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Immunology

Isolation of coordinately regulated genes that are expressed in discrete stages of B-cell development

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ABSTRACT **We have utilized subtractive hybridization to isolate 16 distinct cDNA sequences representing genes expressed in pre-B-cell lines but not myeloma cell or fibroblast lines. These sequences represent RNA transcripts that vary in abundance in pre-B-cell lines from 0.001% to 0.05%. Five of these sequences were not related to any known genes. One was related to but distinct from known myosin regulatory light chain genes and another encoded a protein with lectin domains. Three represented previously identified genes encoding carbonic anhydrase type II, thymosin, and CD2; these genes were not previously known to be specifically expressed in early stages of B-cell development. Other isolated genes corresponded to pre-B-cell-specific or pre-B-cell/B cell-specific genes recently described by others. The isolated cDNA sequences may be divided into two general categories—those representing genes expressed only in the pre-B-cell stage of B-cell development and those expressed in both the pre-B-cell and B-cell stages. The *in vivo* expression patterns of the identified genes suggest that some function specifically in lymphocytes while others may have roles in additional lineages.**

Differentiation of B-lineage cells occurs in three general stages—the precursor B-cell (pre-B-cell) stage, the B-lymphocyte stage, and the plasma-cell stage (1). In mammals, pre-B-cell development occurs in the liver of the fetus and shifts to bone marrow in adults. Pre-B-cells first assemble heavy (H) chain variable (V) region genes from component variable (V_H) diversity (D), and joining (J_H) gene segments and subsequently assemble light (L) chain V region genes from V_L and J_L segments (2). Expression of H and L chains by pre-B-cells leads to the generation of B lymphocytes, which express complete immunoglobulin molecules on their surface (3). B lymphocytes migrate to peripheral lymphoid organs, such as the spleen and lymph nodes (1). Binding of specific antigen to the membrane immunoglobulin can induce a B lymphocyte to proliferate and mature, ultimately generating terminally differentiated, immunoglobulin-secreting plasma cells. T lymphocytes differentiate by a program somewhat analogous to that of B cells; in particular, genes encoding T-cell receptor (TCR) V regions also are assembled in a programmed fashion in pre-T-cells (4). Assembly of immunoglobulin and TCR V region genes is mediated by a shared recombination activity [*V–D–J* recombinase (5)]; recently, a *V–D–J* recombination-activating gene called RAG-1 was isolated and found to be expressed specifically in pre-B- and pre-T-cells (6).

Staging of B-lineage cells often is based on histological analyses, expression of lineage or stage-specific antigens (1, 7, 8), the nature of expressed immunoglobulin, and the status of immunoglobulin H and L chain gene rearrangements (3). Expression patterns of certain genes such as terminal deoxynucleotidyltransferase, germ-line V_H gene segments, and certain protooncogenes also have been correlated with discrete stages of B-cell development (9–12). Most recently, subtractive hybridization/differential screening methods led to isolation of two genes called λ5 and $V_{pre\text{-}B}$; specifically expressed in pre-B-cells (13, 14); a single gene called mb-1, expressed in both pre-B-cells and B cells but not in plasma cells (15); and a B-lineage-specific gene called B29, expressed in all B-cell stages (16). Although no functions have been defined for the λ5, $V_{pre\text{-}B}$, or B29 products, all display homology to immunoglobulin L chain domains. Present evidence suggests mb-1 protein complexes with membrane immunoglobulin (15, 17), while λ5 and $V_{pre\text{-}B}$ proteins may associate with μ H chain proteins (18).

Identification of additional genes expressed specifically in discrete stages of B-cell development should allow further definition of this pathway and elucidation of novel stage-specific processes. To isolate such genes, we have utilized subtractive hybridization techniques (19–21) to isolate sequences specifically expressed in cell lines representing pre-B-cell but not plasma-cell stages. We further optimized this technology to permit isolation of cDNAs representing mRNAs of low abundance, reasoning that rare mRNAs might represent regulatory genes not readily accessible by other methods.

MATERIALS AND METHODS

Cell Lines. The various cell lines and their characteristics have been described (11, 12, 22–27).

cDNA Preparation. Radiolabeled cDNA of relatively low specific activity was made from 6 μg of poly(A)$^+$ RNA pooled from three pre-B-cell lines (38B9, 22D6, and 300-19P) as described (28).

Preparative and Analytical Hybridizations. Details of the subtractive hybridizations, S1 nuclease assays, and fractionation of double- and single-stranded nucleic acids by fractionation on hydroxyapatite were essentially as described (28). The cDNA from the above reaction was added to 50 μg of poly(A)$^+$ RNA from the Ltk$^-$ fibroblast cell and hybridized to an R_0t of about 5000. After hybridization, single-stranded cDNA (about 5–10% of the input cDNA) was isolated by hydroxyapatite chromatography and was hybridized back to 50 μg of pre-B-cell poly(A)$^+$ RNA (to an R_0t of 5000) to render it double-stranded; the fraction of this cDNA that hybridized back to autologous RNA was estimated to be about 60% by S1 nuclease analysis. The cDNA·RNA hybrids were used

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Abbreviations: H, heavy; L, light; V, variable; J, joining; D, diversity; TCR, T-cell receptor; RAG, recombination-activating gene; Ab-MuLV, Abelson murine leukemia virus.

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directly in the conventional second-strand synthesis reaction as described (28). The double-stranded cDNA was inserted into a λ gT10 phage vector as described (28) except that endogenous *Eco*RI sites were not methylated and the cDNA was size-selected on a 1.2% ultrapure agarose gel (IBI) to be >250 base pairs (bp). The resulting library (60,000 recombinants) was amplified, and phage DNA was isolated and used to prepare purified *Eco*RI inserts (size-selected on 1.2% agarose to be 250–1000 bp). The inserts were used to construct a Lambda ZAP (Stratagene) cDNA library, which was used in the following screening procedures.

Conventional cDNA Library Construction. Double-stranded radiolabeled cDNA was synthesized from the pooled poly(A)$^+$ RNA of three pre-B-cell lines (38B9, 22D6, and 300-19P) as described (28). The cDNA was inserted into a Lambda ZAP phage vector (Stratagene) as described (28) to yield ≈2 million recombinant phage.

Subtractive Probe Preparation. Radiolabeled cDNA for making subtractive probes was prepared the same way as the cDNA used for library construction, except that radiolabeled dATP, dCTP, and dGTP (specific activity of 800 Ci/mmol; 1 Ci = 37 GBq) were included in the cDNA synthesis reaction mixture at 12.5 μM each, and the reaction mixture was supplemented to 50 μM with unlabeled dATP, dCTP, and dGTP and to 500 μM with unlabeled dTTP (28). Other details were as described above except that, for probe preparation, a second round of hybridization to non-pre-B-cell mRNA was performed to maximize removal of nonspecific sequences. Analogous subtraction strategies were used to prepare the following four subtracted cDNA probes: pre-B-cell (38B9, 22D6, or 300-19P) minus fibroblast (Ltk$^-$), fibroblast minus pre-B-cell, pre-B-cell minus myeloma cell (MOPC104E), and myeloma cell minus pre-B-cell.

Subtracted Probe Enriched for Rare cDNAs. A probe enriched for rare, pre-B-cell-specific mRNAs was made from the pre-B-cell-minus-myeloma-cell probe by hybridizing the subtracted cDNA to a 10-fold excess of autologous pre-B-cell RNA to a R_0T of 100 and using hydroxyapatite chromatography to select unhybridized cDNA.

Subtractive Library Screening. Twenty-five hundred plaque-forming units from the pre-B-cell-specific Lambda ZAP library were plated onto each of four 243 mm^2 plates. Five duplicate nitrocellulose lifts were prepared from each plate (29), probed with the total pre-B-cell cDNA probe and with the four subtractive probes described above, and exposed to x-ray film (at −70°C with intensifying screen) for 2–3 weeks. Hybridization was performed as described (28); after washing, the hybridized filters were treated with proteinase K [100 mM NaCl/10 mM Tris, pH 8.1/0.1 mM EDTA/0.25% sodium dodecyl sulfate (SDS)/0.2 mg of proteinase K per ml at 37°C for 2 hr] to reduce background and to allow detection of weak signals. Plates were further dried prior to storage (to minimize diffusion of phage) by placing opened plates in sterile hoods for 10–30 min with the air blower in operation. Plates were stored at 4°C until single plaques were isolated. By this procedure, picks of single primary plaques made 2–3 weeks after plating were >95% homogeneous and did not require rescreening.

Screening of cDNA Inserts. High-titer phage plate lysates were prepared from purified phage and slot-blotted (Schleicher & Schuell) onto Nytran filters according to the following procedure (28): 90 μl of the phage stock was incubated in 50% formamide/0.1 M Tris, pH 8.0/10 mM EDTA for 10 min at 68°C, cooled on ice, centrifuged for 5 min in a Microfuge to pellet insoluble debris, and loaded onto the slot-blotter. Duplicate slot-blots were probed with the same probes described above. Desired cDNA inserts were isolated by following standard Lambda ZAP protocols (Stratagene).

RESULTS

Isolation of Pre-B-Cell-Specific cDNA Clones. Pooled poly(A)$^+$ RNA preparations from three well-characterized Abelson murine leukemia virus (Ab-MuLV)-transformed pre-B-cell lines (38B9, 22D6, and 300-19P) were used to prepare cDNA. From this cDNA we subtracted sequences also expressed in the Ltk$^-$ fibroblast line; this subtraction retained all B lineage-specific sequences expressed in pre-B-cells, including those also expressed at later stages in this lineage. The resulting pre-B-cell-minus-L-cell cDNA was used to construct a subtracted cDNA library. This library displayed a 20-fold enrichment for sequences known to be specifically expressed in pre-B-cells but not in fibroblasts (e.g., H chain mRNA); this level of enrichment was consistent with RNA hybridization analyses of purified cDNA probes that showed pre-B-cell lines and fibroblast lines share 90–95% of their mRNA sequences (data not shown). Ten thousand recombinant plaques from the pre-B-cell-minus-L-cell cDNA library were screened with the following five

Table 1. Characterization of gene groups

Group	Homology† (identity*)	mRNA size‡	Abund./ rep.§	Distrib.¶
PB10		1.0	0.02%/2	Pre-B/B BM/Sp/Th
PB11	λ5 gene*	1.2	0.05%/1	Pre-B BM
PB17	Carbonic anhydrase II gene*	1.8	0.02%/1	Pre-B BM/Sp/Br/Kd
PB18	v-*abl**	ND	ND/4	ND
PB20	TCR-γ gene*	1.2	ND/2	ND
PB27	$V_{pre\text{-}B}$*	0.9	0.04%/9	Pre-B ND
PB35	CD2* gene	1.5	0.001%/1	Pre-B Sp/Th
PB36		2.5	0.001%/1	Pre-B/ BM/Br/Th
PB37	Thymosin gene*	0.9	0.05%/20	Pre-B/B Ubiquitous
PB54		2.8	ND/2	Pre-B/B BM/Sp/Th
PB59	CD19 gene*	2.0/2.5	0.05%/2	Pre-B/B BM/Sp
PB65	Myosin L chain 2 gene	0.9/1.5	0.02%/11	Pre-B BM/Th
PB74		1.7	0.02%/2	Pre-B BM/Sp/Th/L
PB98		8–10	0.002%/2	Pre-B Hr/Sp/Th
PB99		3.5	0.006%/2	Pre-B BM/Th
PB104		3.0	0.02%/1	Pre-B/B BM/Sp/Th

†The homologies or identities (*) of indicated clones to known genes were determined from their nucleotide sequences. Nearly full-length sequences were used for PB10, PB59, PB65, PB74, PB99, and PB104. Partial nucleotide sequences of at least 300 bp were examined for all others except PB54, for which only very limited sequence is available. The nucleotide sequences of these clones will be reported separately upon their completion; before then further information can be obtained from the authors.

‡Size is shown in kilobases.

§Approximate message abundance (Abund.) for each group as judged by its representation in a pre-B-cell cDNA library; and its representation (rep.) among the 97 subtracted clones that were screened.

¶Distribution (Distrib.) of specific message in both cell lines (upper line for each group entry) and tissues (lower line). Pre-B, pre-B-cell; Pre-B/B, pre-B-cell/B cell; BM, bone marrow; Sp, spleen; Th, thymus; Br, brain; Kd, kidney; Hr, heart; L, liver; ND, not determined.

subtracted cDNA probes: pre-B-cell minus fibroblast, fibroblast minus pre-B-cell, pre-B-cell minus myeloma cell, myeloma cell minus pre-B-cell, and a pre-B-cell minus myeloma cell enriched for rare cDNAs. The library also was screened with probes for genes already known to be specifically expressed in pre-B-cells versus L cells (e.g., genes for immunoglobulin H chains, N-myc, and terminal deoxynucleotidyltransferase) to avoid their isolation.

Many plaques hybridized to both pre-B-cell-specific and myeloma-cell-specific but not fibroblast-specific probes, indicating that they represented sequences expressed throughout B-cell development but not in fibroblasts. In general, these sequences were not further characterized, but one set was shown to correspond to retroviral-like repetitive sequences (encoding intracisternal A particles; ref. 30) that were upregulated during B-cell differentiation (data not shown). Hundreds of plaques hybridized to the pre-B-cell-specific probes (with varying intensities) but not to the myeloma cell- or fibroblast-specific probes. Some of these plaques were detected only with the pre-B-cell-minus-myeloma-cell probe enriched for low-abundance sequences. We chose 120 pre-B-cell-specific plaques for characterization; rapid slot-blotting analyses demonstrated 97 of these contained inserts that clearly hybridized to pre-B-cell-specific but not myeloma cell-specific probes. Furthermore, several of these represented phage that hybridized preferentially to the pre-B-cell-specific probe enriched for low-abundance cDNAs; subsequent analyses confirmed that they represented low-abundance pre-B-cell-specific sequences (e.g., PB36 and PB99; Table 1).

The Isolated cDNA Clones Represent 16 Distinct Genes. A combination of methods was employed to definitively assign phage cDNA inserts to particular groups. These included cross-hybridization analyses, partial or complete DNA sequence analyses, characterization of corresponding RNA transcript sizes and specificities (see below), and Southern blotting assays of genomic DNA (data not shown). These analyses showed that 66 of the selected 97 pre-B-cell-minus-myeloma-cell-specific phage clones contained inserts that could be categorized into 16 groups, each representing a distinct gene expressed in pre-B-cell lines but not in myeloma cell lines (Table 1). The mRNA abundance for the selected sequences was estimated by representation in the conventional pre-B-cell cDNA library and ranged from 0.001% to 0.05% of total mRNA (Table 1). The remaining 31 phage contained inserts that either did not identify specific transcripts or did identify mRNAs weakly expressed in myeloma cells.

The frequency with which each of the 16 groups was represented in the 97 clones varied from 1 to 20 (Table 1); because 10 of 16 groups had just one or two representatives, other similarly regulated genes are probably represented in the remainder of our library. In this regard, recently identified pre-B-cell-specific sequences BP-1 (31) and RAG-1 (6) were not evident among the 97 purified cDNA clones (see below). However, RAG-1 was relatively abundant among cDNA clones isolated from a conventional thymus library upon screening with a similar subtracted probe (G.R. and F.W.A., unpublished data).

Characterization of Purified cDNA Clones. Complete or partial DNA sequence analyses (data not shown) demonstrated that 6 of the sequences represented by the 16 specific groups displayed no significant homologies to any known genes (Table 1; PB10, PB36, PB54, PB98, PB99, and PB104),

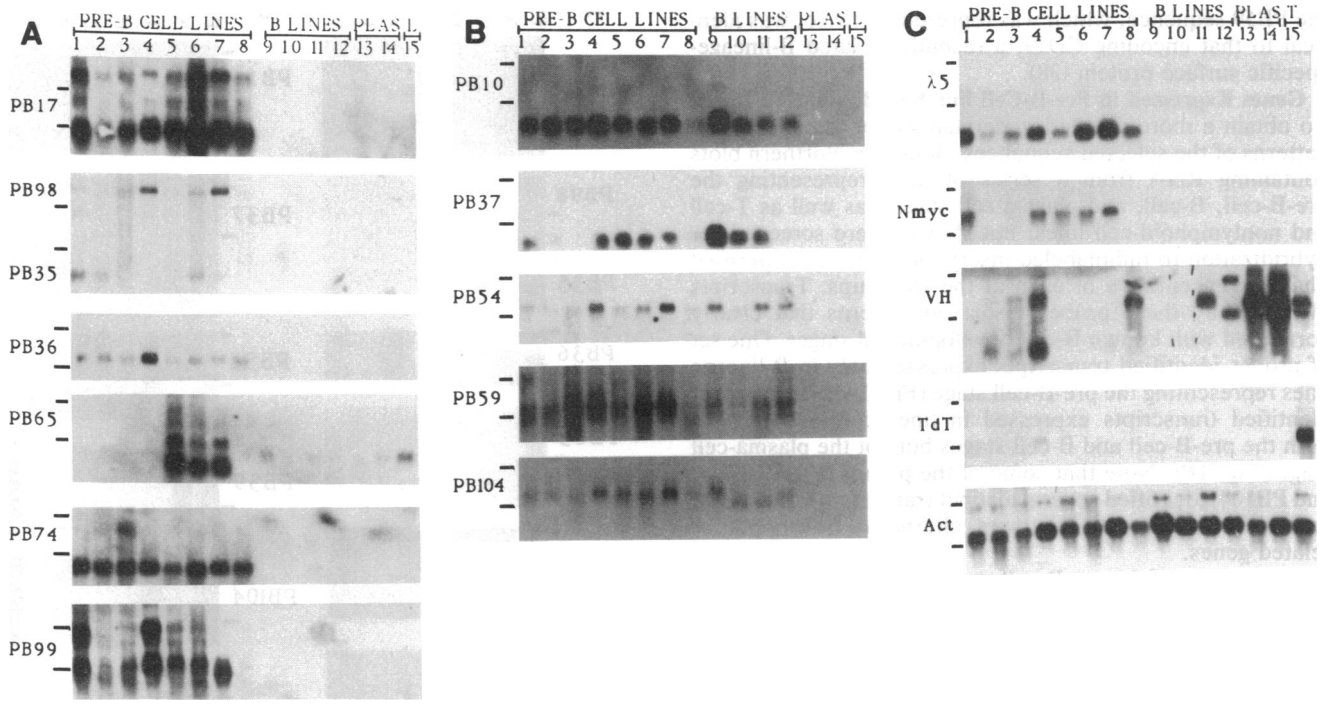

FIG. 1. Expression of subtracted clones in cell lines representing different stages of B-cell development. (*A*) RNA blot-hybridization (Northern) analyses of pre-B-cell-specific genes. Note that the PB65 probe also identifies a slightly larger transcript that is nonspecifically expressed and represents cross-hybridization to a related gene (E.M.O., unpublished data). In addition, the PB35 and PB98 inserts were assayed simultaneously for hybridization; other analyses with the individual probes for each gene indicate that the PB35 probe identifies the 1.5-kilobase (kb) RNA, whereas the PB98-specific transcript is the ≈8.0-kb RNA species. (*B*) Northern analyses of pre-B-cell/B cell-specific genes. (*C*) Northern analyses of previously defined pre-B-cell-specific genes. Note that the larger V_H-hybridizing transcripts in more mature B-lineage lines represent mature H chain mRNA, whereas the transcripts in the pre-B-cell lines represent germ-line V_H transcripts. A β-actin probe (Act) depicted in *C* serves as a control for RNA levels. Bold lines mark positions of 18S and 28S rRNAs. Multiple identical Northern blots were prepared from total cellular RNA isolated from the indicated cell lines. Lanes: 1, BASC6-C2; 2, HAFTL-1; 3, 22D6; 4, 22D10; 5, SC24; 6, 300-19; 7, 18-8; 8, 70Z; 9, WEHI-231; 10, WEHI-279; 11, BCL_1; 12, A202J; 13, 104E; 14, MPC-11; 15, Ltk^- fibroblast sample (L) in *A* and *B* and thymus (T) sample in *C*. PLAS, plasma-cell lines.

and 2 were related to but distinct from known genes (PB65 and PB74). Of these, one referred to as PB98 was isolated twice—once alone and once as a dual insert in the same plasmid with PB35 (Table 1 and Fig. 1). The PB74 sequence also was not highly related to any known gene but encoded a protein with two s-type lectin domains (ref. 32; J.E.B. and F.W.A., unpublished data). Finally, the PB65 sequence, which was represented 10 times, was related to (75% homology) but distinct from known myosin regulatory light chain genes (ref. 33; E.M.O., unpublished data).

Three of the 16 purified cDNA sequences appeared identical to previously characterized genes that were not known to be expressed in pre-B-cells. The most highly represented sequence (designated PB37) among the original 97 phage clones corresponded to the previously defined gene for thymosin (34), a small secreted protein of unknown function. The PB17 sequence, which was represented once, corresponded to the gene for type II carbonic anhydrase (35). PB35, which was present once in the library, represented the gene for CD2 which has been defined as a T cell-specific surface protein (36).

The remaining five characterized sequences represented genes known to be expressed in various B-lineage cell lines that we did not eliminate in our preliminary screening procedures (Table 1). The PB18 group represented v-*abl* sequences, and the PB20 group consisted of TCR γ chain transcripts, both of which are expressed in Ab-MuLV-transformed pre-B-cells and not myeloma cells (37). The PB27 group was represented nine times and corresponded to the previously defined pre-B-cell-specific gene $V_{pre\text{-}B}$ (14). The PB11 group corresponded to the pre-B-cell-specific gene $\lambda 5$ (13); this gene was represented only once despite being expressed at similar abundance to $V_{pre\text{-}B}$ (Table 1). Finally, the PB59 sequence, which was represented twice, was identical to that encoding CD19, a recently isolated B-lineage-specific surface protein (38).

Genes Expressed in Pre-B-Cell but Not Plasma-Cell Lines. To obtain a more detailed understanding of the expression patterns of the selected sequences, duplicate Northern blots containing RNA from a series of lines representing the pre-B-cell, B-cell, and plasma-cell stages (as well as T-cell and nonlymphoid-cell lines; not shown) were screened for hybridization to radiolabeled inserts prepared from purified phage representative of each of the 16 groups. Transcripts identified by these probes displayed patterns that clearly correlated with known B-cell developmental stages. One set of probes identified transcripts expressed only in B-lineage lines representing the pre-B-cell stage (Fig. 1*A*); the other set identified transcripts expressed in lines corresponding to both the pre-B-cell and B-cell stages but not the plasma-cell stage (Fig. 1*B*). Note that some of the probes (PB59, PB99, and PB104) identified several distinct transcripts in cell lines; these might represent precursors, alternative transcripts, or related genes.

Comparison of the pre-B-cell stage-specific genes (Fig. 1*A*) with other previously defined pre-B-cell-specific genes (Fig. 1*C*) revealed certain correlations in expression pattern. Five (PB17, PB36, PB35, PB74, and PB98) had patterns similar to $\lambda 5$ (Fig. 1*C*) and $V_{pre\text{-}B}$ (not shown); they were expressed at a relatively uniform level in most pre-B-cell lines. None of the sequences displayed patterns similar to germ-line V_H transcripts or terminal deoxynucleotidyltransferase (Fig. 1*C*), which are thought to be expressed most highly in cell lines representing early pre-B-cells undergoing H chain but not L chain gene rearrangement. One sequence, PB99, exhibited an expression pattern reminiscent of N-*myc* (Fig. 2*C*) and RAG-1 gene (6), both of which are expressed in all pre-B-cell lines but at much lower levels in the pre-B-cell line 70Z. The 70Z line is an unusual pre-B-cell line in many respects (23); in particular it possesses very low *V–D–J* recombinase activity (24). Finally, one of the pre-B-cell line-specific sequences (PB65) was expressed only in a subset of pre-B-cell lines (Fig. 1*A*); more extensive analyses have demonstrated that PB65 is expressed in bone marrow- but not fetal liver-derived Ab-MuLV-transformed pre-B-cell lines (E.M.O., unpublished data).

The PB98 sequence hybridizes to a RNA transcript that is similar in size (8 kb, Fig. 1*A*) to that encoded by the RAG-1 gene (6) and also maps to the same chromosome as RAG-1 gene (data not shown). However, the limited sequence available (≈800 bp) and the expression pattern (Figs. 1*A* and 2*A*) do not match that of the RAG-1 transcript (ref. 6).

Expression of Stage-Specific B-Lineage Genes *in Vivo*. To further characterize the tissue specificity of the isolated sequences, we assayed expression in murine organs. Genes expressed only in cell lines representing the pre-B-cell stage of B-cell development displayed several different patterns *in vivo*. None had tissue expression patterns that suggested exclusive pre-B-cell specificity (i.e., only in adult marrow and fetal liver) as is observed for the known pre-B-cell-specific gene $\lambda 5$ (PB11, Table 1). However, expression of two of these sequences (PB99 and PB65, Fig. 2*A* and Table 1) was limited to primary lymphoid organs of the B-cell and T-cell lineages (newborn liver, adult marrow, and adult thymus). This expression pattern is similar to that of RAG-1 gene (6) and suggests these two genes are likely to be involved in processes common to pre-B- and pre-T-cells. One sequence that had pre-B-cell specificity in tested B-lineage cell lines was expressed only in primary B- and T-cell lymphoid organs and in brain (PB36; Fig. 2*A*). Thus, this sequence may encode a product that functions specifically in precursor lympho-

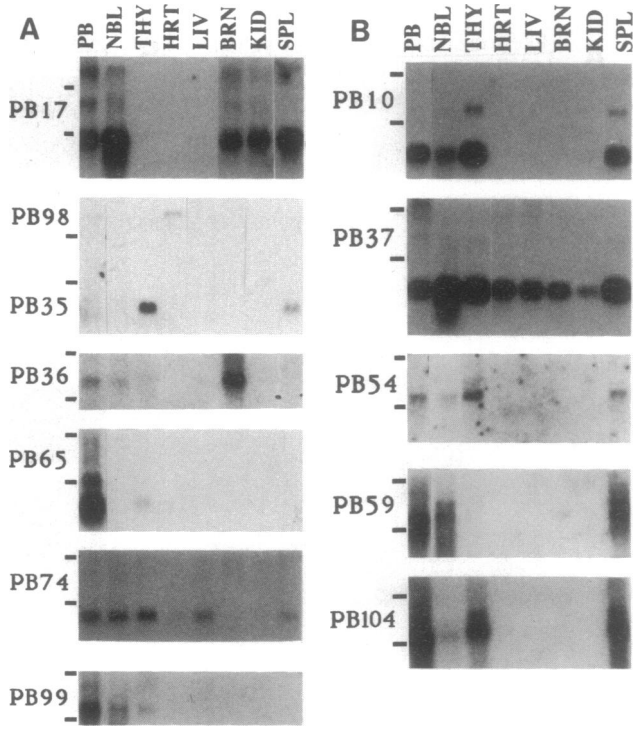

FIG. 2. Expression of subtracted clones in tissues. (*A*) Tissue-specific expression of pre-B-cell-specific genes. The PB35/98 probe was as described in Fig. 1. (*B*) Tissue-specific expression of pre-B-cell/B cell-specific genes. Multiple identical Northern blots were prepared from total cellular RNA isolated from the indicated murine tissues and assayed for hybridization to radiolabeled probes specific to the genes indicated; the ubiquitous PB37 (thymosin gene) probe depicted in *B* serves as a control for RNA levels. Bold lines mark the positions of 18S and 28S rRNAs. Lanes: PB, pre-B-cell line 300-19; NBL, newborn liver; THY, thymus; HRT, heart; LIV, liver; BRN, brain; KID, kidney; SPL, spleen.

cytes and in brain. Finally, four genes expressed in pre-B-cell but not B-cell lines were expressed in both peripheral and primary lymphoid tissues (PB17, PB35, PB74, and PB98; Fig. 2*A*). Three of these also were expressed in nonlymphoid tissues: PB17 (type II carbonic anhydrase) was expressed at substantial levels in brain and kidney, PB74 was expressed in liver, and PB98 was expressed in the heart.

Expression of genes specific to pre-B-cell lines in peripheral lymphoid tissues suggests either that *in vivo* and *in vitro* regulation of these genes differ, that there are splenic B cells expressing these genes that are not represented by the mature B-cell lines screened, or that a non-B-cell lineage or nonlymphoid cell type in adult spleen expresses these genes. For example, splenic T cells may express some pre-B-cell-specific genes not expressed by splenic B cells. However, we note that our analyses of T-cell lines (not shown) suggest that most genes with pre-B-cell specificity in the B-cell lineage are also expressed only in the early stages of T-cell development. Expression of genes only in the precursor stage of lymphocyte development but in multiple nonlymphoid lineages has precedence—for example, the N-*myc* and c-*myb* protooncogenes have such expression patterns (11, 12).

Genes expressed in cell lines representing both the pre-B-cell and B-cell stages of B-cell development generally had very specific patterns *in vivo*. With the exception of the gene for thymosin, which was expressed ubiquitously (PB37; Fig. 2*B* and Table 1), the remainder of the pre-B-cell/B-cell genes (PB10, PB54, PB59, and PB104; Fig. 2*B*) were expressed only in primary and peripheral lymphoid tissues (including thymus). These analyses, together with assays of T-cell lines (not shown), indicate that these sequences are expressed in B- and T-lineage cells.

DISCUSSION

These new subtractive clones compose a more complete set of markers than were previously available for defining B-cell development. Initial characterization of the isolated genes already has provided a number of unusual insights. The striking stage-specific expression of genes for carbonic anhydrase, thymosin, and CD2 in B-lineage cell lines was previously unappreciated and warrants further examination. Expression of the myosin regulatory light chain-related PB65 gene is apparently restricted to adult bone marrow- but not fetal liver-derived pre-B-cell lines, providing the first clear marker for distinguishing these pre-B-cell subsets (E.M.O, unpublished data). Other cDNA clones represent as-yet-undefined genes that must necessarily encode products involved in processes occurring during the earlier stages of lymphocyte development. Such processes include *V–D–J* recombination and its control, regulation of specific gene expression, and response of pre-B-cells and B cells to factors specific for these developmental stages. A more detailed characterization of these genes will clarify their roles, if any, in these various processes. In addition, this group of coordinately regulated genes should facilitate searches for potential transcriptional regulatory elements involved in B-lineage stage-specific gene transcription. Finally, this panel of genes may also provide useful markers for further staging and characterization of human lymphoid tumors.

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