filter was probed with an oligo-labeled 401-bp *Sau*3AI fragment of RR3 and washed at high stringency. The molecular weight markers are as in *B*.

with a repetitive sequence. Restriction fragment length polymorphism between the two different mouse strains bearing different *PC-1* alleles is visible in the *Bam*HI and *Pst* I digests. In the *Bam*HI case, the 2.4- and 2.85-kbp BALB/c DNA fragments were replaced by 3.1- and 4.6-kbp fragments in the (C57BL/6 × DBA/2)F_1 DNA. The *Pst* I polymorphism involves the replacement of the 11-kbp BALB/c DNA fragment by a 9.2-kbp fragment.

The 401-bp *Sau*3AI cDNA fragment of RR3 hybridized only with the 8.6-kbp *Eco*RI fragment of ABPC 20 and the hybrid mouse liver DNA, suggesting the presence of a single *PC-1* gene in the haploid mouse genome (Fig. 4*C*).

DISCUSSION

Potential PC-1 cDNAs were initially identified by their ability to hybridize at low stringency to short oligonucleotide probes, whose sequences were based on protein sequence data (3). Phages λRR3 and λRR20 would appear to contain PC-1 cDNAs, as judged by several criteria. The mRNA recognized by the cDNAs is of sufficient size to encode PC-1, is a low-abundance species (only two PC-1 cDNAs were present in a library of 10^5 recombinant clones), and is present only in the cell lines or tissue found to express the protein. In addition, the cDNAs contain a sequence that predicts the 15-residue PC-1 tryptic peptide [peptide 1 (3)] used to design the oligonucleotides. This tryptic peptide was generated from protein purified by a procedure that utilized a monoclonal anti-PC-1 antibody, IR518 (3). Monospecific rabbit antisera raised against the purified protein recognized the same protein as the IR518 antibody (4), which is evidence that the protein sequenced was PC-1. The purity of the protein sequenced was rigorously monitored (3) and the yield of peptide 1 was high (unpublished data). Hence it is unlikely that this amino acid sequence was derived from a minor contaminant in the PC-1 preparation.

Screening cDNA libraries with oligonucleotide probes has been successfully employed to isolate the genes encoding a number of cell surface proteins (5, 7, 8, 10, 11, 14, 15, 20, 44). The mRNAs encoding these proteins often possess long 3′-untranslated regions (5, 6, 8, 10, 13, 17). Even in cases in which the mRNA does not possess a long 3′ untranslated region, 3′ sequences are often over-represented in libraries primed with oligo(dT), because of the inherent inefficiency of reverse transcription *in vitro*. A probe that recognizes the coding region may therefore detect many fewer clones than expected on the basis of mRNA abundance. The problem of 3′-bias is exacerbated if the mRNA contains particular sequences that are reverse-transcribed with low efficiency, or if N-terminal amino acid sequences are used to design oligonucleotide probes. The location of tryptic peptides is often not known.

To circumvent these potential problems, we constructed a cDNA library in which reverse transcription was primed randomly along mRNA molecules. Providing that the library is of sufficient size, all RNA sequences should be represented with approximately equal probability. This approach may also facilitate the isolation of overlapping clones encoding entire mRNA species, a task that may be difficult or impossible for long mRNAs when oligo(dT)-primed libraries are used (5, 6). Our work and also a recent paper by Ebina *et al.* (45) shows that a randomly primed library can be successfully employed to isolate clones of rare mRNA species.

The PC-1 mRNA was found to be ≈3.5 kilobases in length. There was, in general, a good correlation between the protein and mRNA concentrations in various tissues, but we could not detect PC-1 protein or mRNA in mouse brain, which is in disagreement with previous reports. However, the evidence for the existence of PC-1 in the brain is indirect, because it is based only on antibody absorption studies (1) and biochemical analysis of a neuroblastoma cell line (21). Our failure to demonstrate directly the existence of brain PC-1 raises the possibility that PC-1 may not be present in that tissue. Alternatively, the level of PC-1 in the brain may be below the limits of detectability of the methods employed here.

The level of γ1 mRNA present in the spleens of mice chronically infected with *M. corti* was greater than 15-fold higher than the levels found in normal mice. If this increase in immunoglobulin mRNA were the result of a corresponding increase in antibody-forming cells, as has been suggested (42), and all these cells possess amounts of PC-1 similar to the amount found in normal mice, then a corresponding increase in PC-1 levels would have been expected. In fact, we found that PC-1 mRNA levels in infected mouse spleens were only 2.5-fold higher than in normal mice. If we assume that the mRNA levels reflect protein levels, then it would appear possible that PC-1 is not expressed uniformly on plasma cells *in vivo*, or that its expression on plasmacytoma cells is greater than that of normal plasma cells (10).

The investigation of PC-1 in normal tissues has proven very difficult due to the low levels of PC-1 found *in vivo*. We could not observe a significant population of $PC\text{-}1^+$ cells when normal mouse spleen cells or spleen cells mitogenically stimulated *in vitro* were analyzed by using the fluorescence-activated cell sorter after staining with directly fluoresceinated antibody (unpublished observation). In contrast, plasmacytomas (3) and a neuroblastoma cell line (unpublished observation) express relatively large amounts of this antigen (≈10^5 subunits per cell). The high expression of PC-1 in the plasmacytomas studied here does not appear to be due to gross changes in chromosomal DNA, because only one copy of the gene encoding PC-1 was observed in both plasmacytoma DNA and germ-line DNA, and no rearrangement of PC-1 DNA was observed. The possibility of more subtle changes has not been ruled out.

We have previously identified a rat protein that is present on the surface of antibody-secreting cells and is highly homologous to murine PC-1 (4). Our finding that a rat gene hybridizes to a murine PC-1 cDNA strengthens the argument that rats possess a homologue of PC-1. A similar protein is also present on antibody-secreting cells in the hamster (4). As yet there is no evidence for a human PC-1 homologue. Attempts to find an homologous gene in human DNA were not successful because RR3 cDNA hybridizes to a repetitive sequence in human DNA. Alternative hybridization strategies may overcome this problem.

The cDNA clones described here will facilitate the isolation of further cDNAs that span the entire PC-1 mRNA and genomic DNA. A computer search has so far failed to identify convincing homology with any known protein (3), but determination of the complete sequence will be necessary before this point can be addressed in detail. Recently, it has been discovered that the receptor for low density lipoprotein is made up of a number of domains, some of which are encoded by exons derived from the genes of other proteins (46, 47). These exon-encoded domains probably have similar functions, although the proteins in which they are found are involved in quite different physiological processes. Examination of the genes of proteins whose function is unknown may therefore yield valuable data. If PC-1 is found to share exon-encoded domains with other proteins of defined function, the design of further experiments to elucidate the role of PC-1 in the final stage of B-cell development may be greatly simplified.

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