component of global radiation makes a very minor contribution to the photons accepted by a horizontal, cosine-corrected receiver but plays an important role in determining the light environment within the axis (*9*).

18. The use of $CuSO_4$ (6 g liter^{-1}) filters did not significantly affect elongation of isolated seedlings ($P > 0.5$).
19. We thank O. E. Sala for critical reading of the manuscript. Supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas and the University of Buenos Aires (AG 062).

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Specific Expression of a Tyrosine Kinase Gene, *blk*, in B Lymphoid Cells

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Several pathways of transmembrane signaling in lymphocytes involve protein-tyrosine phosphorylation. With the exception of p56^{lck}, a tyrosine kinase specific to T lymphoid cells that associates with the T cell transmembrane proteins CD4 and CD8, the kinases that function in these pathways are unknown. A murine lymphocyte complementary DNA that represents a new member of the *src* family has now been isolated and characterized. This complementary DNA, termed *blk* (for B lymphoid kinase), specifies a polypeptide of 55 kilodaltons that is related to, but distinct from, previously identified retroviral or cellular tyrosine kinases. The protein encoded by *blk* exhibits tyrosine kinase activity when expressed in bacterial cells. In the mouse and among cell lines, *blk* is specifically expressed in the B cell lineage. The tyrosine kinase encoded by *blk* may function in a signal transduction pathway that is restricted to B lymphoid cells.

THE PROGRAMS OF LYMPHOCYTE proliferation and differentiation that underlie the humoral immune response are mediated by the binding of specific antigens, cell-surface adhesion molecules, and lymphokines to receptors on B and T cells. Protein-tyrosine phosphorylation is implicated as being important in these processes. Stimulation of T cells by specific antigen results in tyrosine phosphorylation of specific substrates, including the zeta chain of the T cell receptor–CD3 complex (*1*). Likewise, within several minutes of exposure to interleukin-2 (IL-2), a number of proteins in responsive cells become phosphorylated on tyrosine residues; the concentration of IL-2 required for phosphorylation is similar to that required for proliferation (*2*). Interleukin-3, which exerts its effects on lymphoid and myeloid progenitor cells, also induces phosphorylation of specific substrates on tyrosine, including a 140-kD protein that may represent a component of the IL-3 receptor (*3*).

By analogy to the receptors for epidermal growth factor and insulin, some of the transmembrane proteins implicated in lymphocyte activation may exert their effects through specific tyrosine kinases. In the growth factor receptor kinases, ligand-binding and kinase domains are covalently linked (*4*). Such an association can also be achieved by noncovalent interactions. The *lck* gene, a member of the *src* family, is expressed predominantly in T lymphocytes (*5*). Its product, a 56-kD tyrosine kinase (p56^{lck}), is specifically associated with two transmembrane proteins implicated in T cell activation, CD4 and CD8; cross-linking of CD4 is accompanied by an increase in the protein-tyrosine kinase activity of p56^{lck} (*6*). Other lymphocyte transmembrane proteins may also couple to tyrosine kinases of the Src-type. Seven members of the *src* family have been described: *src, lyn, yes, fyn, fgr, hck*, and *lck* (*7, 8*). Of these, only *lck* is expressed predominantly in lymphoid cells. The participation of tyrosine phosphorylation in multiple pathways of signal transduction in B and T cells suggested the existence of additional, lymphoid-specific members of the *src* family.

The catalytic domains of Src-type tyrosine kinases are highly conserved (*7, 8*), suggesting that degenerate oligonucleotide probes could be used to identify genes encoding similar proteins. We compared the nucleotide sequences of four tyrosine kinase genes: *lck* (*5*), v-*abl* (*9*), c-*src* (*10*), and v-*yes* (*11*). Two regions of highly conserved nucleotide sequence were identified, and three degenerate oligonucleotides, complementary to the coding strand within these regions, were synthesized (*12*). A mixed B and T cell cDNA library was screened with a pool of these probes, and seven independent cDNA clones were isolated. On the basis of DNA hybridization, clones 54, 96, 100, 108, and 109 were uniquely represented in the set, whereas clones 102 and 103 appeared to be closely related. By nucleotide sequence, clones 54, 96, and 108 were found to represent *src, lck*, and *abl*, respectively (*13*). Clones 100 and 109 are likely to be murine homologs of *yes* and *fyn* because partial nucleotide sequence analysis revealed >95% identity with the human genes (*13*). The nucleotide sequences of clones 102 and 103 confirmed that they were overlapping cDNAs and indicated that they were derived from a new member of the *src* family, which we have called *blk*.

On rescreening the library with *blk* cDNA probes, three additional 5′ overlapping clones, designated 201, 205, and 215, were obtained. These five cDNA clones define a DNA sequence spanning 2094 bp. An open reading frame of 499 codons extends from a Met codon at nucleotides 350 to 352 to an amber stop codon at nucleotides 1847 to 1849 (Fig. 1). The Met codon at positions 350 to 352 occurs in a context favorable for translation (*14*). Four termination codons occur upstream from this Met codon in the same reading frame. Five other potential Met codons lie upstream from nucleotide 350, but these are followed by in-frame termination codons (Fig. 1A). Thus, the entire *blk* coding sequence is represented within the region included in the clones. The most 3′ terminal clone, 103, contains 245 bp of 3′ untranslated region and is devoid of a polyadenylated tract or a polyadenylation signal (*15*); it is likely that this clone does not include the complete 3′ untranslated sequence of *blk*.

To prove the integrity of the large open reading frame predicted by the nucleotide sequence, we synthesized *blk* transcripts in vitro, and translated them in a cell-free system (Fig. 2). The complete sense transcript directed the synthesis of a polypeptide of apparent molecular mass 55-kD, in agreement with the molecular mass predicted by the nucleotide sequence (Fig. 2B, lane 1). A truncated transcript that includes 314 codons of *blk* open reading frame and 6 additional codons derived from the bacterial cloning vector (Fig. 2A), directed the synthesis of a 33-kD polypeptide (Fig. 2B, lane 2), thus verifying that the major translation product is initiated at or near the Met codon at nucleotides 350 to 352. No polypeptides of comparable size were detected in reactions directed by antisense transcripts (Fig. 2B, lanes 3 and 4) or in reactions devoid of exogenous RNA (Fig. 2B, lane 5).

The protein encoded by *blk* (p55^{blk}) represents a new member of the Src family of

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protein tyrosine kinases (Fig. 1B) on the basis of the following observations: (i) The amino acid sequences of 64 out of 65 known protein kinases are invariant at 14 positions (7); these amino acids are found at the corresponding locations in p55^{blk} (Fig. 1B). Two of these residues, Gly^{240} and Gly^{242}, have been implicated in nucleotide binding. Another of these invariant residues, corresponding to Lys^{261} in p55^{blk}, interacts with analogs of adenosine triphosphate and is essential for enzymatic activity (*16*). Leu^{516} of p60$^{c\text{-}src}$, corresponding to Leu^{484} of p55^{blk}, is also critical for activity (*8*). (ii) The protein encoded by *blk* contains two consensus sequences, DLRAAN (residues 354 to 359) and PIKWTAPE (residues 392 to 399), that distinguish protein-tyrosine kinases from protein-serine/threonine kinases (7). (iii) The *blk*-encoded protein has 53% amino acid sequence identity to the *src* product and greater similarity to proteins encoded by *hck* and *lck* (63 and 61% identity, respectively). The tyrosine residue at position 383 of p55^{blk} corresponds to a site of autophosphorylation (Tyr^{416}) in p60$^{c\text{-}src}$ (*17*). The tyrosine at position 495 of p55^{blk} corresponds to Tyr^{527} of p60$^{c\text{-}src}$, Tyr^{505} of p56^{lck}, and Tyr^{501} of p59^{hck}; mutations in *src*, *lck*, and *hck* that convert this Tyr to Phe confer increased kinase activity and the ability to transform fibroblasts (*18*). (iv) The protein products of *src* and *lck* are myristoylated through an amide linkage to a Gly residue at position 2. The protein encoded by *blk* also contains Gly at position 2, in the context of amino acid residues that favor N-myristoylation (*19*). (v) Between residues 7 and 57, the amino acid sequence encoded by *blk* is not homologous to that encoded by any other member of the *src* family; in this region the sequences of the other protein products of the *src* family also diverge (7). (vi) The *blk* gene has been assigned to mouse chromosome 14 (*20*), which distinguishes it from *src* (chromosome 2) (*21*) and *lck* (chromosome 4) (*22*).

To demonstrate that p55^{blk} is a tyrosine

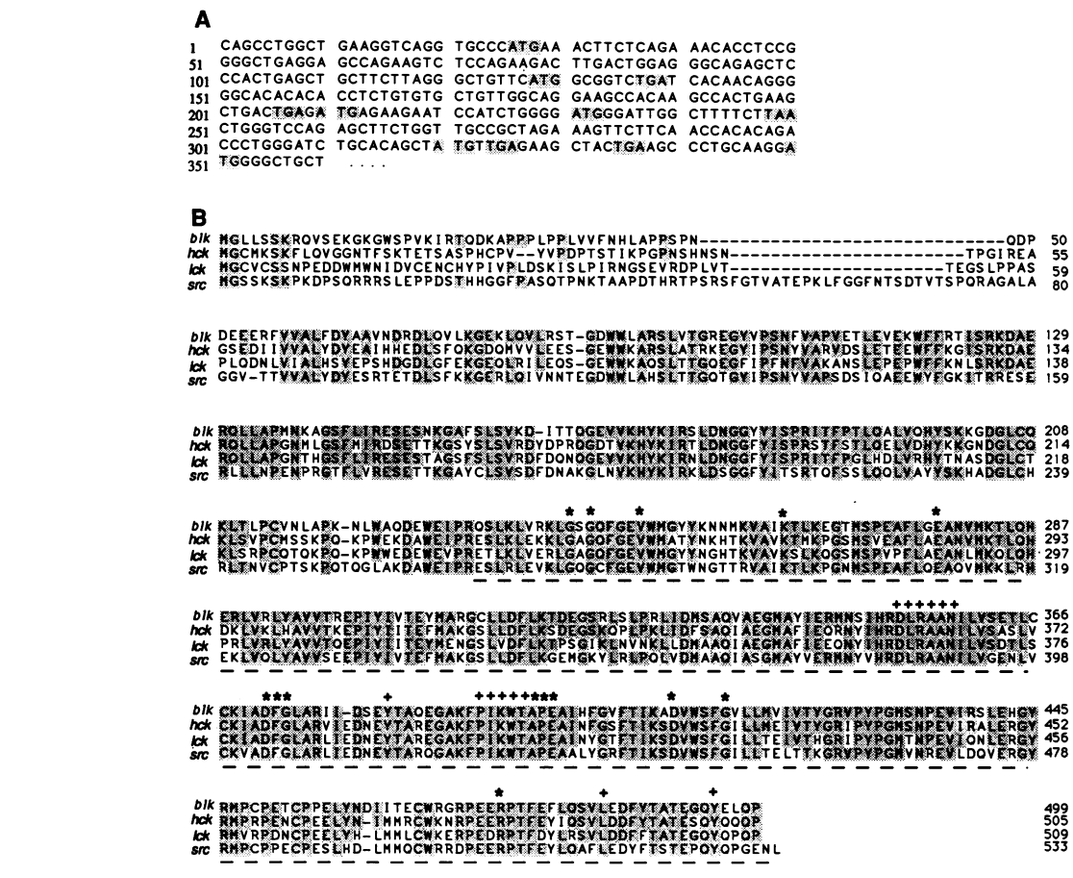

Fig. 1. Deduced amino acid sequence of the *blk* product. A cDNA library representing polyadenylated RNA from a mixed culture of a bovine insulin-specific T cell hybridoma (71.67) (*34*) and a bovine insulin-primed B cell hybridoma (LB27.4) (*35*) was constructed in λgt10 (*36*). The *blk* cDNA clones 102 and 103 were identified by hybridization to ^{32}P-labeled oligonucleotides as described (*37*). Hybridization to pooled oligonucleotides SD11, SD12, and SD13 (1×10^9 to 2×10^9 cpm/μg; 36, 51, and 194 p*M* per degeneracy, respectively) was performed for 36 hours at 37°C, and washes were performed at 50°C. Positive clones were isolated by two additional rounds of screening. The 5′ overlapping *blk* clones 201, 205, and 215 were identified in subsequent screens of the same library with *blk* cDNA probes as described (*38*). Nucleotide sequence was determined by the dideoxynucleotide chain termination method (*39*). (**A**) Nucleotide sequence of clone 205 in the 5′ untranslated region. Nucleotides are numbered at left. Potential initiator Met codons within the 5′ untranslated region and their nearest in-frame termination codons are shaded, as is the first Met codon of the translated sequence at nucleotide positions 350 to 352. (**B**) Alignment of the deduced p55^{blk} amino acid sequence with sequences of other Src-family tyrosine kinases. Conceptual translation of the single long open reading frame of *blk* is shown in single-letter amino acid code (*40*). Identical residues encoded in human *hck* (*28*), murine *lck* (*5*), and avian c-*src* (*10*) are shaded. (*) Residues conserved among protein kinases; (+) additional residues characteristic of tyrosine kinases. The dashed underline identifies the catalytic domain (*7, 8*). Amino acids are numbered at right. Dashes are placed within sequences to maximize overlap.

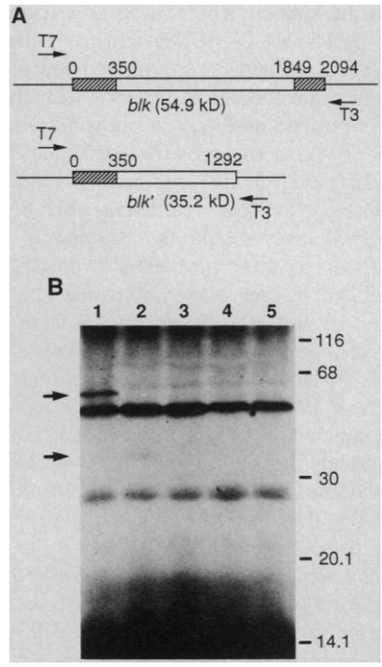

Fig. 2. Translation of *blk* transcripts in vitro. (**A**) Templates for in vitro transcription. A plasmid containing the complete *blk* coding sequence was constructed in pBluescript (SK+) from the 1202-bp Pst I fragment of clone 205 [nucleotides (nt) 1 to 1202] and the 892-bp Pst I fragment of clone 103 (nt 1203 to 2094). The structure was verified by nucleotide sequence analysis. A 3′ truncated construct (*blk'*) contained the insert from clone 205 (nt 1 to 1292). Open bars, coding sequence; shaded bars, untranslated regions. Conceptual translation predicts a *blk* polypeptide of 54,890 daltons and a *blk'* product of 35,200 daltons. (**B**) Products of in vitro translation. Constructs were transcribed in sense and antisense directions with T7 and T3 RNA polymerases in the presence of a 5′ 7mGppp5′G cap analog (Stratagene), and transcripts (2.5 μg) were translated by a rabbit reticulocyte lysate (Promega) in the presence of [^{35}S]methionine. Products were fractionated on a 15% SDS-polyacrylamide gel, and visualized by autoradiography. (Lane 1) *blk* sense transcript, (lane 2) *blk'* sense transcript, (lane 3) *blk* antisense transcript, (lane 4) *blk'* antisense transcript, and (lane 5) no exogenous RNA. The specific products of *blk* (~55 kD) and *blk'* (~35 kD) are denoted by arrows. Molecular masses of standards are indicated in kilodaltons.

kinase, the intact *blk* coding region was placed under control of a bacteriophage T7 promoter in the *Escherichia coli* expression vector pET8c (*23*). The resulting plasmid, pET8c-*blk*, was introduced into the *E. coli* strain BL21(DE3) (*23*), which expresses bacteriophage T7 RNA polymerase when grown in the presence of isopropyl-β-*D*-thiogalactoside (IPTG). After induction of T7 RNA polymerase by IPTG, a 55-kD protein was expressed in cells harboring pET8c-*blk*, but not in cells carrying the vector alone or in cells grown without IPTG (*13*). Thus, pET8c-*blk* supports expression of $p55^{blk}$. To determine whether $p55^{blk}$ exhibits tyrosine kinase activity when expressed in *E. coli*, which lacks endogenous tyrosine kinases, we cultured cells containing pET8c-*blk* or pET8c with or without IPTG, and lysates were assayed for phosphotyrosine-containing proteins with an affinity-purified antibody to phosphotyrosine (*24*). Two reactive proteins (molecular mass 85 kD and 55 kD) appeared after IPTG induction in cells containing pET8c-*blk* (Fig. 3, lanes 4 and 6). Binding of the antibody to these proteins was specifically inhibited by free phosphotyrosine (Fig. 3, lanes 10 and 12). These species were not detected in cells containing the vector alone (Fig. 3, lanes 1 and 2) or in cells grown without IPTG (Fig. 3, lanes 1, 3, and 5). We conclude that $p55^{blk}$ is a tyrosine kinase. Although the size of the 55-kD species is consistent with autophosphorylation of $p55^{blk}$, demonstration of this property awaits isolation of $p55^{blk}$.

The sequence of the 5′ untranslated region of *blk* was confirmed by analysis of a murine genomic clone (*25*). Five ATG codons occur within this region. Transcripts of most mammalian genes do not contain Met codons upstream of the initiator codon. Among members of the *src* family, however, 5′ ATG codons are common. In the case of *lck*, their presence greatly reduces translational efficiency; removal of these elements may in part be responsible for the overexpression of $p56^{lck}$ in the transformed cell line LSTRA (*26*).

Genes that function in specialized pathways of signal transduction are often expressed preferentially in cells that employ those pathways. We examined the expression of *blk* in normal mouse tissues. A 2.5-kb RNA was detected in spleen but not in thymus, brain, heart, lung, kidney, liver, or intestine (Fig. 4A). The absence of expression in thymus suggested that B cells were primarily responsible for the expression observed in spleen. To investigate this, we assayed splenic mononuclear cells for *blk* expression before and after selective depletion of B cells. In the depleted population, B cells constituted at most 5% of cells, as assessed by staining with antibody to immunoglobulin, in comparison to about 63% of cells in the untreated sample (*27*). The 2.5-kb *blk* transcript was observed in RNA from unfractionated splenocytes (Fig. 4B, lane 2). Removal of B cells was accompanied by the loss of *blk* transcripts (Fig. 4B, lane 3) and a large reduction in immunoglobulin kappa RNA (Fig. 4C); in contrast, T cell receptor beta chain transcripts were undiminished in the B cell–depleted population (Fig. 4D).

We also examined *blk* expression in cell

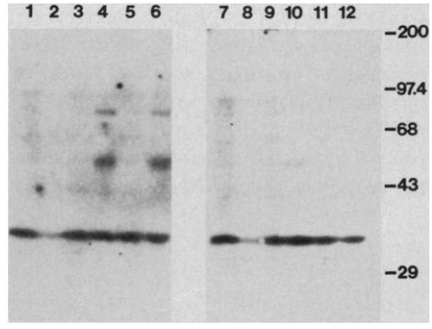

Fig. 3. The *blk* gene encodes a protein-tyrosine kinase. To construct the expression plasmid pET8c-*blk*, an Nco I restriction site was introduced immediately upstream of the *blk* coding region, which was inserted between the Nco I and Bam HI restriction sites of pET8c (*23*). In pET8c-*blk*, the *blk* coding sequence begins at nt 63 relative to the transcription startpoint. Plasmids pET8c-*blk* and pET8c were propagated in *E. coli* strain BL21(DE3) in 10 ml of M9ZB medium at 37°C as described (*23*). When cultures reached an optical density at 650 nm of ~0.5, IPTG (0.8 m*M* final concentration) was added to half of each culture. After a further 2.5 hours at 37°C, 0.5 ml was removed from each culture. Cells were centrifuged at 12,000*g* for 2 min, suspended in 0.25 ml of SDS sample buffer, and heated to 100°C for 5 min. Samples (25 μl) were fractionated in duplicate on a single SDS-polyacrylamide gel, and transferred to nitrocellulose as described (*41*). After transfer, the filter was cut into identical halves and blocked with tris-buffered saline (TBS) containing 0.1% Tween-20. The filters were incubated for 2 hours at room temperature with a 1:500 dilution of affinity-purified antibody to phosphotyrosine (*24*) in TBS, in the absence or presence of 200 μ*M* *O*-phospho-*L*-tyrosine (P-Tyr). Filters were rinsed with TBS and incubated for 1 hour at room temperature with ^{125}I-labeled protein A (1.6 dpm/ml) in TBS and 0.1% Tween-20. Filters were washed in TBS and autoradiographed for 3 days at −70°C with an intensifying screen. (Lanes 1 to 6) Antibody reaction without P-Tyr and (lanes 7 to 12) reaction in the presence of P-Tyr. (Lanes 1, 2, 7, and 8) Cells containing pET8c and (lanes 3, 4, 9, and 10 and 5, 6, 11, and 12) two independent isolates of cells containing pET8c-*blk*. (Lanes 1, 3, 5, 7, 9, and 11) No IPTG added and (lanes 2, 4, 6, 8, 10, and 12) IPTG added. Molecular masses of standards are shown in kilodaltons.

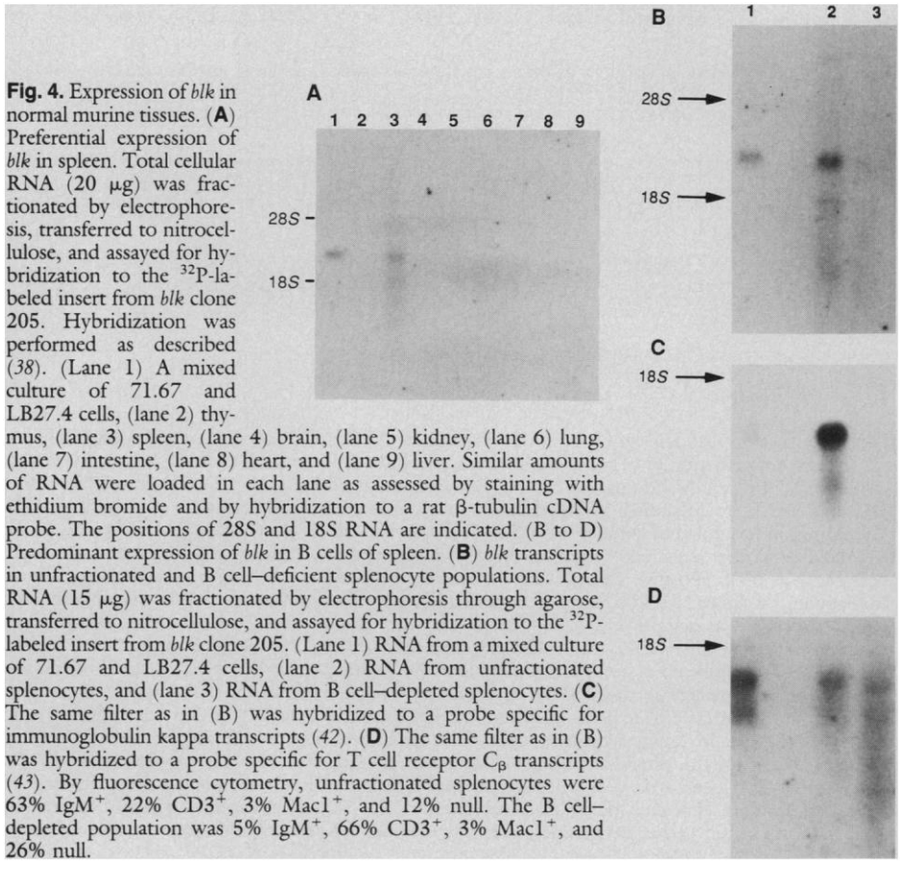

Fig. 4. Expression of *blk* in normal murine tissues. (**A**) Preferential expression of *blk* in spleen. Total cellular RNA (20 μg) was fractionated by electrophoresis, transferred to nitrocellulose, and assayed for hybridization to the ^{32}P-labeled insert from *blk* clone 205. Hybridization was performed as described (*38*). (Lane 1) A mixed culture of 71.67 and LB27.4 cells, (lane 2) thymus, (lane 3) spleen, (lane 4) brain, (lane 5) kidney, (lane 6) lung, (lane 7) intestine, (lane 8) heart, and (lane 9) liver. Similar amounts of RNA were loaded in each lane as assessed by staining with ethidium bromide and by hybridization to a rat β-tubulin cDNA probe. The positions of 28S and 18S RNA are indicated. (B to D) Predominant expression of *blk* in B cells of spleen. (**B**) *blk* transcripts in unfractionated and B cell–deficient splenocyte populations. Total RNA (15 μg) was fractionated by electrophoresis through agarose, transferred to nitrocellulose, and assayed for hybridization to the ^{32}P-labeled insert from *blk* clone 205. (Lane 1) RNA from a mixed culture of 71.67 and LB27.4 cells, (lane 2) RNA from unfractionated splenocytes, and (lane 3) RNA from B cell–depleted splenocytes. (**C**) The same filter as in (B) was hybridized to a probe specific for immunoglobulin kappa transcripts (*42*). (**D**) The same filter as in (B) was hybridized to a probe specific for T cell receptor C_β transcripts (*43*). By fluorescence cytometry, unfractionated splenocytes were 63% IgM^+, 22% $CD3^+$, 3% $Mac1^+$, and 12% null. The B cell–depleted population was 5% IgM^+, 66% $CD3^+$, 3% $Mac1^+$, and 26% null.

lines representing diverse lineages (Fig. 5). Expression of *blk* was detected in a wide variety of B lymphoid cell lines (Fig. 5A, lanes 1 to 26), but not in cell lines of T lymphoid, myeloid, erythroid, fibroblastoid, neuronal, or hepatocellular origin, nor in a mammary carcinoma cell line (Fig. 5A, lanes 27 to 31, and Fig. 5B, lanes 1 to 8) (*13*). The steady-state level of *blk* expression showed no obvious correlation with the mode of B cell transformation. Expression in these cell lines is therefore not an aberrancy related to a particular process of transformation, nor is it likely to be completely responsible for the transformed phenotype. Expression of *blk* was observed in B cell precursors as well as in cell lines representative of mature B cells, suggesting that in B cell ontogeny, *blk* is expressed before the appearance of surface immunoglobulin.

The B lymphoid cell specificity of *blk* expression distinguishes it from the three other members of the *src* family that are preferentially expressed in hematopoietic cells: *lck*, *fgr*, and *hck*. In murine organs, *lck* is expressed predominantly in thymus, and in murine cell lines its expression is specific to the T cell lineage (*5*); *hck* and *fgr* are expressed predominantly in cells of the myeloid lineage (*28*). B lymphocytes contain a tyrosine kinase activity with a substrate specificity and phosphorylation kinetics that are distinct from the major activity in T cells (*29*); a principal endogenous substrate for tyrosine phosphorylation in B cells has an apparent molecular mass of 55 kD (*29*). The B cell specificity of *blk* and the predicted size of its product are consistent with these observations.

A specific association (*6*) has been demonstrated between $p56^{lck}$ and CD4 (*30*), a transmembrane protein of T cells that functions in concert with the T cell receptor in antigen–major histocompatibility complex recognition. This association is presumably mediated by a physical interaction between the cytoplasmic domain of CD4 and the unique NH_2-terminal amino acid residues of $p56^{lck}$. The protein encoded by *blk* resembles $p56^{lck}$, but possesses a distinctive NH_2-terminal amino acid sequence. On the basis of structural similarity between $p55^{blk}$ and $p56^{lck}$, the specific association between $p56^{lck}$ and CD4, and the B lymphoid specificity of *blk* expression, it is possible that $p55^{blk}$ functions in association with a B lymphoid cell–specific transmembrane protein.

The leukocyte common antigen CD45, a family of hematopoietic cell surface glycoproteins, represents a group of closely related protein-tyrosine phosphatases (*31*). As signal transduction through CD4 is amplified by specific crosslinking to CD45, a model has been proposed in which CD45 activates $p56^{lck}$ by tyrosine dephosphorylation (*32*). The existence of a B cell–specific isoform of CD45 (B220) suggests the possibility of an analogous relationship between B220 and a Src-related tyrosine kinase, perhaps $p55^{blk}$ (*33*).

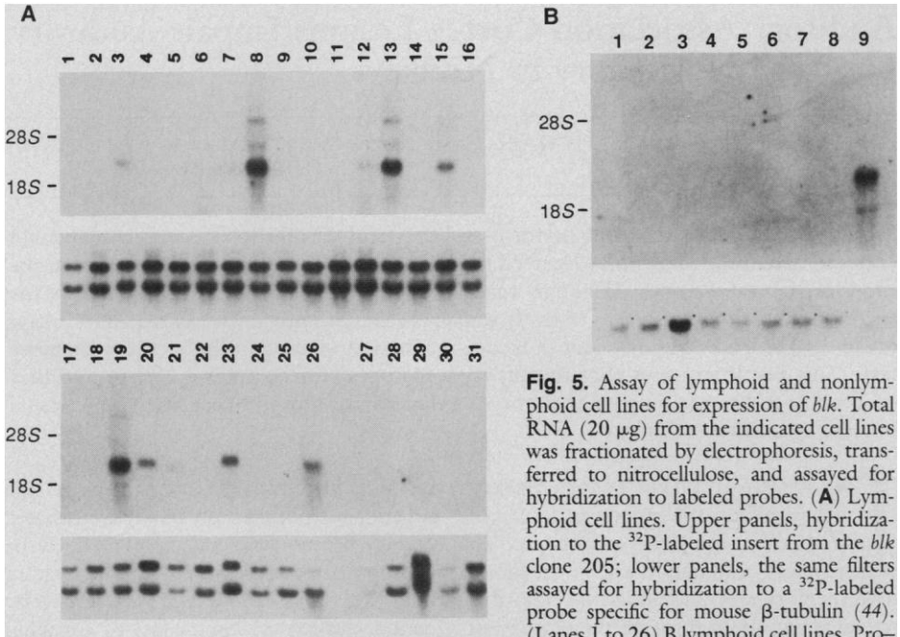

Fig. 5. Assay of lymphoid and nonlymphoid cell lines for expression of *blk*. Total RNA (20 μg) from the indicated cell lines was fractionated by electrophoresis, transferred to nitrocellulose, and assayed for hybridization to labeled probes. (**A**) Lymphoid cell lines. Upper panels, hybridization to the ^{32}P-labeled insert from the *blk* clone 205; lower panels, the same filters assayed for hybridization to a ^{32}P-labeled probe specific for mouse β-tubulin (*44*). (Lanes 1 to 26) B lymphoid cell lines. Pro–B cell lines: (lane 1) Ba/F3 (lane 2) HAFTL-1-14.5, (lane 3) HAFTL-1-14.6, (lane 4) HS1C5, (lane 5) HCR3, (lane 6) HAC6, (lane 7) H58C1, (lane 8) JP2, (lane 9) JP4, (lane 10) JP7, (lane 11) 2.1A3753, (lane 12) FE1NC3, (lane 13) FE2NC1, (lane 14) KFFTL-1, (lane 15) BAFTL-1, (lane 16) BASC2/C6, (lane 17) 22D6.5, and (lane 18) 38B9.7. Pre–B cell lines: (lane 19) PD31 and (lane 21) 70Z/3. Mature B cell lines: (lane 20) 1-7, (lane 22) M12.14.5, (lane 23) A20/2J, and (lane 26) LK. Plasma cell lines: (lane 24) SP2/0 and (lane 25) S194. The B lymphoid cell lines HAFTL-1-14.5, HAFTL-1-14.6, H58C1, HSIC5, HRC3, and HAC6 are transformed with the Harvey murine sarcoma virus; JP2, JP4, JP7, and 2.1A3753 with the murine Rous sarcoma virus; FE1NC3 and FE2NC1 with the Snyder-Theilen feline sarcoma virus; KFFTL-1 with the Kirsten sarcoma virus; BAFTL-1 and BASC2/C6 with the BALB/c murine sarcoma virus; and 22D6.5, 38B9.7, 1-7, and PD31 with the Abelson murine leukemia virus (A-MuLV) (Lanes 27 to 31) T lymphoid cell lines: (lane 27) CTLL, (lane 28) HT-2, (lane 29) EL4, (lane 30) R1.1, and (lane 31) 2B4.11. The positions of 28*S* and 18*S* RNA are indicated. (**B**) Nonlymphoid cell lines. Upper panel, hybridization to a labeled restriction fragment from *blk* clone 201; lower panel, the same filters assayed for hybridization to a rat β-tubulin probe. (Lane 1) IMEA, (lane 2) Hepa I, (lane 3) BALB/c hepatoma, (lane 4) Neuro-2a, (lane 5) C127, (lane 6) L(tk-), (lane 7) NIH3T3, (lane 8) Friend-MEL, and (lane 9) RNA from the B lymphoid cell line PD31 as a positive control. Lymphoid, myeloid, and erythroid cell lines, except for LK, 2B4.11, CTLL, and HT-2, were grown in RPMI 1640 supplemented with 10% fetal bovine serum and 50 μ*M* 2-mercaptoethanol. LK and 2B4.11 were maintained in RPMI 1640 and Eagle's Hanks amino acid (EHAA) media (1:1) supplemented with 10% fetal bovine serum and 50 μ*M* 2-mercaptoethanol. CTLL and HT-2 were grown in RPMI 1640 supplemented with 10% fetal bovine serum and murine IL-2 (5 units per milliliter). The cell lines NIH 3T3, L(tk-), Neuro-2a, and C127 were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Hepatocyte lines were grown in minimal essential medium supplemented with 10% fetal bovine serum. All cell lines are of murine origin except for the rat hepatocellular lines IMEA and Hepa I.

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12. The nucleotide sequences of murine *lck*, v-*abl*, chicken c-*src*, and v-*yes* were aligned within a highly conserved region corresponding to codons 398 to 410 and 416 to 426 of *lck*. Three degenerate oligonucleotides, complimentary to codons 400 to 409 and 418 to 424 of *lck* and the equivalent positions in

c-*src*, v-*yes*, and v-*abl* were synthesized by the phosphotriester method on an automated synthesizer (Applied Biosystems 380B). The sequences of the oligonucleotides were: 5'GG (T/G)GC (T/G/A/C)GT CCA (C/T)TT (A/G)AT (G/C)GG3' (SD11); 5'GT CCA (C/T)TT (A/G)AT GGG (A/G)AA (T/C)TT (G/A)GC3' (SD12); and 5'CCA (C/G)AC (G/A)TC (T/C)GA CTT GAT (G/T)G3' (SD13). For screening of the cDNA library, oligonucleotides were labeled with [γ-^{32}P]adenosine triphosphate by T4 polynucleotide kinase to specific activities of 1×10^9 to 2×10^9 cpm/μg.
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Auditory Association Cortex Lesions Impair Auditory Short-Term Memory in Monkeys

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Monkeys that were trained to perform auditory and visual short-term memory tasks (delayed matching-to-sample) received lesions of the auditory association cortex in the superior temporal gyrus. Although visual memory was completely unaffected by the lesions, auditory memory was severely impaired. Despite this impairment, all monkeys could discriminate sounds closer in frequency than those used in the auditory memory task. This result suggests that the superior temporal cortex plays a role in auditory processing and retention similar to the role the inferior temporal cortex plays in visual processing and retention.

DAMAGE TO THE INFERIOR TEMPORAL cortex, the highest order visual cortex, impairs performance on both visual discrimination and visual short-term memory tasks (*1*), indicating that this region is important for both the perception and memory of visual information. Although there is some evidence that damage to the superior temporal cortex, the highest order cortex of the auditory system, impairs auditory discriminative functions (*2*), there has been no convincing evidence that such lesions cause deficits in auditory short-term memory (*3*).

In the few studies that have addressed this issue, monkeys were trained on tasks in which the sample stimuli were acoustic and the comparison stimuli were either visual or spatial. The absence of any clear auditory memory impairments after lesions to the superior temporal cortex may have resulted because the monkeys coded and retained the appropriate comparison stimulus rather than the sample stimulus during the delay period, thereby solving the task by engaging visual or spatial, rather than auditory, memory processes (*4*). To ensure that auditory memory is being assessed, both the sample and comparison stimuli must be auditory. Although most attempts at training monkeys in this manner have met with failure (*5*), we succeeded by using monkeys that had extensive experience discriminating complex auditory stimuli (*6*). We report now that when monkeys are trained in such a task, lesions of the superior temporal cortex severely impair their auditory short-term memory, yet have no effect on their visual memory.

Three *Cebus apella* monkeys, ranging in age between 7 and 9 years, were trained on a successive auditory delayed matching-to-sample (DMS) task with a 3676-Hz high-frequency tone (HT) and a 243-Hz low-frequency tone (LT) as stimuli (*7*). At the end of a 20-s intertrial interval the sample

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