Inducing the Loss of Immunoglobulin \( \lambda \) Light Chain Production and the Rearrangement of a Previously Excluded Allele in Human Plasma B Cell Lines with Concanavalin A*

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We investigated the expression of differential \( \lambda \) light chains in human B cell lines secreting immunoglobulin (Ig). When these cell lines were cultured with concanavalin A for a long period of time, a subpopulation of some but not all of these cell lines was induced to express new \( \lambda \) light chains replacing the original \( \lambda \) chain (light chain shifting). Production of the new \( \lambda \) chain, which replaces the original \( \lambda \) chain, results from a V\( J \) rearrangement at a previously excluded allele and a dramatic reduction of the original \( \lambda \) chain transcript, although no difference was found in the level of heavy chain transcript. Recombination activating genes RAG-1 and RAG-2, which are normally expressed during specific early stages of lymphocyte development, were expressed in not only the light chain-shifting-inducible lines but also in the non-inducible cells. Treatment of these Ig secreting cell lines with dibutyryl cAMP, which is known to enhance expression of the RAG genes, could not induce the creation of new \( \lambda \) light chain-producing cells from the inducible lines, suggesting that the expression of the two RAG genes is not sufficient for inducing new \( \lambda \) light chain production. Concanavalin A induced a gradual but significant production loss of the original \( \lambda \) chain in a subpopulation of the light chain shifting-inducible cells but not in the non-inducible cells. Association of new \( \lambda \) light chain production with loss of original \( \lambda \) chain raises the possibility that, when the RAG genes are expressed, concanavalin A may act on a novel intracellular mechanism controlling \( \lambda \) light chain allelic exclusion in these plasma cell lines.

During the differentiation of B lymphocytes, gene segments on the immunoglobulin (Ig) heavy and light chain loci assemble in a defined ordered manner (1). The differentiation of B lymphocytes progress from stem cells to B cell precursors (pro-B), undergo heavy chain Ig rearrangement (pre-B), and light chain Ig rearrangement (surface IgM*, light chain-expressing mature B cells), and finally become immunoglobulin secreting cells (plasma). Each B cell encodes for a single heavy chain and a single light chain. This so-called "allelic exclusion" is thought to be important in conferring on the B cell receptor a high degree of antigen specificity, which is critical for providing antibody monospecificity. Expression of membrane-bound \( \mu \) heavy chains mediates allelic exclusion at the Ig heavy chain loci via feedback inhibition (2, 3). Although light chain rearrangement is believed to be blocked by the expression of cell surface Ig (sIg), which results from the assembly of an \( \mu \) heavy chain and a light Ig chain (4, 5), recent findings suggest that light chain gene rearrangement often continues in pre-B and immature B cells (6–8). It remains to be elucidated when secondary rearrangement in the light chain loci occurs. The molecular mechanisms of allelic exclusion for the Ig light chain have not been well characterized.

Recently, when screening for variant Ig-secreting cells that are resistance for the cytotoxic effect of concanavalin A (ConA) to isolate a glycosylation mutant, we found that secretion of various new \( \lambda \) light chains which replaces the original \( \lambda \) chain occurs at a high incidence, and that this differential \( \lambda \) light chain expression can lead to an alteration of antigen binding specificity (9). In addition, the nucleotide sequence of one of the new light chain's \( V \) \( \lambda \) gene segment is 90% homologous with a \( \text{V}^{\text{VIII}} \) germline sequence which was recently recognized as a new \( V \) region subgroup of the human \( \lambda \) light chain (44). The functional importance of \( \text{V}^{\text{VIII}} \)-related molecules has been shown in their preferential association with certain types of autoantibodies, i.e. rheumatoid factors (45). In this report, we investigated the molecular basis for differential \( \lambda \) light chain expression in human plasma cell lines. Several factors have been shown to be involved in the timing of when Ig gene rearrangement occurs. Components of the recombinational machinery necessary for Ig rearrangement include the recombinating genes RAG-1 and RAG-2 (10). These genes are normally expressed only when B cells rearrange the Ig heavy and light chain loci during the early stages of B cell differentiation, but not in mature and plasma cells (11, 12). Recently, RAG gene expression has been found in mature B cells, indicating that the control of the expression of these two genes is complex and not well understood (43). RAG-1 and RAG-2 genes are expressed in the HB4C5 and its donally related cell lines including the Ig-secreting fusion partner line NAT-30. However, expression of the RAG genes alone was shown to be not sufficient to induce new \( \lambda \) light chain expression in these Ig-secreting cell lines. Other factors such as short regions of homology between the two recombining coding ends, quality of the octamer motif in the promoter, and transcriptional activation of a rearranging gene segment have also been shown to be a requirement for Ig rearrangement (38–41).

Here we describe a new phenomenon for \( \lambda \) light chain expression in Ig-secreting cells whereby the loss of original \( \lambda \) light chain production, which results from a reduction of the transcript level, is inducible by ConA and may trigger rearrangement of new \( \lambda \) light chain gene on a previously excluded allele. Stimulation with ConA may act on the mechanism controlling the expression of a \( \lambda \) light chain from an originally rearranged allele in the Ig-secreting cell lines. Our findings provide a
possible mechanism by which the original \( \lambda \) light chain is replaced with a new \( \lambda \) light chain in antibody-secreting cells.

### MATERIALS AND METHODS

**Cells**—The human hybridoma HB4C5, which secretes an antibody reactive to human lung adenocarcinoma cells, was generated by fusing a B lymphocyte with a NAT-30 cell (13), which secretes IgM and was originally established from a human Burkitt lymphoma cell line Namalwa (14). Concanavalin A-resistant clones of the HB4C5 were isolated originally established from a human Burkitt lymphoma cell line Nama-

**Conjugation**—Anti-human IgM and CA4 were dissolved in deionized water.

**Cell Stimulation**—Cells were cultured in a 5% CO\(_2\) atmosphere at 37°C in the presence of either 2 \( \mu \)g/ml ConA or 1 \( \mu \)g/ml dibutyryl cAMP (Bt2cAMP). The cells were then harvested at specific time points. ConA and Bt2cAMP were purchased from Sigma, ConA and Bt2cAMP were dissolved in deionized water.

**Ig Secretion Assay**—Cells were plated in triplicate at 5 \( \times \) 10\(^5\) cells/ml in a 35-mm culture dish. Culture supernatants were harvested at 3 and 5 days and assayed for the presence of IgM using the enzyme-linked immunosorbent assay using horsedradish peroxidase (HRP)-\(^1\)conjugated antibody to human IgM (Biosource).

**Western Blot Analysis**—Cytoplasmic extracts were prepared essentially as described (18). Briefly, cell extracts from each cell line were prepared by one cycle of freeze-thaw lysis (1 \( \times \) 10\(^5\) cells) in phosphate-buffered saline (PBS) containing 1 mM phenylmethylsulfonyl fluoride. The resulting lysate was spun for 5 min at 4°C, and the supernatant containing the whole cell extract was stored for further use. Cell extract samples from supernatant of each cell extract were bled for 5 min in sample buffer, electrophoresed on SDS-polyacrylamide gels (10%), and trans-ferred to a nitrocellulose membrane (19). The blotted membranes were washed in PBS containing 0.05% Tween 20 and incubated in a 1:500 dilution of HRP-conjugated goat anti-human \( \lambda \) light chain for 1 hour. The membranes were washed in PBS containing 0.05% Tween 20 and developed with 1\( \times \) 4-chloro-1-naphthol, 0.01% \( \mathrm{H}_2\mathrm{O}_2\) in PBS with 20% methanol.

**Flow Cytometric Analysis**—Cultured cells were washed in PBS, then stained on ice with the appropriate monoclonal antibodies for 30 min in 100 \( \mu \)l of PBS and the relative fluorescence intensity was detected by flow cytometry (Coulter). Antibodies used were as follows: mouse anti-CD19 conjugated to fluorescein isothiocyanate (FITC) (Becton Dickinson & Co.), mouse anti-CD21/FITC (Biosource), mouse anti-CD23/FITC (Nichirei Corp., Tokyo, Japan), and goat anti-human \( \mu, \kappa, \) and \( \lambda \) chain antibodies/FITC (Biosource). FITC-conjugated mouse or goat IgG (Bio-source) alone was used as a negative control.

**PCR Amplification**—Total RNA from equal numbers of cells was subjected to \( \beta \)DNA synthesis, and 1 \( \mu \)g of \( \beta \)DNA was amplified using specific primers. Each \( \beta \)DNA was synthesized from total RNA using a kit (Life Sciences) according to the instructions provided by the manufacturer. Each \( \beta \)DNA was amplified using the appropriate primer pairs: for the RAG-1 gene, 5'-RAG1 and 3'-RAG1 (12), 5'-ACTACTCGAG-GCTTCGACGTGTA(GTCTAC-3') (sense) and 5'-ACTAAAGCTTCT-GAGGTTGTCATG-3' (antisense); for the RAG-2 gene, primers 5'-RAG2 and 3'-RAG2 (20), 5'-ATACCTGGTGTACGGGAAA-3' (sense) and 5'-CAAGCCCTTTTGTCAAGAAG-3' (antisense); for the \( \beta \)-actin gene, 5'-ACT-5'-GAAATCGTGTCGATGACATAG-3' (sense) and 3'-Act, 5'-CCAAGGTTACTACAGGAGGAG-3' (antisense).

**DNA Analysis**—Genomic DNA was prepared as described (22). One ng of genomic DNA was used for the PCR assay. PCR to detect V\( \lambda \)S to \( J \) \( \lambda \) or V\( \lambda \)A to \( J \) \( \lambda \) coding joint was done using the following primers: PV\( \lambda \)C5 (\( \lambda \)C5-CDR1 region specific), 5'-AACAGCTCTCAACATTGG-GAA-3' (sense) or PV\( \lambda \)CA2 (CDR1 region of \( \lambda \)CA2 specific), 5'-ACTCT-CTCAGGATGGGACG-3' (sense), and \( \lambda \)J (conserved 3' of \( \lambda \)J), 5'-TCAAGTTATCCCTTGCCC-3' (antisense). PCR reactions were done as described above for reverse transcriptase-PCR. PCR product sizes are for V\( \lambda \)C5-J \( \lambda \), 251-bp, and for V\( \lambda \)CA2-\( \lambda \), 258-bp. Reaction products were run on 0.8% agarose gels, followed by Southern blot hybridization. The PCR products were transferred to a nylon transfer membrane (Hybond-N\( ^{-} \), Amersham) and hybridized with \( ^{32} \)P-radiolabeled probes. DNA fragments coding for either \( \lambda \)CS or \( \lambda \)CA2, which were used for analyzing the variable region sequence, was digested from their respective vectors with EcoRI and PstI restriction enzymes. The resulting 0.8-kb paternal fragment containing the appropriate \( \lambda \) gene was used as probes for detection of the V\( \lambda \)C5-\( \lambda \) or V\( \lambda \)CA2-\( \lambda \) coding joint. To check for possible Taq polymerase errors, all PCR products were sequenced and compared with defined sequences (9).

**Results**

### Differential Expression of \( \lambda \) Light Chains Induced by ConA in a Namalwa Subline

We have previously shown that various new \( \lambda \) light chains are expressed by ConA-resistant variant clones derived from HB4C5 cells (termed CA1, CA2, CA3, and CA4) as shown in Fig. 1 (9). All of these new \( \lambda \) light chain producing variant cells secrete only one new light chain species

![Fig. 1. Secretion of \( \lambda \) light chains by Namalwa and its clonally-related cell lines](image)
which is different in size from the original species. HB4C5 is a hybriroma line generated by the fusion of a B lymphocyte with the NAT-30 cell line, which is a 6-thioguanine-resistant variant clone of the human Burkitt lymphoma line Namalwa. Namalwa, NAT-30, and HB4C5 cell lines secrete the same 32-kDa light chain protein, of which its relatively larger size is due to a N-glycosylation located at the Asn-25 site in the variable region (21). To find out if expression of a differential λ light chain can be expressed in Namalwa and NAT-30 cells as well as in HB4C5 cells, selection for ConA-resistant cells was performed on both lines as described previously (15). The cells were seeded in 96-well culture plates and cultured in ConA-containing medium for 1 month. Supernatants from wells containing ConA-resistant cells were chosen randomly and run on SDS-polyacrylamide electrophoresis gels followed by immunoblotting using an anti-λ chain antibody (Fig. 2). ConA resistant NAT-30 cells not only secreted the original light chain species, but also λ chains of different sizes (Fig. 2A). To determine whether a single cell clone can secrete more than one λ light chain, we subcloned these new λ chain-expressing cells using the limiting dilution method. No ConA resistant NAT-30 subclone was able to produce a new λ light chain together with the original 32 kDa-λ chain species (data not shown). Similar results have been found in the ConA-resistant HB4C5 subclones (9). These distinct light chain forms are a result of size differences between the core peptides, not from N-glycosylation as evaluated by lectin blot analysis (data not shown). In contrast, differential λ light chain expression was not observed in Namalwa cells even after being cultured under the same conditions (Fig. 2B).

Immunologic Phenotypes of the Differential λ Light Chain Expressing Variant Clones and Other Clonally Related Cell Lines—All of these cell lines secrete IgM in amounts ranging from 46 to 1400 ng/ml in 5 days (Table I). In contrast, the amount of Ig in the culture supernatants of the mature B cell line Raji was shown to be very low (≈0.5 ng/ml). These results indicate that all of the cell lines used in this study are in the plasma cell stage with regard to the Ig secretion capability, although the different Namalwa sublines are shown to be arrested in varying states of cellular maturity which include the plasma state (24). The cell surface expression of differentiation-specific antigens was examined with various antigen-specific antibodies (Table I). In all cell lines related to Namalwa cells, sIgM was expressed, whereas CD21 and CD23 were not. Both CD21 and CD23 have been shown to be negative on cells in the plasma state (25). These differences in cell surface antigen expression are in part consistent with states analogous to different stages in the B cell differentiation pathway (26). In fact, the expression pattern of CD21 and CD23 shown in Raji (mature B) and LICR-LON-HMy2 (plasma B) cell lines were consistent with the observations described above. These results also support that Namalwa and related cell lines used in this study are in the plasma stage.

Secondary Rearrangement for the Expression of a New λ Light Chain Occurs on a Previously Excluded Allele in New λ Light Chain-secreting Variant Clones—To determine the genetic event responsible for the expression of the new λ light chain and the replacement of the original λ light chain, we analyzed the λ light chain gene rearrangement in NAT-30, HB4C5, and the new λ light chain producing clones. PCR was carried out to amplify the genomic DNA from each cell line using primers specific to the CDR1 of λC5 chain and J λ regions, then the reaction products were assayed by Southern blotting (Fig. 3). The combination of these primers provide a way to detect the original V λC5 to J λ coding joint in the Igλ loc. The original coding joint formation was detected in the genomic DNA of all of the cell lines tested (Fig. 3B). Furthermore, authenticity of the PCR products from these cell lines was confirmed by nucleotide sequencing. The presence of the V λJ λ coding joint for λC5 implies that the rearrangement for a new light chain does not occur on the original allele. We examined for the presence of a new V λJ λ coding joint formation for λCA2, a new λ chain produced by CA2 cells, using primers specific to the CDR1 of the V λCA2 and J λ regions. PCR product was detected only in the genomic DNA from CA2 cells (Fig. 3C). This coding joint formation for λCA2 and the retained VJ λ region for λC5 indicates that the new λ chain expression in CA2 cells result from a secondary light chain gene rearrangement on a previously excluded allele.

Loss of Original λ Chain Production in the New λ Chain-producing Clones Is a Result of a Significant Reduction of the Original Transcript—To understand the molecular basis for the loss of original λ light chain secretion in the new λ chain
producing clones, we examined the possibility of whether expression of the original λ chain is inhibited before secretion by assaying the cytoplasmic fraction of NAT-30 and CA2 cells on an immunoblot using an anti-human λ chain antibody (Fig. 4A). Although the λC5 protein is 32 kDa large, before it undergoes post-translational processing its core polypeptide is 26 kDa (9). The size of the λCA2 protein is 28 kDa. As shown in Fig. 4A, NAT-30 cells produced the λC5 protein only, and CA2 cells produced only the λCA2 protein in both the supernatant and cytoplasmic fractions. Similarly, in the other new λ chain producing clones, only the new λ light chain protein was expressed by cells in both the cytoplasm and supernatant (data not shown). We, furthermore, analyzed Namalwa, NAT-30, HB4C5, and the new λ chain-producing sublines (CA1, CA2, CA3, and CA4) for the mRNA expression of the original λ light chain λC5 and the original μ chain originally found in NAT-30 cells (μNAT-30) by Northern blot analysis (Fig. 4, B and C). V gene segments are unique to each heavy and light chains, thus can be used to define oligonucleotide probes characteristic to each transcript. Therefore, oligonucleotides specific to the variable region sequences of λC5 and μNAT-30 were used as probes to assess the level of these transcripts. The amount of total RNA isolated was normalized against the amount of β-actin transcript (Fig. 4D). No differences were seen in the expression level of the μNAT-30 transcript (Fig. 4B). Transcript encoding for the original λ chain λC5 was expressed in Namalwa, NAT-30, as well as in the HB4C5 cells (Fig. 4C). Conversely, the level of the λC5 transcript in all of the new λ chain producing clones was dramatically reduced when compared to the parental HB4C5 cells. These results suggest that loss of original λ light chain production in the new λ chain-secreting variants originates mainly at the transcriptional level.

RAG Genes Are Expressed in the Ig-secreting Cells, But Is Insufficient for Inducing Expression of a New λ Light Chain—VJ recombination activity requires the expression of the recombination-activating genes RAG-1 and RAG-2 (10, 12). To test whether a relationship exists between expression of the differential λ light chains and the expression of RAG-1 and RAG-2, we examined for the expression of the RAG genes in Namalwa cells and related cell lines (Fig. 5). Interestingly, RAG-1 and RAG-2 genes were expressed in not only the light chain-shifting-inducible NAT-30 and HB4C5 cell lines but also in the non-inducible Namalwa cells. The two RAG genes were also expressed in the new λ light chain-producing variants. Although NAT-30 and HB4C5 cells had been maintained for over 3 months in normal ConA-free medium, differential λ chain expression was not seen in either cell lines. These results suggest that the constitutive expression of the RAG genes is not sufficient to bring about expression of differential λ light chains. To examine whether enhancement of the expression of the RAG gene in NAT-30 and HB4C5 cells could initiate expression of differential λ light chains, we examined the effect of Bt2cAMP, which is known to directly increase the expression level of the RAG genes and to enhance V(D)J rearrangement (28). Although the expression of RAG-1 increased in both cell lines exposed to the reagent (Fig. 6A), differential λ chain
expression was not detected in either NAT-30 or HB4C5 cells at day 3 or day 60 of continuous culture (Fig. 6B). These results suggest that the enhancement of RAG expression is not sufficient for inducing expression of differential \( \lambda \) light chains. The RAG genes were still expressed in the new \( \lambda \) light chain-producing clones, suggesting that secondary rearrangement and expression of new \( \lambda \) light chains cannot terminate expression of the RAG genes. Furthermore, none of the new \( \lambda \) light chain-producing clones secreted any other new \( \lambda \) chain although both RAG genes were continuously expressed. These findings indicated that other factors in addition to the expression of RAG genes are necessary to induce expression of new \( \lambda \) light chains.

ConA Induced the Appearance of a Surface Ig-negative Subpopulation from a Ig-secreting Cell Line—Striking features of ConA that induced differential \( \lambda \) light chain expression include the fact that none of the new \( \lambda \) chain-secreting subclones produced the original \( \lambda \) chain species simultaneously with a new \( \lambda \) light chain, and that several clones do not produce any \( \lambda \) light chain at all (9). Production of the light chain is required for the slg expression. In fact, the light chain-negative subclones of HB4C5 cells were also shown to be slg-negative while the new \( \lambda \) chain-secreting subclones as well as the parental HB4C5 cells were all slg positive (data not shown). Therefore, cells that have lost production of the original light chain are also manifested as the slg-negative state. To assess the relationship between ConA stimulation and the loss of the original \( \lambda \) light chain production, HB4C5 and Namalwa cells were cultured in the presence of ConA, and the effect of ConA treatment on both the level of original \( \lambda \) chain transcript produced and the expression of slg and \( \lambda \) chain (slg\( \lambda \)) were examined (Fig. 7). The transcript level for the original \( \lambda \) and \( \mu \) chains produced from ConA-treated HB4C5 and Namalwa cells did not significantly differ from non-treated cells (Fig. 7A), which suggests that ConA does not inhibit \( \lambda \)5 gene transcription directly in these cell lines. However, when HB4C5 cells are subjected to continuous culture with ConA, a small slg-negative population (0.12%) was detected at day 7 (data not shown), and the slg-negative population increased to 8.57% after a 4-week culture in ConA. Interestingly, a slg-negative population was not inducible in Namalwa cells (Fig. 7B). This value coincides with the result that 2 non-\( \lambda \) chain producing clones out of 21 total sublines from ConA-treated HB4C5 cells were found (9). After a 4-week culture in the absence of ConA, untreated HB4C5 and Namalwa cells are shown to be mainly slg-positive, and the slg-negative population did not increase in either bulk populations (Fig. 7B). These results suggest that ConA may induce loss of original \( \lambda \) chain production leading to a slg-negative subpopulation and that the inducible effect of ConA depends on the cell type. Taken together, these results suggest that at least expression of both RAG genes and loss of original \( \lambda \) chain production are necessary to induce expression of new \( \lambda \) chain in human plasma cells. ConA acts on the later process although we do not know at present why the original \( \lambda \) chain mRNA level is decreased only in the new \( \lambda \) chain-producing clones although the overall new \( \lambda \) light chain productivity is comparable to the parental HB4C5 cells.

**DISCUSSION**

We have previously found that an original \( \lambda \) light chain can be replaced with a new \( \lambda \) chain which may lead to an alteration of antigen binding (we call this process "light chain shifting"). This phenomenon occurs in ConA resistant Ig-secreting cells at a high incidence (9). This finding is in contrast to other published findings which state that ongoing variant light chain expression is found in pre-B and slg\( ^- \) B cells in vivo and in vitro, but not in Ig-secreting cells (40, 41). After examining the expression of a differential \( \lambda \) light chain from our Ig-secreting cells, we found that the loss of original \( \lambda \) chain production is due to a significant reduction of its transcript level, and that the expression of the new \( \lambda \) light chain results from a secondary rearrangement of the \( \lambda \) light chain locus on a previously excluded allele. In addition, we showed that not only new light chain expressing variants but also the parental HB4C5 cells and NAT-30 cells express the RAG-1 and RAG-2 genes. The constitutive expression of the RAG genes and light chain shifting by ConA are the most striking features of these Ig-secreting cell lines.

Ig rearrangement activity and expression of the two RAG genes have been known to be strongly linked in vivo and in various in vitro cell lines (10, 43). The RAG genes are expressed precisely when B cells rearrange the Ig heavy and light chain loci during the early stages of B cell differentiation, but not in mature and plasma cells (11). Therefore, the expression of RAG genes in our Ig-secreting cell lines is unusual, suggesting a possible link between RAG gene expression and secondary rearrangement in these plasma cells. With regard to RAG gene expression in human mature B cell lines, it has been postulated that loss of slg expression not only interrupts a signal required to terminate RAG expression, but also triggers the up-regulation of RAG gene expression (31). However, since our Ig-secreting cell lines expressed RAG genes constitutionally without loss of original \( \lambda \) chain expression, we excluded this up-regulation mechanism as an explanation for expression of the RAG genes in these cell lines. The expression of the RAG genes in the Namalwa and its related plasma cell lines open new possibilities for investigation. A recent study suggests that variable expression of the Epstein-Barr virus membrane protein gene
controls the expression of RAG genes in slg⁻ cells (43). The Namalwa cell line is also one of Epstein-Barr virus-bearing B cell lines. Taken together with our results, these findings suggest that slg expression and RAG gene expression are not exclusive, and that slg expression is not directly involved in the regulation of RAG gene transcription.

Although RAG genes were expressed in the Namalwa as well as in the NAT-30 and HB4C5 cell lines, expression of new λ light chains from secondary rearrangement occurred in the HB4C5 and the NAT-30 cells but not in the parental Namalwa cells. In addition, NAT-30 and HB4C5 cells produce only the original 32-kDa λ light chain species when cultured in ConA-negative medium. Therefore, the constitutive expression of both RAG genes was shown to be insufficient to induce light chain shifting. Continuous rearrangement of the VL genes have been observed in mature B cells (6, 37). This phenomenon was documented when studying variants, which either altered their slg idiotype or completely lost slg expression through additional rearrangements or spontaneous mutations, that were selected by immunoselection from bulk cultured cells. In contrast, production of a new λ chain in the HB4C5 and NAT-30 cells occurs at a high rate when the cells were cultured in medium containing ConA. Enhancement of RAG genes expression has been known to elevate V(D)J rearrangement activity (28). Although we have tested agents that increase expression of the RAG genes and elevate V(D)J recombination activity, expression of new λ chains was not induced. These data suggest that expression of the differential λ chains in NAT-30 and HB4C5 cells is not a result of an increased expression of RAG genes. It has been established that ConA affects several intracellular second messenger pathways such as increasing the intracellular calcium concentration and activating protein kinase C in mature B cells and thymocytes (35, 36). However, these signals have been shown to decrease V(D)J rearrangement activity (28). Although the RAG genes have been speculated to be of fundamental importance for Ig rearrangements, DNA repair activity and DNA-dependent kinase and transcriptional activation of a rearranging gene segment are also shown to be required (32–34). It is possible that ConA affects one of the other intracellular factors that work in association with the RAG genes products to induce light chain shifting.

The striking feature of light chain shifting by ConA treatment is the fact that production of the original λ chain protein is lost in the new λ chain producing clones even though the originally rearranged VJ coding joint formation is retained. This loss of original λ chain production is explained by the significant reduction of its transcript level. It was demonstrated that ConA also induced the appearance of a significant slg-negative subpopulation in light chain shifting-inducible HB4C5 cells but not in non-inducible Namalwa cells. These results suggested that one of the major effects of ConA treatment on light chain shifting in our Ig-secreting cell lines is to induce a reduction of the original λ light chain transcript level which leads to loss of the original λ chain production. Noteworthy is the fact that none of the new λ chain-secreting subclones produced both the original and new light chains, and that light chain-negative subclones were found (9). If the loss of original λ chain production occurs after production of the new λ chain, double (original and new) λ chain producers should have been detected. Similarly slg-negative subpopulations should not have been detectable because either original or new λ chain or double λ light chain producing cells would all be slg-positive. These findings indicate that loss of the original λ light chain production, which is manifested as a slg negative condition, may precede expression of a new λ light chain. This manifestation could play a role in providing an induction signal for new λ light chain gene expression. Taken together, these results suggest that both the loss of original λ chain production and expression of the RAG genes are necessary for the expression of new λ light chains in our Ig-secreting cells. Although ConA may trigger the light chain shifting process, we do not know at present why subclones which reduce the level of original λ chain transcript are inducible by ConA treatment. There are many molecules including slg on the surface of B cells that act as signal transducing receptors. Since lectins, which include ConA, are polyreactive agents, the induction of ConA-driven light chain shifting has led to the hypothesis that cross-linking of receptor molecule(s) on the surface membrane may control the reduction of the original light chain transcript. Analysis of the differences between Namalwa and HB4C5 cells should be useful in obtaining insights into the mechanism responsible for the loss of original λ light chain production by ConA.

It is interesting to speculate that the expression mechanism of this new λ light chain is somewhat analogous to the receptor editing model that has been described for autoantibody regulation (29, 30). Receptor editing is a process where a B cell which expresses an autoantibody changes into a cell expressing a non-autoantibody antigen through secondary rearrangement of the light chain. This process is thought to occur at the immature B cell stage but not at the mature stage (29). Conversely, the data presented in this paper raises the possibility that plasma B cells can be altered to improve antigenic recognition or to impair autoreactive antigen binding by inducing an alteration in the structure and specificity of the produced antibodies. This may also explain the generation of some autoantibodies. Indeed, we isolated new λ light chain producing var-
iant clones that secrete antibodies reactive to double-stranded DNA (data not shown). Clarifying how the original λ chain transcript level was dramatically reduced in the new λ chain-producing clones may allow us to study the mechanism that controls the replacement of the original light chain in plasma cells.

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