Therefore, on $\alpha_H\gamma_R$ cells the receptor is likely expressed as an oligomer of α and γ chains, whereas for the $\alpha_H(\beta\gamma)_R$ cells, only about 20% of their expressed receptors are of the $\alpha_H(\beta\gamma)_R$ type under the conditions of our transfection. Thus, the binding properties of the cells collected in Table 1 reflect the binding properties of the mixture of $\alpha_H(\beta\gamma)_R$ and $\alpha_H\gamma_R$.

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18. The numbers of RBL cells and transfected cells in the mixtures were adjusted so that each cell type would contribute an approximately equal number of receptors. After being washed, the mixed cells were suspended in the detergent to obtain a final concentration of 2×10^7 cells per milliliter. This is a lower concentration than we usually use because the COS cells are substantially larger than the RBL cells. An appropriate cell concentration is important in order to obtain both solubilization and a cell lipid/detergent ratio that will maintain the receptors' integrity (*11, 12*). Complete medium consisted of Dulbecco's modified Eagle's medium (Biofluids, Rockville, MD) to which had been added 17% fetal bovine serum (Gibco, Grand Island, NY) and 2.0 m*M* glutamine.

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Activation of an Excluded Immunoglobulin Allele in a Human B Lymphoma Cell Line

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Mature B cells that express surface immunoglobulin (Ig) are usually committed to their original Ig product. It was shown that such a cell can replace its light chain by rearranging and expressing a new light chain from the other allele. Anti-idiotype antibodies were used to isolate idiotypic variants from a surface $IgM^+\lambda^+$ human B cell tumor line. The variants expressed a new λ light chain. Both the original and the new λ transcripts were present in the variant cells, but only the new one was expressed as a protein on the cell surface. Therefore, although the cell exhibited allelic exclusion and had only one Ig receptor at a time, the commitment to a particular light chain gene was reversible.

FUNCTIONAL IG GENES ARE CREATED during B cell differentiation by the rearrangement of discrete germ line gene segments (*1*). Once formed, these rearranged Ig genes can be further altered during clonal evolution to give rise to a diverse repertoire of antibody-producing cells. Three different mechanisms have been described by which the combining site of the Ig product of a B cell may be modified. These include ongoing Ig gene rearrangement (*2*) and replacement of the variable (V) region of previously rearranged Ig genes (*3*) in pre–B cells and point mutation in B cells (*4*). We now report an additional genetic mechanism of variation occurring in a human B lymphoma cell line, the activation and expression of the other allele.

We isolated rare variants of a surface $IgM^+\lambda^+$ cell line derived from a patient with a diffuse large cell lymphoma (*5*) that had altered idiotypic structures. Three phenotypic groups of clonally related variants were isolated by two-color cell sorting. Variants showed either a complete loss of reactivity, a partial loss of reactivity, or varied reactivity with a panel of different anti-idiotype antibodies. To confirm that these variants were indeed unique, their Igs were examined by two-dimensional gel electrophoresis. A representative clone from each of the three groups expressed heavy chains that were indistinguishable, but light chains of slightly different apparent molecular size and isoelectric mobility (Fig. 1A). Variation in the λ light chain gene was also documented by DNA blot analysis of wild-type and variant DNA with a Cλ probe. The three variants shared a rearranged band not present in the DNA of the parental cell (Fig. 1B).

To understand the molecular basis for this light chain heterogeneity, we cloned and sequenced the V-region cDNA from the parental cell and from two idiotype-negative variants. A transcript (T1) was expressed by the parental cell that coded for an unusual λ light chain protein, in that position 88 contained aspartic acid rather than the invariant cysteine residue. An identical transcript was also found in each of the variants. However, the variant cells expressed, in addition, a second transcript (T2). The V and joining (J) regions of T1 and T2 were different (Fig. 2). In addition, the constant (C) region of T2 differed at amino acid 119 from the C region of T1 by one nucleotide of the 80 bases sequenced (CCA instead of CCG).

To determine whether a T2 was expressed in the parental cell, we made probes from V-region fragments unique to each transcript (indicated in Fig. 2). These probes distinguished between V regions when tested on phage DNA containing these genes. Messenger RNA from the parental cell and from three idiotype-negative clones was examined by RNA blot analysis with T1- and T2-specific probes and with a human actin control probe (Fig. 3). T1 was expressed by all three idiotype-negative clones, as well as by the parental cell. When corrected for differences in mRNA loaded on the gel, as indicated by the actin probe, no differences were seen in T1 expression in the variants as compared to the parental cell. In contrast, a T2 was expressed only by the idiotype-negative variants and not by the parental cell.

Further evidence that the variants were expressing a λ Ig transcript different from that of the parental cell was obtained by a serological analysis of the protein. Flow cytometry was performed on the parental cell and on variants C5 and C14 with monoclonal antibody 14 D1, which differentiates between λC region allotypes $Kern^-Oz^-$ and $Kern^-Oz^+$ (*6*). The two variants both reacted with 14D1, which indicated that they used λC region Oz^-, whereas the parental cells were unreactive (Fig. 4).

All idiotype-negative clones expressed T2 even though the variants were phenotypically distinct and produced light chain proteins that migrated differently on two-dimensional gels. We compared the sequences of T2 from idiotype-negative variants C14 and C5 (Fig. 2). The two clones had identical V, J, and C regions. The sequences differed from each other by seven nucleotides at the V-J junction, resulting in two amino acid differences between the two proteins. Except for this junctional difference, the V-region sequences of the T2 transcripts were identical. Likewise, V-region sequences of T1 transcripts from the parental cells, from an idiotype-positive clone, and from the two variants were all identical. Therefore, there was no evidence for somatic hypermutation in this cell line.

We have shown that a mature B cell can rearrange a second light gene at a different allele even though it had originally produced a functional surface Ig product. Although rearrangements often occur during B cell development on both Ig alleles, the expressed Ig protein is usually produced from only one allele (*7*). This phenomenon has been referred to as allelic exclusion (*8*). In

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the case of the light chain, expression of a surface Ig molecule has been postulated to mediate a feedback inhibition on subsequent rearrangements (*9*). However, recent experiments in transgenic mice have shown that an endogenous λ chain can be expressed in lymphoid cells that express a κ transgene, thus challenging the negative feedback theory (*10*). Similarly, endogenous μ chain expression has been documented in a cell expressing a rearranged transgenic heavy chain (*11*). In the cell line described here only one Ig light chain protein was produced by the cells. The parental cell expressed the protein encoded by T1, whereas the variant cells expressed the protein encoded by T2 (Fig. 4), even though the two transcripts were both present in the variants (Fig. 3). Thus, at the protein level, allelic exclusion was obeyed. We do not know why a second light chain gene rearrangement occurred in the variants. However, one possible explanation is that surface Ig-negative cells arose, releasing the feedback inhibition on subsequent rearrangement. Indeed, we have observed surface Ig-negative intermediates in a mature murine B cell line that undergoes continuing light chain gene rearrangement (*12*).

The new rearrangements carried out by two different idiotype-negative clones were independent events because the V-J junctions were different (Fig. 2). The enzyme terminal deoxynucleotide transferase (TdT) (*13*) has been postulated to add extra bases at both heavy chain (*14*) and light chain (*15*) rearrangement joints. However, TdT was not detectable in these cells either at the protein or the RNA level. It is possible that these differences were derived from the 3′ end of the gene segment and retained during the V-J joining process (*16*).

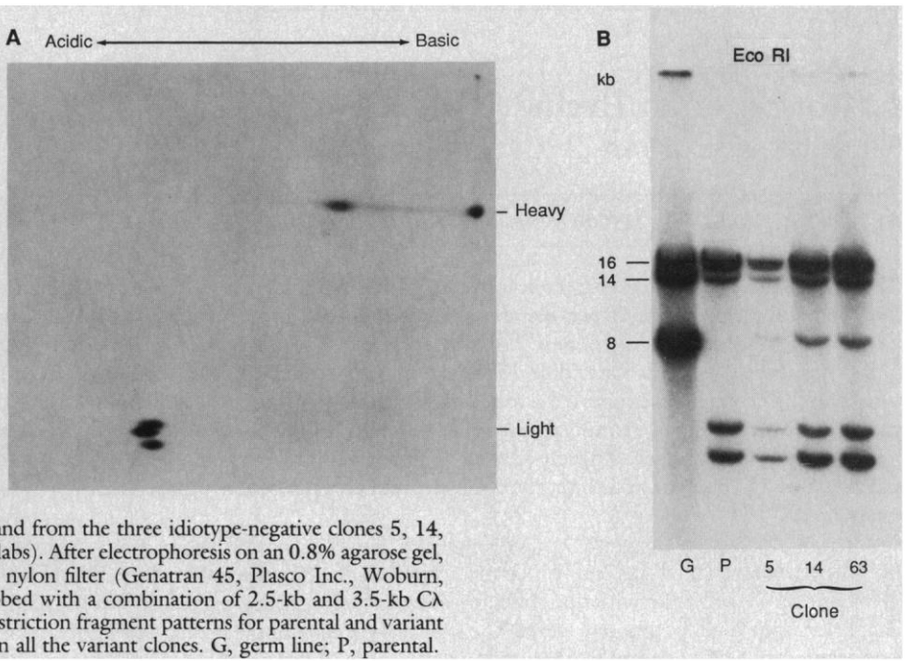

Fig. 1. Light chain differences between parental and idiotype-variant cells. (**A**) Two-dimensional gel electrophoresis of Igs from variant clones. Three idiotype-negative variants were labeled with ^{125}I by the lactoperoxidase (Sigma) method (*19*). After they were labeled, cells were lysed in buffer containing 0.5% NP-40, 10 m*M* tris, *p*H 8.0, 0.2% NaN_3, 1 m*M* phenylmethylsulfonyl fluoride, 1.0 μg of pepstatin A per milliliter, and 0.1 unit of aprotinin per milliliter. Cell lysates were immunoprecipitated with a staphylococcal protein A–antibody to human μ complex, and separated by polyacrylamide gel electrophoresis (PAGE) in a nonequilibrium *p*H gradient (ampholine range *p*H 3.5 to 10.0) followed in the second dimension by reducing SDS–12.5% PAGE. Autoradiographs of three gels were superimposed. Heavy chains and a more basic contaminant from all three variants migrated identically, but light chains migrated differently in both dimensions. (**B**) DNA blot analysis of λ light chain locus. DNA was extracted (*20*) from parental cells and from the three idiotype-negative clones 5, 14, and 63 and digested with Eco RI (New England Biolabs). After electrophoresis on an 0.8% agarose gel, DNA was transferred (*21*) to a Genatran-activated nylon filter (Genatran 45, Plasco Inc., Woburn, Massachusetts), baked at 80°C, hybridized, and probed with a combination of 2.5-kb and 3.5-kb Cλ region probes (*22*) labeled with ^{32}P (*23*). Identical restriction fragment patterns for parental and variant clones were seen, except for a new 8-kb band seen in all the variant clones. G, germ line; P, parental.

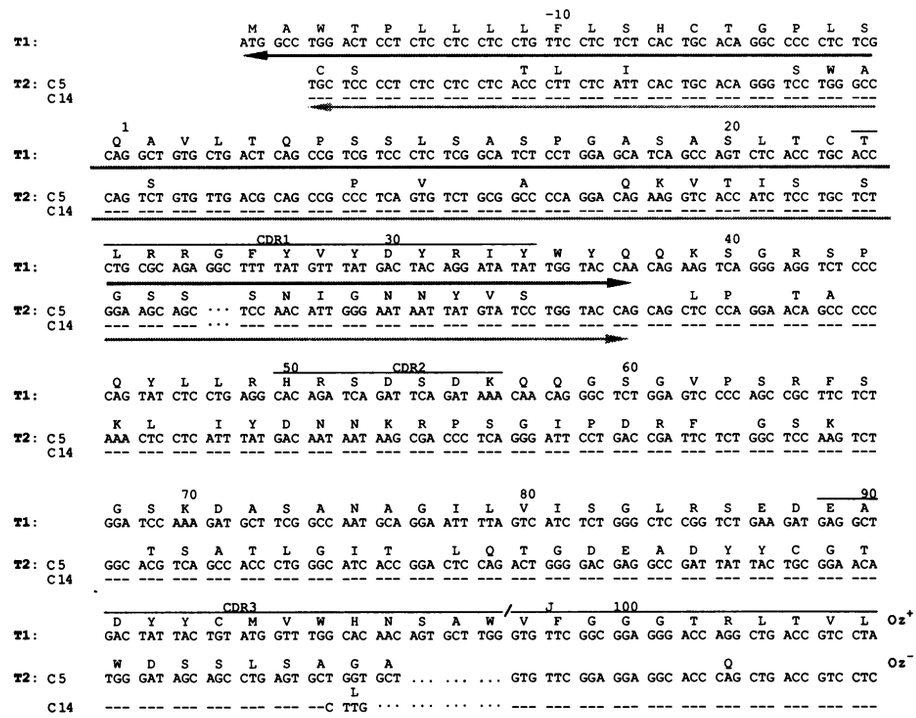

Fig. 2. Sequences of λ light chain V region genes expressed in cell clones. Total RNA was isolated from the parental population and from two idiotype-negative clones by the guanidinum isothiocyanate method (*24*). Polyadenylated mRNA was selected by passage over an oligo(dT)-cellulose (Collaborative Research) column (*25*). Complementary DNA was produced by using a 16-nucleotide 5′ λC region primer as previously described (*26*) and ligated into M13mp19 or M13mp18 (*27*). Plaques were screened with ^{32}P-labeled, 2.5-kb and 3.5-kb Cλ-, T2-, or T1-specific probes. Positive clones were sequenced by the dideoxy method (*28*). Sequences for T1 isolated from parental cells, idiotype-positive clone, and idiotype-negative clone 14 were all identical. T1- and T2-specific probe fragments are underlined by arrows. Amino acids are numbered in accordance with Kabat *et al.* (*29*). Complementarity determining regions (CDRs) are indicated as well as the J region. Reactivity of the T1 and T2 protein products with a monoclonal antibody distinguishing Oz^+ and Oz^- is indicated.

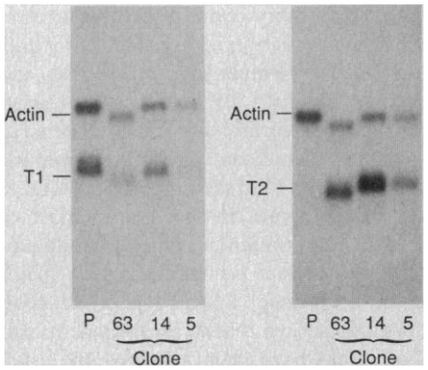

Fig. 3. Expression of T1 and T2 by cell clones. Polyadenylated RNA was separated by electrophoresis on a 2.2*M* formaldehyde denaturing gel and transferred to nitrocellulose (*30*). After baking and prehybridization, the filter was probed successively with ^{32}P-labeled T2, T1, and actin and was washed at 80°C for 30 min in 0.1% SDS and 0.1% saline sodium citrate (SSC) between probes. RNA hybridizing with T1 was present in parental cells and in idiotype-negative clones, but T2 was expressed only in idiotype-negative clones. P, parental.

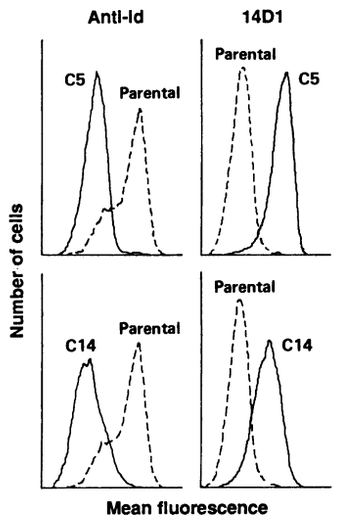

Fig. 4. Expression of Oz and idiotypic determinants by cell clones. Parental cells (dashed lines) and variants C5 and C14 (solid lines) were examined by indirect immunofluorescence with anti-idiotype (anti-id) and monoclonal antibody 14D1 (directed against Oz$^-$) and analyzed with the FACS 440 (Becton Dickinson). Fluorescence intensity is displayed on a log scale.

The two different idiotype-negative clones rearranged the same V-region gene. This implies that Vλ gene usage by the tumor variants was restricted. Preferential V gene usage has been described for the heavy chain locus in normal B cells during murine (*17*) and human (*18*) fetal development. In these cases the preferred heavy chain V-region genes (V_H genes) were those proximal to the J region gene cluster. Mapping of the human Vλ genes may provide an explanation for the repetitive use of one particular Vλ gene by this tumor.

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Regulation of Lymphokine Messenger RNA Stability by a Surface-Mediated T Cell Activation Pathway

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Quiescent T cells can be induced to express many genes by mitogen or antigen stimulation. The messenger RNAs of some of these genes undergo relatively rapid degradation compared to messenger RNAs from constitutively expressed genes. A T cell activation pathway that specifically regulates the stability of messenger RNAs for the lymphokines interleukin-2, interferon-γ, tumor necrosis factor–α, and granulocyte-macrophage colony-stimulating factor is induced by stimulation of the CD28 surface molecule. This pathway does not directly affect the steady-state messenger RNA level, transcription, or messenger RNA half-life of other T cell activation genes, including c-*myc*, c-*fos*, IL-2 receptor, and the 4F2HC surface antigen. These data show that stimuli received at the cell surface can alter gene expression by inducing specific changes in messenger RNA degradation.

ALTHOUGH MUCH HAS BEEN learned about the transcriptional regulation of gene expression (*1*), relatively little is known about how mRNA stability contributes to gene expression (*2*). In both prokaryotic and eukaryotic cells some mRNAs are relatively short-lived while other mRNAs are extremely stable (*2*). In bacteria, many of the genes that encode short-lived mRNAs are responsive to growth conditions (*3*), a short mRNA half-life possibly allowing for rapid modulation of the expression of cell growth–associ-

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