SEQUENCE ANALYSIS AND EXPRESSION OF AN X-LINKED, LYMPHOCYTE-REGULATED GENE FAMILY (XLR)

By JEFFREY N. SIEGEL,* C. ALEXANDER TURNER,‡ DENNIS M. KLINMAN,¶ MILES WILKINSON,§ ALFRED D. STEINBERG,¶ CAROL L. MacLEOD,§ WILLIAM E. PAUL,‖ MARK M. DAVIS,‡ AND DAVID I. COHEN*

*From the *Laboratory of Chemical Biology, National Institute of Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892; the ‡Department of Medical Microbiology, Stanford University Medical School, Stanford, California 94305; the §University of California Cancer Center and the Department of Medicine, University of California, San Diego, California 92093; the ‖Laboratory of Immunology, National Institute of Allergy and Infectious Diseases; and the ¶Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, Maryland 20892*

The existence of X-linked immune deficiency diseases in humans and mice indicates that genetic loci on the X-chromosome play an important role in the growth and differentiation of lymphoid cells. Certain of these diseases, such as Bruton's agammaglobulinemia (1) in humans or *xid* disease in mice (2) primarily affect the B cell lineage, while certain others, such as severe combined immunodeficiency disease (SCID)[1] and Wiskott-Aldrich syndrome also affect T lymphocytes. The nature of the gene defects causing these diseases has previously been uncertain. Experiments in the mouse X-linked immunodeficiency disease, *xid*, have demonstrated a growth disadvantage selectively in B lymphocytes carrying the *xid* gene (3, 4). Similar experiments in human X-linked agammaglobulinemia have addressed the question of whether there was selective expression of the defective gene in the affected cell type, i.e., B lymphocytes. It was found that, in heterozygous carriers, circulating B lymphocytes had predominantly inactivated the abnormal X chromosome, while T lymphocytes and other peripheral blood cells had a random inactivation of either X chromosome. This observation has been confirmed using isoenzyme analysis (5) as well as methylation-sensitive restriction fragment length polymorphisms (6). These data suggest that certain X-linked genes are expressed in a cell type–specific manner, and play a role critical for lymphocyte development. In the mouse, an X-linked gene family has been identified, termed XLR, which is transcribed selectively in

This work was supported in part by grants from the Clayton Foundation for Research, California Division (to C. L. MacLeod), the National Institutes of Health (CA-37778, to C. L. MacLeod and AI-20320, to M. M. Davis), and the March of Dimes Birth Defects Foundation. M. Wilkinson is supported by NIH grant T32-H107107. C. L. MacLeod is a Clayton Foundation Investigator, and M. M. Davis is a Pew Foundation Scholar. J. N. Siegel's current address is Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892.

[1] *Abbreviations used in this paper:* IF, intermediate filaments; SCID, severe combined immune deficiency.

lymphoid cells (7, 8). XLR expression is highly correlated with characteristics of a mature phenotype in B lymphoid cells. Analysis of restriction fragment length polymorphisms indicates that the *xid* immune deficiency mutation and members of the XLR gene family are tightly linked (7).

Here we report further studies characterizing expression of the XLR gene family in lymphoid cells. By analyzing XLR cDNA clones generated from B-lineage tumors and from thymic tissue, we find a single major XLR transcript expressed by both B and T lymphocytes. Sequence analysis demonstrates that this XLR transcript is capable of coding for a protein of 24,000 mol wt. The predicted amino acid sequence displays significant homology to the nuclear envelope constituents lamins A and C, and to the intermediate filament protein keratin. Studies of clonal T lymphocyte tumors arrested at different stages of maturation suggest that expression of XLR is developmentally regulated in the T cell lineage in a manner analogous to that described for tumors of the B cell lineage (8).

Materials and Methods

Preparation of RNA and DNA. Total cellular RNA was prepared from cell lysates homogenized in guanidinium thiocyanate using the method of Chirgwin et al. (9). mRNA was obtained by passing total RNA over an oligo(dT)-cellulose column and eluting the poly(A)$^+$ RNA with a low-salt buffer (10). High-molecular-weight DNA was prepared from mouse livers by the method of Blin and Stafford (11).

Northern and Southern Blotting. Northern blots were carried out by electrophoresis of 5 μg of poly(A)$^+$ RNA per lane on 1% agarose/formaldehyde gels and transferring to nitrocellulose filters (12). For Southern blot analyses, 5 μg of high-molecular-weight DNA was digested with a fivefold excess of restriction enzyme for 12–16 h at 37°C. Electrophoresis of digested DNA was on 0.8% agarose gels, after which the DNA was transferred to nitrocellulose filters (13). Probes were ^{32}P-labeled by the random hexamer priming method (14), and filters were hybridized in 50% formamide, 10% dextran sulfate, 5× SSPE, 1× Denhardt's solution with 0.1% SDS at 42°C for 12–16 h, and washed to a final stringency of 0.2× SSPE with 0.1% SDS at 60°C. Filters were exposed to Kodak XAR-5 film at −70°C using an intensifier screen for 24 h, or as stated. Probes used in hybridizations are as stated in the text. The T3δ probe used was from pGEM-T3δ, kindly provided by Cox Terhorst (Dana-Farber Cancer Institute, Boston, MA) (15).

cDNA Libraries. Double-stranded cDNA was synthesized from 5 μg of poly(A)$^+$ RNA (16, 17) and blunt-ended using T4 DNA polymerase. Eco RI linkers were added and cDNA was ligated into λgt10. The BALB/c adult thymocyte cDNA library was generously provided by C. Benoist (Institut de Chimie Biologique, Strasbourg, France), and the MOPC 167 library by S. Kim (Massachusetts Institute of Technology, Cambridge, MA). The BXSB.*xid* whole spleen and L10.A libraries were made in our laboratory. XLR cDNA clones were screened by hybridizing to the entire pM1 Eco RI insert, unless otherwise indicated. Positively hybridizing isolates were subcloned into pUC8 for restriction enzyme mapping. Clones were sequenced by the Sanger dideoxy chain-termination technique in M13 bacteriophage (18), or by the technique of Maxam and Gilbert (19). Sequences were analyzed using the SEQ program (20) and homologies of the predicted amino acid sequences with other known proteins were analyzed using the DFASTP program of Pearson and Lipman (21), and the Dayhoff protein sequence data bank. Hydropathicity analysis was performed using the method of Kyte and Doolittle (22).

Mice. MRL-*lpr/lpr*, MRL-*lpr/lpr.xid*, BXSB, BXSB.*xid*, CBA/N, and CBA/Ca mice were obtained from breeding colonies at NIH. The *xid* congenic strains were bred so that >90% of their genetic material was derived from the background strain.

Cell Lines. The B lymphoma cell line L10A was obtained from R. Asofsky (NIH). The

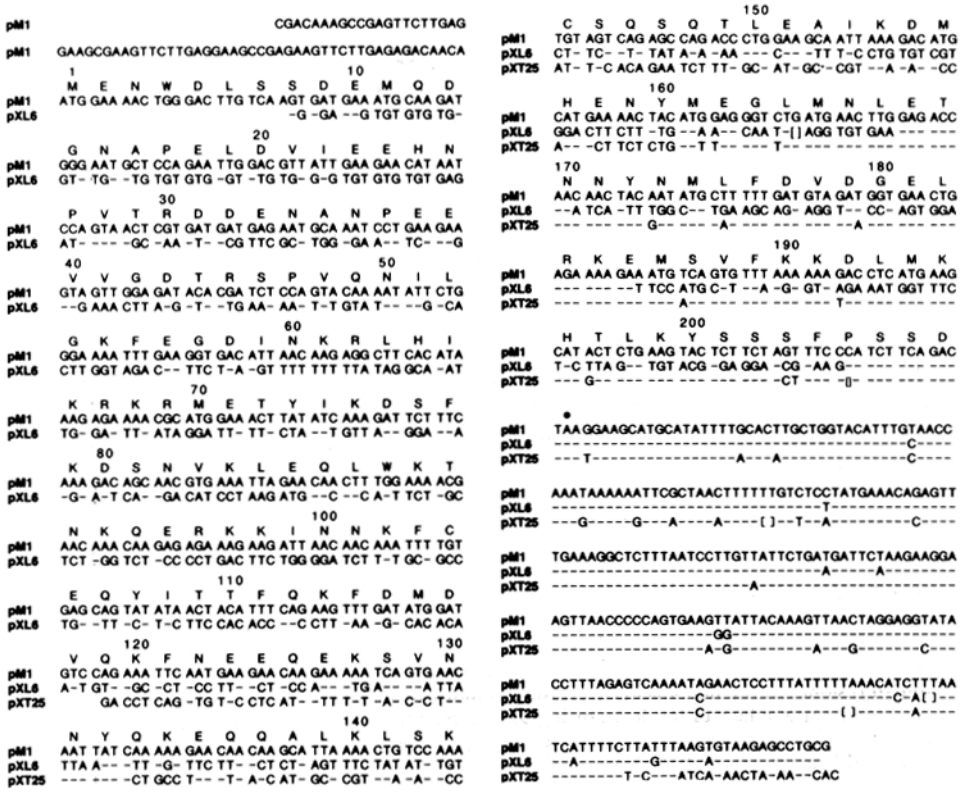

FIGURE 1. Comparison of the sequence of cDNA clone pM1 with pXL6 and portions of pXT25. The 208-amino-acid open reading frame of pM1 is shown in single-letter code above the nucleotide sequence. Neither pXL6 nor pXT25 has an open reading frame. pXL6 and pXT25 share a 3′ region with >95 and 90% homology with pM1, respectively, while the 5′ regions have no significant homology. Asterisk indicates a 13 bp insertion in pXT25: GTTTGTATCCTTG. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00639.

S49 thymoma cell line was obtained from Robert Hyman (Salk Institute, San Diego, CA). The SL12 cell lines have been described (23–25).

Results

Southern blots probed with pX310, the cDNA clone originally used to define XLR, demonstrate a family of 15–20 crosshybridizing bands (7). To determine which of these genes are expressed and the nature of the XLR gene product, we used cDNA libraries in λgt10 from a variety of cell types, and probed them for XLR cDNA clones. One clone, termed pM1, was derived from a cDNA library from the plasmacytoma MOPC167. The clone is 954 bp in length, which is several hundred nucleotides shorter than the major 1.3 kb mRNA detected on formaldehyde Northern gels (8). We interpret the difference in length to be most likely attributable to the poly(A) tail present in the mRNA but not in the cDNA clone. The DNA sequence was determined from both strands, and is shown in Fig. 1. As indicated, pM1 has a single open reading frame beginning with the ATG at base 63 and extending through base 699 with a 3′ untranslated

FIGURE 2. Hydropathicity plot of the predicted amino acid sequence of pM1 showing its highly hydrophilic nature analyzed by the technique of Kyte and Doolittle (22). Hydrophilic residues are shown above the axis; hydrophobic residues are shown below. Note that there is no extended stretch of hydrophobic residues compatible with a typical transmembrane region.

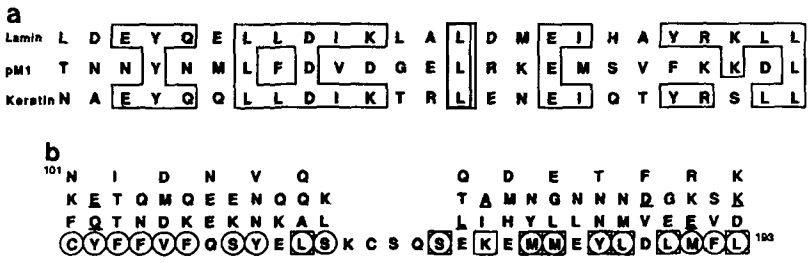

FIGURE 3. (*a*) Comparison of amino acid sequence of human lamin C (26, 27) to the predicted amino acid sequence of pM1 and to keratin (28). This region of lamin C extends from residues 356 to residue 420. Homologous residues are enclosed in boxes. (*b*) Schematic α-helical structure of pM1 emphasizing the potential hydrophobic face and its similarities with the repeating heptad structure of lamin C. The amino acid sequence is arranged with the first and fourth residues of the heptads in the lower positions. In an intact α helix, these residues would be aligned on one face of the helix owing to its 3.5-residue periodicity. Hydrophobic residues at first and fourth positions of a heptad are enclosed in a circle, and identical residues between pM1 and lamin C are underlined or enclosed in a box. While 73% of the residues of the first and fourth positions in the heptads are uncharged, only 45% are uncharged overall ($p < 0.001$) suggesting that pM1, like lamin C, may have a hydrophobic face in this α-helical domain. Note that the heptad pattern of pM1 undergoes a phase shift after residue 142 at the same position as lamin C (26).

region of 255 bases. The predicted amino acid sequence corresponding to this open reading frame is 208 amino acids, with a predicted molecular weight of 24,000. A hydropathicity analysis performed by the method of Kyte and Doolittle (22) (Fig. 2) demonstrates that pM1 encodes a predominantly hydrophilic protein with 22% acidic residues and 15% basic residues, suggesting a somewhat acidic protein (especially as there are no *N*-linked glycosylation sites). The absence of any extended hydrophobic regions or signal peptide indicates that the pM1 protein could represent a cytoplasmic or nuclear protein but not a transmembrane protein.

A computer search using the program of Lipman and Pearson revealed significant homology between the predicted pM1 protein sequence and the nuclear structural proteins lamins A and C (26, 27) and with the structural protein mouse epidermal keratin II (28), both of which are members of the intermediate filament (IF) family of proteins. Overall, there is 14.5% homology with lamin in 166 residues of overlapping sequence. The homology between the carboxy terminal α helix of lamin C, termed coil 2 (26), and pM1 is illustrated in Fig. 3*a*. 6 of 25, or 24% of the residues in this region are conserved between pM1 and lamin. Five of the six are also shared with keratin.

To determine whether the putative pM1 protein might share structural fea-

tures with IF proteins, we asked whether it had preserved the repeating heptad structure seen in all IF proteins. In the α-helical coils of IF proteins, the first and fourth residue in each heptad are hydrophobic, generating a hydrophobic face to the α helix, which has 3.5 residues per turn. Adjacent IF proteins can interact via these hydrophobic faces, allowing them to coil around each other to form a structure termed a coiled coil (26). In Fig. 3*b*, we have represented the predicted amino acid sequence of the region of pM1 corresponding to coil 2 of lamin C. The amino acid sequence has been organized by heptads analogous to the heptad structure of lamin C, with the first and fourth residues of each heptad depicted as adjacent, as they would fold in an α helix. Interestingly, it can be seen that 9 of the 16 residues shared by pM1 and lamin C occur in the first and fourth positions of each heptad. This is significantly greater than expected ($p < 0.05$) when analyzed by a two-tailed t test. Furthermore, 19 out of 26, or 73% of the residues in the first and fourth positions are uncharged, while overall only 42 out of 93, or 45%, are uncharged. This represents a highly significant difference ($p < 0.001$, $\chi^2 = 11.36$) by χ^2 analysis with one degree of freedom. The maintenance of the potential heptad structure and the conservation of hydrophobic residues suggest that portions of the pM1 protein share significant structural features with lamin C and other members of the IF family of proteins, and may on this basis assume a coiled-coil configuration in this domain.

T Cell vs. B Cell Specificity. As reported earlier, XLR mRNA is present in B cell tumors that represent mature stages of differentiation, and in normal thymus (data not shown) (8). To determine whether more than one member of the XLR gene family was transcribed in different lineages or stages of differentiation, cDNA libraries were screened with a full-length pM1 probe, and positively hybridizing isolates were characterized. Of eight XLR isolates from a BALB/c adult thymocyte cDNA library, all were of a similar length; ~1,000 bp. In addition, seven had identical restriction maps. One of these seven clones was sequenced and determined to be identical to pM1 (100% homology). To examine XLR transcripts in a B cell at a presecretory stage of differentiation, we made a cDNA library from the presecretory B cell lymphoma L10A. Of four XLR cDNA isolates examined, sequencing showed that three were identical to pM1 in the entire coding block and the untranslated flanking regions present, although the untranslated regions of the clones were truncated compared to pM1.

Rare cDNA clones were isolated which shared homology with pM1 but appeared distinct by size and by restriction mapping. pXL6 was one of four cDNA isolates from the L10A library. It was sequenced in its entirety and is compared to pM1 in Fig. 1. It is similar in length to pM1, but unlike pM1 has no open reading frame. The 3′ portion (as defined by the open reading frame of pM1) of 300 bases is >95% homologous to pM1, but has a number of nucleotide differences. The sequence similarity ends at base 690 of pM1. However, two shared octanucleotides 61 and 105 bases upstream from the region of homology were found. Based on this sequence data, probes specific for pXL6 and pM1 were designed using the Acc I–Xmn I 388 bp fragment of pXL6 and the Eco RI–Nsi I 540 bp fragment of pM1 (Fig. 4*a*). Southern blots using these specific probes were carried out using high-molecular-weight liver DNA from a variety of strains and related *xid* congenics. As seen in Fig. 4*b*, the Nsi I–Eco RI

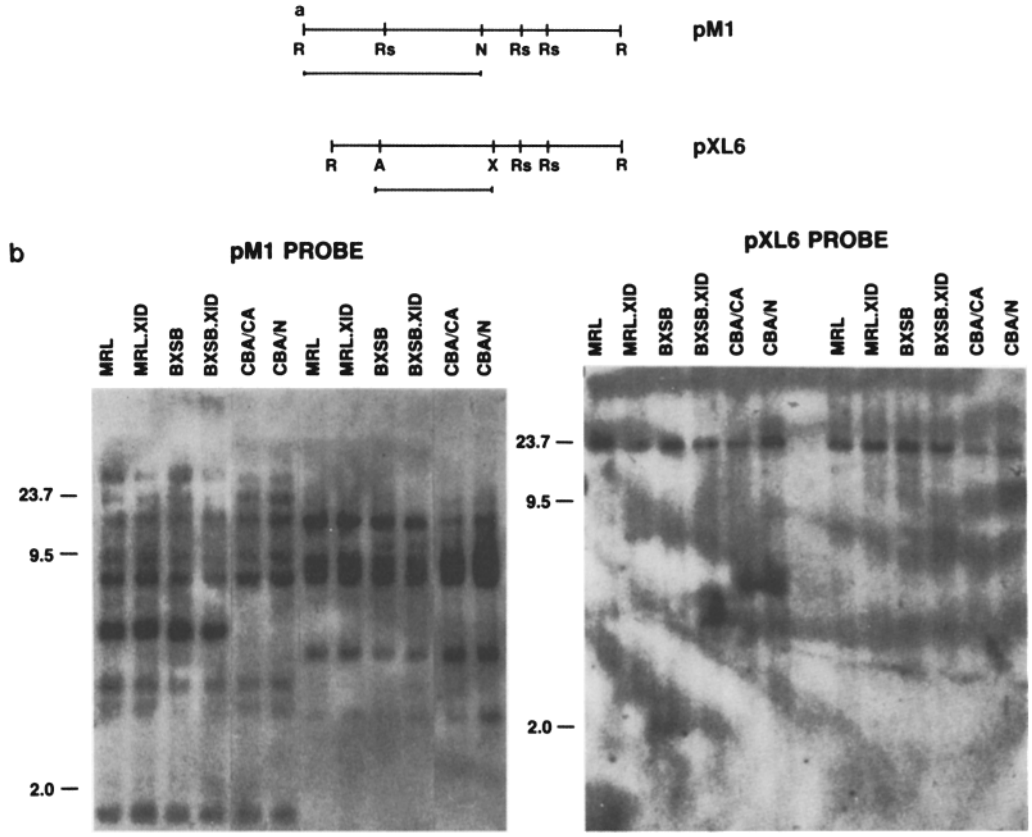

FIGURE 4. (*a*) Restriction map of pM1 and pXL6 showing the sites used to generate probes specific for each isolate. *R*, Eco RI; *Rs*, Rsa I; *N*, Nsi I; *A*, Acc I; *X*, Xmn I. (*b*) Southern blots of high-molecular-weight liver DNA from the designated strains probed with pM1-specific and pXL6-specific probes. The pM1-specific probe detects nine and six bands in the Pvu II and Bam HI digests, respectively, while the pXL6-specific probe detects only one band in each. There is one band detected with the pM1 probe at 7.5 kb in the Pvu II digest, which is polymorphic between CBA/N and normal strains. This polymorphic band is not conserved in the *xid* congenics. This finding contrasts with other polymorphisms detected using the XLR probe pX310 (4), which are retained in the *xid* congenic strains. In this figure, the abbreviations MRL and MRL.*xid* designate the strains MRL.*lpr/lpr* and MRL *lpr/lpr.xid* respectively. The filters were exposed to XAR5 film at −70°C for 3 d.

pM1 probe recognized at least nine bands in Pvu II–digested DNA, and six bands in Bam HI digests. While this probe defined a 7.5 kb Pvu II band that is polymorphic between CBA/N and other strains, this polymorphism was not retained by the *xid* congenic strains. This indicates that this particular member of the XLR gene family is not closely linked to *xid*, in contrast to other members, which are (7). Southern blots probed with the pXL6 specific probe showed only a single 23 kb band, and no polymorphisms were seen between any strains.

When these pM1-specific and pXL6-specific fragments were used to probe northern blots of poly(A)$^+$ RNA from a secretory plasmacytoma, a presecretory myeloma, and T cell tumors expressing XLR, bands were seen with the pM1 probe, but no hybridization was seen with the pXL6 probe (data not shown)

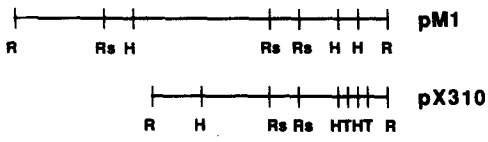

FIGURE 5. Restriction map of pM1 compared with the truncated cDNA clone pX310 derived from the T-T hybridoma 3.3T. pX310 retains two internal Rsa I sites present in pM1, but also has two new Taq I sites and one new Hinf I site, demonstrating that it is distinct from pM1. *R*, Eco RI; *Rs*, Rsa I; T, Taq I; H, Hinf I.

even in the presecretory myeloma from which the clone was derived. We conclude that pXL6 represents a very rare transcript derived from a unique element of the XLR gene family.

One of the eight cDNA clones from the thymic library appeared distinct from pM1 by restriction mapping. Sequencing of this clone, pXT25, showed that overall it is only 40% homologous to pM1 but that the central portion of the clone has a region with 90% homology (Fig. 1). This homology ends abruptly at base 560 of pM1. The two octanucleotides in the 5′ region shared between pXL6 and pM1 are in the region of homology between pM1 and pXT25 and are hence also present in pXT25. The 3′ end of pXT25 extends beyond the termination of pM1. No open reading frame was found in pXT25. The specificity or heterogeneity of these minor transcripts, while interesting, remains unproven.

In our original studies we described a cDNA clone, pX310, derived from the murine T cell hybridoma, 3.3T (7). Unfortunately, pX310 is only a 580 bp fragment of XLR, and, despite repeated attempts, we were not able to isolate its full-length equivalent. Partial nucleotide sequence analysis of pX310, however, reveals several interesting properties. First of all, its overall nucleotide homology to pM1 is 92%; this homology is global, unlike the specific regional homologies of XL6 and XT25 to pM1. This imperfect homology is unlikely to be due to the fact that pM1 and pX310 were isolated from different mouse strains (BALB/c vs. AKR) because BXSB and BALB/c do not differ for pM1 (see below). These differences in nucleotide sequence can be confirmed by specific differences in restriction sites (Fig. 5); therefore, pX310 is likely to be the product of a gene distinct from pM1. Second, the 5′ sequence of pX310 has a high degree of similarity to the open reading frame of pM1 and might encode a protein. A truncated open reading frame extends from the first nucleotide of pX310 to residue 324. Comparison of the 107 residues of this putative peptide with the related sequence of pM1 reveals five predicted amino acid substitutions and two gaps, of five and eight residues, respectively (data not shown). We conclude that T and B lymphocytes at various stages of differentiation which transcribe XLR apparently use one major common member, but also transcribe other members, at least one of which may encode a related but distinct protein.

XLR Clones from the xid Background. Earlier studies demonstrated a tight linkage between members of the XLR gene family and *xid*. Furthermore, XLR mRNA was not present in several NZB.*xid* plasmacytomas examined, whereas all plasmacytomas from nonmutant backgrounds expressed XLR (8). These findings suggested that the *xid* mutation altered the normal expression of XLR in B cell tumors, and raised the possibility that XLR transcription either might not occur or might be aberrant in the B cells of *xid* mice. We chose to analyze an *xid* congenic strain BXSB.*xid*, where the *xid* mutation had been bred onto the BSXB background relatively recently, unlike CBA/N and CBA/Ca, which are

somewhat more distantly related. We reasoned that any differences between XLR in the BXSB.*xid* strain and previously sequenced XLR genes could be compared with XLR isolates from the BXSB strain to assure that these differences were due to *xid* and not to genetic drift. A cDNA library was constructed from splenic RNA from a BXSB.*xid* mouse. One isolate hybridizing to pM1, termed pB1, was found to be of a size consistent with a full-length transcript. Sequencing showed that pB1 was identical to pM1. These results rule out the possibility that the *xid* mutation is associated with any direct change in the sequence of the major XLR transcript identified by cDNA cloning. However, they neither address the issue of XLR gene regulation in B cells, because pB1 may be derived from T cells, nor do they address the possibility of other transcripts difficult to isolate by this technique. These studies also demonstrate a high degree of sequence conservation between the BALB/c strain (from which pM1 was derived) and the BXSB strain, although XLR sequences are not particularly well conserved in other mammals, such as humans, because no bands are detected on Southern analysis of human DNA under conditions of moderate stringency (data not shown).

XLR Gene Expression in T Lymphocyte Differentiation. Previous work demonstrated that XLR is transcribed in some but not all B lymphocyte tumors. Early-stage tumors such as Abelson-transformed pre-B cell lines rarely express XLR, whereas late-stage tumors like plasmacytomas invariably express XLR. XLR expression was most reliably correlated with the absence of the B cell marker 14G8, and with the transcription of J chain mRNA; both characteristics of late-stage B cells. When XLR expression was examined in T cell tumors, we found a marked degree of heterogeneity. The T lymphomas BW5147, EL4, and S49 transcribe XLR, while SIA expresses only trace amounts of XLR mRNA (data not shown). To determine whether the pattern of XLR expression in T cells could be correlated with the stage of differentiation, we examined a series of cloned T lymphoma cell lines derived from a single AKR thymoma SL12 (23). Three of these cloned lines were used, representing cells arrested at different stages of differentiation. The clone SL12.3 is the most primitive. It is positive for Pgp-1, a marker present on early thymic precursor cells and prothymocytes but not on mature thymocytes (29), and stains brightly for surface Thy-1 but does not transcribe detectable T cell receptor β chain (TCR_β), T3δ, or T3ϵ RNA. SL12.4 is a more mature subclone of SL12 which has lost Pgp-1 expression. Unlike SL12.3, SL12.4 cells transcribe T3ϵ, T3δ, and a truncated 1.0 kb TCR_β mRNA. The clone RS4.2 was subcloned from SL12.4 by selecting in vitro for dexamethasone-resistant variants. It is more mature than SL12.4 because it transcribes a fully rearranged 1.3 kb TCR_β mRNA and stains much less brightly for Thy-1 (24, 25, 30).

A Northern blot using poly(A)$^+$ RNA from the T thymoma subclones was probed with a full-length pM1 probe. As shown in Fig. 6, there was a striking correlation between XLR mRNA expression and the stage of differentiation. The most primitive subclone had no detectable XLR mRNA of the major 1.3 kb size. Both the most mature subclone RS4.2 and the intermediate-stage clone have substantial amounts of the 1.3 kb message. Thy-1 mRNA is expressed in a reciprocal manner compared with XLR mRNA, as predicted for thymocytes at

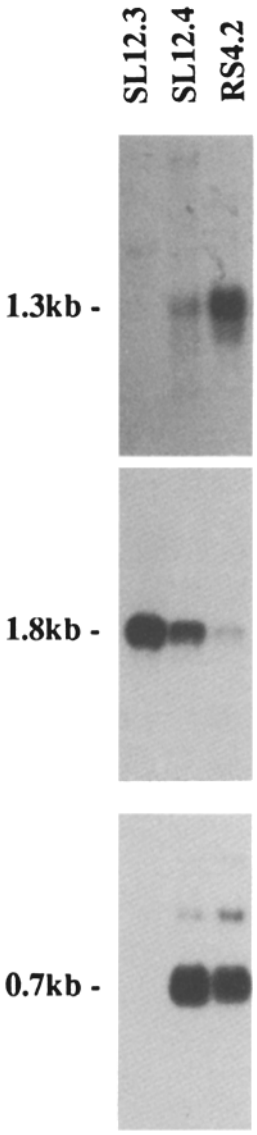

FIGURE 6. Northern blots of poly(A)$^+$ RNA from subclones of the SL12 AKR T lymphoma with phenotypes corresponding to different stages of differentiation. As detailed in the text, SL12.3 has characteristics of an early stage thymocyte, while SL 12.4 resembles intermediate and RS4.2 a later stage thymocyte. The same blot was hybridized to a full-length pM1 insert to detect XLR mRNA, then, after stripping, it was rehybridized to probes for T3δ and Thy-1. XLR mRNA expression increases with the stage of differentiation of the lymphoma lines along with levels of T3δ mRNA while Thy-1 expression decreases.

later stages of maturation (31). We conclude that, in this T thymoma lineage, XLR expression correlates with the later stages of maturity of these cells.

Discussion

In this paper, we examine the transcription of an X-linked gene family XLR, which is expressed selectively in lymphoid cells. Sequencing of several cDNA clones derived from different tissues indicates that there is a single common XLR transcript, represented by the clone pM1, which is transcribed in thymus, in B and T cell tumors, and is also represented in spleen cells. pM1 was found to have an open reading frame capable of coding for a protein of 208 amino

acids, 24,000 mol wt. Hydropathicity plots indicate that the pM1 gene product has no transmembrane or leader sequence, favoring an intracellular localization of the pM1 protein product. Computer searches for amino acid homology showed a distant but significant match with lamins A and C—protein constituents of the nuclear envelope—and with keratin, all members of the intermediate filament family of proteins. The region of homology centered on an α-helical segment of lamin comprising coil 2. This region is characterized by a pattern of repeating heptads, the first and fourth positions of which are composed of predominantly uncharged residues. This repeating motif confers a hydrophobic face on the α helix, allowing it to interact with other lamin proteins to produce a coiled-coil structure. The significance of the homologies among pM1, lamin, and keratin is strengthened by the fact that many of the conserved residues are located at these positions. These sequence similarities suggest that pM1 may also have a conformation allowing interactions with other proteins to produce coiled coils.

Studies with *xid* congenic strains have indicated a close linkage with certain members of the XLR gene family. Since *xid* disease and a number of X-linked immune deficiency diseases in humans are associated primarily with an abnormality in B lymphocytes, we asked whether there was a difference in the XLR transcripts present in T and B lymphocytes. We isolated and characterized cDNA clones from a variety of different cell lines and tissues. In the case of both normal thymus and the surface Ig-positive presecretory B cell lymphoma L10A, we found that the predominant transcript represented in cDNA libraries was pM1. Other cDNA clones were found as a minor component in these libraries, but probes specific for these genes demonstrated that they are transcribed at extremely low levels. Furthermore, sequencing these clones showed that they lacked an open reading frame. These other XLR cDNA clones represent low-level transcription of other members of the XLR gene family because specific probes localize these transcripts to unique XLR fragments on Southern analysis. S1 analysis and RNase protection experiments failed to detect T or B cell transcripts distinct from pM1 (data not shown). This result suggests that neither XL6, XT25, or any other distant member of the XLR gene family is likely to be a quantitatively important transcript in lymphocytes. However, transcripts, like pX310, that are very closely related to pM1 (such as pX310) cannot be distinguished from pM1 in this assay, and may either be rare or, for some technical reasons, difficult to clone by the procedures we used. Protein studies will be required to evaluate the presence of proteins distinct from but very closely related to pM1. While we have no evidence for a functional role for the rare transcripts XL6 and XT25, such a role cannot be ruled out, since other developmentally regulated genes like the homeotic gene bithorax are functionally important despite the absence of an open reading frame (32).

The precise relationship of the XLR gene family to the *xid* mutation remains under active investigation. Mice carrying only the mutant *xid* gene clearly do not have a deletion of the gene encoding the major XLR transcript pM1. Furthermore, an XLR cDNA clone isolated from the BXSB.*xid* strain was sequenced and found to be identical to pM1. Several possibilities remain for explaining the genetic linkage of *xid* to the XLR gene family and the abnormal expression of XLR in *xid* plasmacytomas: (*a*) *xid* may represent an abnormality

in an XLR transcript distinct from pM1 (*b*) *xid* may represent a mutation in a promoter or regulatory element of the pM1 gene or, alternatively, (*c*) *xid* may cause abnormal expression of a gene, linked to XLR, that encodes a regulatory factor that influences expression of pM1. Genetic mapping studies may help to differentiate these possibilities. In this regard the discovery of a pM1-specific restriction fragment length polymorphism between CBA/N and normal mice that does not map to *xid* (see above) may help to further localize *xid* when studied in conjunction with other XLR polymorphisms that do map to *xid*.

The expression of XLR transcripts has been shown to be regulated in B cell development. Expression is predominantly observed in B cell tumors of a mature phenotype. We sought to determine whether the pattern of expression followed the same developmental sequence in T cells as in B cells. To address this question, we studied a series of subclones derived from the AKR T lymphoma SL12. As detailed above, phenotypic markers indicate that SL12.3 is the least differentiated, SL12.4 is an intermediate phenotype, and RS4.2 is the most differentiated, based on their expression of transcripts and surface markers. RS4.2, unlike the earlier-stage clones, transcribes a mature 1.3 kb TCR_β mRNA and, on induction with phorbol esters, expresses TCR_α as well (25). Northern blot hybridization using XLR showed that only the more differentiated clones SL12.4 and RS4.2 transcribed detectable amounts of the predominant XLR message. Since these clones were all derived from a single tumor line and were grown under identical conditions, these data suggest that XLR expression may be correlated with maturation in normal thymocyte development as well as in B cells.

While an understanding of the function of the pM1 gene product will have to await further studies, the observation that the gene is expressed in both T and B lymphocytes is not inconsistent with its playing an important role in determining the phenotype of late-stage lymphocytes. While a number of differentiation markers have appeared initially to be restricted to either T or B lymphocytes, closer study has revealed that many are in fact shared. Responsiveness to IL-2 (33) and IL-4 (B cell–stimulatory factor 1) (34), expression of T cell–activating protein (TAP) (35), and even the ability to transcribe IgH (36), TCR_α, and TCR_β (37) genes are all traits now known to be characteristic of both T and B lymphocytes under certain circumstances. These data suggest a shared aspect to the control of the lymphocyte genetic repertoire. It is conceivable that a developmentally regulated gene like XLR could play a role in controlling the expression of other genes responsible for the differentiated phenotype.

In conclusion, we have studied expression of the gene family XLR in T and B lymphocytes by cloning and sequencing cDNA isolates from a variety of sources. In spite of the presence of many bands on Southern blots using a variety of restriction enzymes, we find a single gene, represented by the clone pM1, to be the major transcript in cDNA libraries from thymus, spleen, and from a secretory and a nonsecretory myeloma. Some of the additional bands appear to encode rare transcripts in T and B cells, and it is uncertain whether the remaining bands encode nontranscribed pseudogenes or transcripts in cells other than those we examined. The amino acid sequence of the predicted pM1 protein shows a distant but clear homology to lamins A and C and to other intermediate filament proteins, suggesting that the pM1 product might also form a multichain complex

with a coiled structure. Further information regarding the role of this gene family in lymphoid development will be aided by the isolation of the XLR protein from cells and the definition of its subcellular localization. We expect that a further understanding of the genomic structure and conserved regions of this gene family will lead to the isolation of a human probe that may aid in the understanding of human X-linked immunodeficiencies.

Summary

The XLR gene family consists of ~10 X-linked genes, the expression of which is regulated in lymphocyte development. Certain members of the gene family are closely linked to the murine *xid* immune deficiency mutation. Sequence analysis of a cDNA clone pM1 derived from the plasmacytoma MOPC167 showed an open reading frame capable of coding for a protein of 208 amino acids and mol wt 24,000. The lack of a signal peptide or transmembrane region indicates a probable cytoplasmic or nuclear localization for the predicted pM1 protein. The predicted protein shares significant homology with lamins A and C and other members of the intermediate filament family of proteins, and shares features important for the coiled-coil structure proposed for these proteins. Analysis of cDNA clones derived from a presecretory lymphoma and from adult thymus indicates that B and T lymphocytes transcribe a common major mRNA identical to pM1, while other rare transcripts were also identified by these studies. A series of clonal T lymphoma lines representing distinct stages of thymic differentiation showed that, as with B lymphoid tumors, XLR expression is correlated with the maturation of the thymomas.

We thank Cox Terhorst for providing the probe for T3δ and Stuart Kim for making available to us his cDNA library from MOPC167. We thank Andrew Siegel (University of Washington, Seattle, WA) for assistance with the statistical analysis of data. We also thank Mrs. Laura Barry for excellent secretarial assistance.

Received for publication 14 April 1987.

References

1. Waldmann, T. A., W. Strober, and R. M. Blaese. 1980. T and B cell immunodeficiency disease. *In* Clinical Immunology. Saunders, Philadelphia. 1:314.
2. Scher, I. 1982. The CBA/N mouse strain: an experimental model illustrating the influence of the X-chromosome on immunity. *Adv. Immunol.* 33:1.
3. Nahm, M. H., J. W. Paslay, and J. M. Davie. 1983. Unbalanced X chromosome mosaicism in B cells of mice with X-linked immunodeficiency. *J. Exp. Med.* 158:920.
4. Paige, C. J., P. W. Kincade, M. A. S. Moore, and G. Lee. 1979. The fate of fetal and adult B-cell progenitors grafted into immunodeficient CBA/N mice. *J. Exp. Med.* 150:548.
5. Conley, M. E., P. Brown, A. R. Pickard, R. H. Buckley, D. S. Miller, W. H. Raskind, J. W. Singer, and P. J. Fialkow. 1986. Expression of the gene defect in X-linked agammaglobulinemia. *N. Engl. J. Med.* 315:564.
6. Fearon, E. R., J. A. Winkelstein, C. I. Civin, D. M. Pardoll, and B. Vogelstein. 1987. Carrier detection in X-linked agammaglobulinemia by analysis of X-chromosome inactivation. *N. Engl. J. Med.* 316:427.
7. Cohen, D. I., S. M. Hedrick, E. A. Nielsen, P. d'Eustachio, F. Ruddle, A. D. Steinberg,

W. E. Paul, and M. M. Davis. 1985. Isolation of a cDNA clone corresponding to an X-linked gene family (XLR) closely linked to the murine immunodeficiency disorder *xid*. *Nature (Lond.)*. 314:369.

8. Cohen, D. I., A. D. Steinberg, W. E. Paul, and M. M. Davis. 1985. Expression of an X-linked gene family (XLR) in late-stage B cells and its alteration by the *xid* mutation. *Nature (Lond.)*. 314:372.
9. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*. 18:5294.
10. Aviv, H., and P. Leder. 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. *Proc. Natl. Acad. Sci. USA*. 69:1408.
11. Blin, N., and D. W. Stafford. 1976. A general method for the isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Res.* 3:2303.
12. Lehrach, H., D. Diamond, J. M. Wozney, and H. Boedtker. 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. *Biochemistry*. 16:4743.
13. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503.
14. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6.
15. Van den Elsen, P., B.-A. Shepley, M. Cho, and C. Terhorst. 1985. Isolation and characterization of a cDNA clone encoding the murine homologue of the human 20K T3/T-cell receptor glycoprotein. *Nature (Lond.)*. 314:542.
16. Huynh, T. V., R. A. Young, and R. W. Davis. 1985. Constructing and screening cDNA libraries in λgt10 and λgt11. *In* DNA Cloning: A Practical Approach. D. Glober, editor. IRL Press, Oxford. 1:49.
17. Gubler, U., and B. J. Hoffman. 1983. A simple and very efficient method for generating cDNA libraries. *Gene. (Amst.)*. 25:263.
18. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA*. 74:5463.
19. Maxam, A. M., and W. Gilbert. 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* 65:499.
20. Brutlag, D. L., J. Clayton, P. Friedland, and L. H. Kedes. 1982. SEQ: a nucleotide sequence analysis and recombination system. *Nucleic Acids Res.* 10:279.
21. Lipman, D. J., and W. R. Pearson. 1985. Rapid and sensitive protein similarity searches. *Science (Wash. DC)*. 227:1435.
22. Kyte, J., and R. R. Doolittle. 1982. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 157:105.
23. MacLeod, C. L., S. E. Weinroth, C. Streifinger, S. M. Glaser, and E. F. Hays. 1985. SL12 murine T-lymphoma: A new model for tumor cell heterogeneity. *J. Natl. Cancer Inst.* 74:875.
24. MacLeod, C. L., L. Minning, D. P. Gold, C. Terhorst, and M. Wilkinson. 1986. Negative trans-regulation of T-cell antigen receptor/T3 complex mRNA expression in murine T-lymphoma somatic cell hybrids. *Proc. Natl. Acad. Sci. USA*. 83:6989.
25. Wilkinson, M., and C. MacLeod. Induction of T cell receptor mRNA in SL12 T lymphoma clones. *EMBO (Eur. Mol. Biol. Organ.) J.* In press.
26. McKeon, F. D., M. W. Kirschner, and D. Caput. 1986. Homologies in both primary and secondary structure between nuclear envelope and intermediate filament proteins. *Nature (Lond.)*. 319:463.
27. Fisher, D. Z., N. Chaudhary, and G. Blobel. 1986. cDNA sequencing of nuclear

lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. *Proc. Natl. Acad. Sci. USA.* 83:6450.

28. Geisler, N., E. Kaufmann, S. Fischer, U. Plessmann, and K. Weber. 1983. Neurofilament architecture combines structural principles of intermediate filaments with carboxy-terminal extensions increasing in size between triplet proteins. *EMBO (Eur. Mol. Biol. Organ.) J.* 2:1295.
29. Trowbridge, I. S., J. Lesley, J. Trotter, and R. Hyman. 1985. Thymocyte subpopulation enriched for progenitors with an unrearranged T-cell receptor β-chain gene. *Nature (Lond.).* 315:666.
30. MacLeod, C. L., E. F. Hays, R. Hyman, and S. Bourgeois. 1984. A new murine model system for the in vitro development of thymoma cell heterogeneity. *Cancer Res.* 44:1784.
31. van Ewijk, W., P. van Soest, and G. van den Engh. 1981. Fluorescent analysis and anatomic distribution of mouse T-lymphocyte subsets defined by monoclonal antibody to the antigens Thy-1, Lyt-1, and T200. *J. Immunol.* 127:2595.
32. Gehring, W. J. 1985. The homeo box: a key to the understanding of development? *Cell.* 40:3.
33. Zubler, R. H., J. W. Lowenthal, F. Erard, N. Hashimoto, R. Devos, and H. R. MacDonald. 1984. Activated B cells express receptors for, and proliferate in response to, pure interleukin 2. *J. Exp. Med.* 160:1170.
34. Fernandez-Botran, R., V. M. Sanders, K. G. Oliver, Y.-W. Chen, P. H. Krammer, J. W. Uhr, and E. S. Vitetta. 1986. Interleukin 4 mediates autocrine growth of helper T cells after antigenic stimulation. *Proc. Natl. Acad. Sci. USA.* 83:9689.
35. Yeh, E. T. H., H. Reiser, B. Benacerraf, and K. L. Rock. 1986. Expression of T-cell-activating protein in peripheral lymphocyte subsets. *Proc. Natl. Acad. Sci. USA.* 83:7424.
36. Grosschedl, R., D. Weaver, D. Baltimore, and F. Constantini. 1984. Introduction of a μ immunoglobulin gene into the mouse germ line: Specific expression in lymphoid cells and synthesis of functional antibody. *Cell.* 38:647.
37. Calman, A. F., and B. M. Peterlin. 1986. Expression of T cell receptor genes in human B cells. *J. Exp. Med.* 164:1940.