Restoration of Ig Secretion: Mutation of Germline-Encoded Residues in T15L Chains Leads to Secretion of Free Light Chains and Assembled Antibody Complexes Bearing Secretion-Impaired Heavy Chains

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Restoration of Ig Secretion: Mutation of Germline-Encoded Residues in T15L Chains Leads to Secretion of Free Light Chains and Assembled Antibody Complexes Bearing Secretion-Impaired Heavy Chains

Elizabeth A. Whitcomb, Tammy M. Martin, and Marvin B. Rittenberg

We previously described T15H chain mutants that were impaired in assembly with L chain and in ability to be secreted from the cell. The unmutated T15L chain is unusual in that it is secretion-impaired in the absence of assembly with H chain. The T15L chain preferentially pairs with T15H in vivo, suggesting that if we introduced mutations that would allow secretion of free T15L chain, they might also lead to the secretion of the complex with the defective H chain. We mutated four positions in the germline T15L that had amino acids infrequently found in other κ-chains. Mutation to the most frequently occurring amino acid at three of the four positions allowed secretion of free L chain, while the combination of two secretion-restoring mutations was synergistic. Coexpression of secretion-restored mutant L chains with the secretion-defective mutant H chains rescued secretion of the assembled H₁L₂ complex, suggesting that during somatic hypermutation in vivo, deleterious mutations at the H chain may be compensated by mutations on the L chain. To our knowledge, this is the first example of mutations in IgL chains that are able to restore secretion-defective H chains to secretion competence in mammalian cells. The Journal of Immunology, 2003, 170: 1903–1909.
Ala (K\(^{17} > E + T^{44} > A\)), the second mutation was added to the pTZ18U clone containing the single T\(^{44} > A\) mutation. All mutants were sequenced in pTZ18U before subcloning into pSV2-neo for transfection.

SP2/0, a mouse hybridoma fusion partner line that does not express endogenous H or L chains (5), was transfected with the T15L mutant construct by lipofectin (Life Technologies, Grand Island, NY) or electroporation (6), and stably transfected clones were selected by G418 resistance (Geneticin; Life Technologies). SP2/0 expressing the wild-type (wt) T15L chain was previously described (7). Lines expressing the low secretor H chains were previously described (8) and were transfected using lipofectin. All cells were propagated in IMDM (Life Technologies) containing 20% FBS (HyClone, Logan, UT), as described (1).

Quantitation of Ig secretion was performed essentially as described (7). Briefly, 1 \(\times 10^6\) cells from transfectants were washed and cultured in 1 ml of fresh IMDM at 37°C. After 4 h, supernatants were collected and cells were lysed in 1 ml of PBS containing 25 mM iodacetamide, 20 \(\mu\)g/ml soybean trypsin inhibitor, 50 \(\mu\)g/ml PMSF, and 0.25% Nonidet P-40 (all reagents from Sigma-Aldrich, St. Louis, MO). Dilutions of lysates and supernatants were assayed for Ig content by sandwich ELISA. For L chain-only determinations, goat anti-mouse \(\kappa\) antiserum was used to coat the plates, and alkaline phosphatase (AP)-coupled goat anti-\(\mu\)M-\(\kappa\) antiserum (Southern Biotechnology Associates, Birmingham, AL) was used for detection. For H chain determinations, rabbit anti-IgG2b and AP-coupled anti-IgG2b (Zymed Laboratories, South San Francisco, CA) were used. For determination of assembled Ig, eluted by heating in 40 mM Tris-HCl, pH 6.8, 0.005% bromphenol blue) containing 100 mM DTT.

Secretion assay

Cytosol supernatants isolated from the transfectants containing both the mutant H and L chains were assayed for binding to PC-containing Ags by ELISA using AP-coupled anti-IgG2b for detection. Binding to PC-histone or Streptococcus pneumoniae (R36A) was performed as described (9). Binding to Trichinella spiralis or Ascaris suum Ags was performed as described (10). Percentage of wt binding was determined by calculating the concentration of mutant Ab needed to give an OD of 0.5 in the ELISA, then comparing that figure with the concentration of wt Ab needed to get the same OD. Binding to PC-histone was inhibited using free PC and the PC analogs, nitröphenolphosphocholine and \(\alpha\)-glycerophosphocholine, at concentrations ranging from 1 \(\times 10^{-2}\) to 1 \(\times 10^{-6}\) M.

Metabolic labeling and immunoprecipitation

Cells were plated at a density of 2.5 \(\times 10^4\) to 1 \(\times 10^4\)/ml and allowed to grow to 60–80% confluence in six-well culture plates (Falcon; BD Biosciences, Franklin Lakes, NJ). Cells were starved of methionine and cysteine by incubation in methionine/cysteine-free DMEM (Sigma-Aldrich) for 30 min. Cells were pulse labeled for 15 min with 0.4 mCi \(^{35}\)S Express protein labeling mix (PerkinElmer Life Sciences, Boston, MA) in methionine/cysteine-free DMEM supplemented with 10% FBS. After the pulse, cells were washed in PBS and chased into complete IMDM. At appropriate chase time points (see Results), the supernatants were harvested and cells were washed with PBS before lysis in Tris, sodium, azide buffer (10 mM Tris-HCl, pH 8.0, 140 mM NaCl, 0.025% NaN3) supplemented with 50 \(\mu\)g/ml PMSF and 1% Nonidet P-40. Lysates were incubated on ice for 1 h, and cell debris was pelleted and discarded.

Lysate and supernatant samples were immunoprecipitated using rabbit anti-mouse \(\kappa\) antiserum (Cappel/ICN, Costa Mesa, CA, or Cortex Biochemicals, San Leandro, CA), followed by protein A-Sepharose (Amersham Pharmacia Biotech, Piscataway, NJ). For assembly of IgG2b, supernatants were harvested and precipitated with protein A-Sepharose. Immunoprecipitates were washed twice in lysis buffer, followed by one wash in Tris, sodium, azide and a final wash in 50 mM Tris, pH 6.7. For analysis of samples under reducing conditions, immunoprecipitates were eluted by heating in 40 \(\mu\)l SDS sample buffer (2% SDS, 10% glyceral, 60 mM Tris, pH 6.8, 0.005% bromphenol blue) containing 100 mM DTT.

Samples analyzed under nonreducing conditions were eluted in 40 \(\mu\)l of SDS sample buffer without DTT. Samples were heated to 85°C for 10 min before analysis on 10 or 12% SDS polyacrylamide gels.

Pulse-chase experiments were quantitated using NIH Image software (version 1.61; National Institutes of Health, Bethesda, MD) for autoradiographs or IP Lab Gel software (version 1.5; Analytics, Vienna, VA) for phosphorimager scans.

Statistics

Statistical significance was determined by unpaired Student’s t test using Multistat software (version 1.12; Biosoft, Ferguson, MO).

Modeling of Ig proteins

Fig. 4 was created using the model of T15 (9) with RasMol software (version 2.7.1; RasMac Molecular Graphics, University of California, Berkeley, CA).

Results

Single point mutations partially restore secretion of the T15L chain: a synergistic effect of two mutations

A number of mutations at particular residues have been shown to affect secretion of free L chains (11–14). Although T15L preserves the secretion-permissive residues previously described with one exception, a conservative change from Gly to Ser at position 16, it cannot be secreted unless assembled with H chain. T15L expresses the unmutated V\(^{e922}\) gene rearranged to Jw5; thus, we compared the germline V\(^{e922}\) sequence (15) with the database of over 5000 expressed and germline V\(\kappa\) sequences and identified a number of positions containing residues present in less than 2% of all V\(\kappa\) genes (4). Mutations were made at four positions: two in framework region (FW) 1, S\(^{166} > G\) and K\(^{17} > E\); one in CDR2, I\(^{56} > S\); and one in FW3, T\(^{84} > A\). Position 16 was shown previously to have an effect on a L chain secretion, in which a nonconservative change from Gly to Arg abolished secretion of free L chains (11). Residue frequency in other \(\kappa\)-chains as well as the most commonly occurring residue and frequency are shown in Fig. 1A. Single point mutations as well as the double mutants I\(^{56} > S + T^{84} > A\), S\(^{166} > G + K^{17} > E\), and K\(^{17} > E + T^{84} > A\) were made and stable transfectants were generated.

At least four individual transfectants expressing the mutations were isolated and L chain secretion was initially analyzed using a quantitative ELISA. Percentage of secretion was determined by comparing the amount of L chain in the supernatant with the total amount of L chain in the lysate plus supernatant. Although less than 1% of the total wtT15L chain was in the supernatant after a 4-h incubation in fresh medium, all transfectants expressing the single mutations S\(^{166} > G\), K\(^{17} > E\), or T\(^{84} > A\) secreted a significant amount of L chain in the supernatant (\(p < 0.001\) vs wt, Fig. 1B). The percentage of secretion for each mutant was similar in different transfectants expressing intracellular L chain at a wide range of concentrations; thus, the increase in L chain in the supernatant was due to the effect of the mutations and was independent of intracellular L chain concentration. The I\(^{56} > S\) mutation had no significant impact on secretion on its own and may have an antagonistic effect when paired with the secretion-restoring mutation T\(^{84} > A\). Although the secretion of the double mutant I\(^{56} > S + T^{84} > A\) is significantly above wt or the single mutant I\(^{56} > S\) and below that of the single mutant T\(^{84} > A\), this putative antagonistic effect was not consistently observed in all the clones, as nearly one-third of the clones (6 of 19) were above the minimum secretion observed for T\(^{84} > A\). The combination of two secretion-restoring mutations was synergistic with the double mutants (K\(^{17} > E + T^{84} > A\) and S\(^{166} > G + K^{17} > E\)) secreting >2.5 times more L chain than was predicted if the effects of the individual mutations were simply additive (predicted S\(^{166} > G + K^{17} > E\) = 10.1%, observed = 29%; predicted K\(^{17} > E + T^{84} > A\) = 9.5%, observed = 24.8%; Fig. 1B). Other secretion-competent \(\kappa\) (non-V\(^{e922}\)) and A L chains exhibit a range of secretion in this assay (12–65% of total L chain (lysate + supernatant) in supernatant after 4 h, \(n = 4\) different L chains; data not shown). Thus, the degree of rescue of T15 mutant L chains resembles that of...
other secretion-competent L chains expressed by myeloma and hybridoma cells.

To confirm that the increases in secretion observed in the double mutants S16 > G + K17 > E and K17 > E + T84 > A were indeed synergistic, a pulse-chase analysis was performed to determine the secretion of L chain labeled during the pulse. Transfectants expressing steady state levels of intracellular L chain similar to the wtT15L cell line were pulse labeled for 15 min and chased up to 6.5 h. Cell lysates and supernatants were immunoprecipitated and analyzed under reducing SDS-PAGE conditions (Fig. 2A). The pulse-chase experiments were performed to determine the secretion of L chain labeled during the pulse. Transfectants expressing steady state levels of intracellular L chain similar to the wtT15L cell line were pulse labeled for 15 min and chased up to 6.5 h. Cell lysates and supernatants were immunoprecipitated and analyzed under reducing SDS-PAGE conditions (Fig. 2A). The percentage of label in the supernatant was determined by comparing the amount of labeled L chain in the supernatant with the amount of labeled L chain detected immediately after the pulse (t = 0). The S16 > S mutant (not shown) was at background for all time points.

**FIGURE 2.** Secretion profile of L chains. Cells were pulse labeled with [35S]methionine/cysteine for 15 min and chased up to 6.5 h. Lysates and supernatants were immunoprecipitated with rabbit anti-mouse κ antisera, followed by protein A-Sepharose. Immunoprecipitates were analyzed by autoradiographs or phosphorimager scans of pulse-chase analysis. B. Quantitation of L chain label in the supernatant for experiments shown in A. Percentage of label in the supernatant was determined by comparing the amount detected in the supernatant with the total labeled L chain detected immediately after the pulse (t = 0). The S16 > S mutant (not shown) was at background for all time points.

**Mutations that restore secretion of free L chains can complement secretion-impairing H chain mutations**

We previously described mutations in the CDR2 of the T15H chain that impaired Ab secretion (7). Because somatic mutation in vivo can occur at both the H and L chain loci, we asked whether the secretion-restoring mutations in the T15L chain could compensate for the H chain secretion defect. The restored mutant L chain S16 > G + K17 > E was expressed in cells with the low secretor H chains. Stable transfectants were obtained, and secretion was determined by incubating cells in fresh medium for 4 h. The amount of Ig was quantified by sandwich ELISA. The amount of assembled H + L chain in the supernatants of transfectants expressing either the wtT15L chain or the S16 > G + K17 > E mutant L chain is shown in Fig. 3, A–C. The secretion-restored mutant L chain was able to rescue secretion of the three mutant H chains tested; multiple transfectants were screened, and restored secretion was consistently observed. The M153 transfectant expressing the wtT15L chain did consistently secrete a low amount of Ig into the supernatant, but the level was 10 times less than the transfectant expressing the mutant L chain, although similar secretion assays, which compared the amount in the supernatant with the steady state level of intracellular L chain (Fig. 1B).

**FIGURE 1.** Infrequently occurring amino acids can affect the secretion of T15L. A. The germline Vc22 sequence (15) was compared with the database of expressed and germline Vκ sequences (4). The residues in T15L at positions 16, 17, 56, and 84 occur in less than 2% of other Vκ sequences. The percentage in other κ-chains (shown in parentheses) as well as the most commonly occurring residue and its frequency (4) are shown. B. Secretion of SP2/0 transfectants expressing wt or mutant L chains. Transfectants were cultured in fresh medium for 4 h, and amount of L chain present in the supernatant or in the cell lysate was determined by quantitative ELISA. Percentage of secretion was determined by comparing the amount of L chain in the supernatant with the total amount in the supernatant plus the amount in the cell lysate. Data represent the average (± SD) of at least four separate transfectants. *p < 0.001 vs wtT15L; #, p < 0.001 vs either single mutant.
Fully assembled IgG2b consists of an H2L2 heterotetramer. The low secretor mutants expressing the wtT15L chain are impaired in assembly, arresting at the H2L stage (8); thus, we wished to determine whether the secretion-restored mutants were secreting fully assembled IgG2b. Cells were pulse labeled for 15 min and chased for 4 h. Ig from the supernatant was immunoprecipitated with protein A-Sepharose, which will precipitate H chains along with any assembled L chains. Fully assembled H2L2 IgG2b was detected in the restored mutants running at the same electrophoretic mobility as the wtT15 (Fig. 3D); thus, the mutations in the L chain allow complete assembly with the mutant H chains and restore transport of completely assembled IgG2b. Free H chain was observed in the supernatant of the M153 transfectant. This is most likely due to labeled H chains assembling with, but not disulfide linked to, unlabeled L chains. The intracellular t½ of L chains is 1–2 h (6), while the intracellular t½ of the secretion-impaired H chains can be >20 h (8); thus, it is likely that L chains synthesized after the pulse are assembling with H chains synthesized during the pulse. Indeed, unpaired H and L chains were detected in supernatants collected from this mutant by Western blot under nonreducing conditions after immunoprecipitation with protein A-Sepharose (data not shown).

We also tested whether the secreted Ab expressing the double-mutant L chain could bind Ag. The secretion-restored M153 mutant expressing the double-mutant L chain S16G + K17E was able to bind the PC-containing Ags: PC-histone, S. pneumoniae (R36A), A. suum, and T. spiralis, although at less than 10% of the wtT15 Ab. The secretion-restored M164 mutant did not bind any of the PC Ags, while the secretion-restored M241 did show detectable binding to PC-histone and S. pneumoniae (R36A), although at a level <1% of wtT15 binding (Table I). These results suggest that while the secretion-competent L chains may fully assemble and allow the mutant H2L2 complex to be secreted, the combining site is not identical with that of the wt Ab. Nevertheless, binding of the secretion-restored M153 Ab to PC-histone was inhibitable by free PC and the PC analogs, nitrophosphocholine and α-glycerophosphocholine, suggesting that its fine specificity still resembles that of wtT15 and was not altered appreciably by the mutations in either the H or L chains (data not shown).

Discussion
In this study, we report that mutation of uncommon residues conserved in the T15L germline sequence permits the secretion of free T15L chains and also allows the L chains to assemble with and restore the secretion of impaired mutant H chains. The T15L chain is unusual for several reasons: 1) it is secretion impaired in the absence of H chain assembly; 2) to our knowledge, it has been

Table I. Ag-binding characteristics of secretion-restored Ig a

<table>
<thead>
<tr>
<th>H Chain Mutant</th>
<th>M153</th>
<th>M164</th>
<th>M241</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>PC-histone</td>
<td>7.8%</td>
<td>N.B.</td>
</tr>
<tr>
<td></td>
<td>S. pneumoniae</td>
<td>2.8%</td>
<td>N.B.</td>
</tr>
<tr>
<td></td>
<td>T. spiralis</td>
<td>4.1%</td>
<td>N.B.</td>
</tr>
<tr>
<td></td>
<td>A. suum</td>
<td>&lt;10%</td>
<td>N.B.</td>
</tr>
</tbody>
</table>

a Binding shown is percentage of wt. The concentration of wtT15 Ig needed to obtain OD450 = 0.5 was calculated and compared with the amount of mutant Ig needed to obtain the same OD. Wt and mutant Ig were compared at the same time point on the same plate.

b No detectable binding at 100 ng/ml. Binding of the wtT15 was detectable at 0.76 ng/ml for PC-histone, S. pneumoniae, and T. spiralis, and 12.5 ng/ml for A. suum.

c Detectable binding at 100 ng/ml, however, too low to accurately compare with wtT15.
observed in vivo only in the immune response to PC-containing Ags; and 3) it has been observed in vivo associated only with the T15H chain. In this study, we set out to determine the molecular basis for the secretion impairment of T15L. Mutation of three single residues restored secretion modestly, and the combination of two secretion-restoring residues was synergistic; thus, multiple residues contribute to the secretion impairment of T15L. Additionally, we asked whether the secretion-restoring L chain mutations could affect the assembly of T15L with mutant H chains that are secretion impaired because T15H and T15L associations appear to be preferred in vivo. We found that secretion-restored T15L chains could assemble with and allow the secretion of H chains that could not fully assemble with the unmutated secretion-impaired T15L chain.

**Mutation of germline residues permits secretion of free L chains**

Although most L chains can be secreted in the absence of assembly with H chain, a handful have been described that are secretion impaired (11–13, 16). Most of these secretion-impaired L chains are defective as a result of replacement mutations. Several residues in FW1 and FW3 have been identified as critical for L chain secretion: Gly16 in FW1 (11), and Phe62 and Tyr/Phe87 in FW3 (12, 13). Other residues in FW3 have also been suggested to be involved in L chain secretion (aa 57–65) (12). T15L, which has no mutations from the germline Vx22, is secretion impaired in the absence of H chain assembly, yet preserves the amino acids previously identified to affect secretion of free L chains in FW3 (positions 57–65 and 87) and has a conservative substitution at position 16 from Gly to Ser, suggesting involvement of other residues in L chain secretion and the T15L defect. We demonstrate that secretion of T15L can be achieved by the replacement of its infrequently occurring amino acids. Mutation of three positions to the most commonly occurring residue (S16 > G, K17 > E, T84 > A) allowed secretion of free L chain, while one mutation (I56 > S) had no effect by itself, but may be antagonistic when combined with a secretion-restoring mutation (T84 > A). Thus, in addition to stability of expression in E. coli (3), our data indicate that sequence statistics can often, but not always, predict an effect on secretion in mammalian cells. Additionally, the combination of two secretion-restoring mutations can synergistically increase L chain secretion, confirming that multiple amino acids contribute to the secretion impairment of wtT15L.

**Structural consequences of secretion-restoring mutations**

Two mutations in FW1 of the T15L chain allow modest secretion of free L chain on their own and act synergistically to increase secretion when expressed together. The conservative change at S16 > G is at the pivot point of the β turn in FW1 (Fig. 4, A and B); the preferred Gly would most likely be more flexible. Mutation of this position in a A L chain from Gly to Arg prevented secretion (11). Conservation of Gly16 is high (97%), indicating its importance in L chain structure (4). Position 17 is an acidic amino acid in the majority of L chains (79%), but a Lys in T15L; an acidic residue at this position may influence L chain stability by forming a hydrogen bond with Ser/Thr at position 14 (Fig. 4B) (17). Thus, converting Lys to Glu in T15L restores the potential for this apparently critical bond. Mutations at position 17 have been observed in pathogenic or unstable L chains (17). The observation that the double-mutant L chain with both FW1 mutations is secreted robustly provides further evidence that the stability of FW1 is important for overall L chain secretion function.

The FW3 mutation T84 > A permits modest secretion on its own and acts synergistically when expressed with the K17 > E mutation. Position 84 is most often Ala, with Gly the second most frequently observed residue (4); thus, small hydrophobic residues appear preferred. Position 84 comprises part of the hydrophobic core of the V_L domain (18); thus, the larger polar Thr at residue 84 is likely to require a major alteration in the overall folding of the V_L domain. Ala84 was shown to be one of the earliest residues to
become inaccessible during folding of another murine κ-chain Fv (19). Additionally, Thr in place of the conserved Ala at position 84 in a human κ-chain proved to be destabilizing (20). Phe also contributes to the stabilization of this hydrophobic core (18), and others have shown replacing a polar Ser for the conserved Phe impairs L chain secretion (see Fig. 4C) (12).

**Secretion-restoring L chain mutations restore H chain secretion**

We previously described T15H chains with mutations in CDR2 that were impaired in Ab assembly and secretion (7). Because introducing secretion-competent L chains D16L or J558L did not restore secretion in the T15 HCDR2 secretion-impaired mutants (21), it was surprising that mutations in T15L that allowed secretion of free L chains could restore the H chain mutants. The low secretor H chains are unable to fully assemble with the wtT15L chain arresting at the H2 L intermediate (8). However, assembly of the H2L2 complex is complete when the low secretor H chains are paired with the secretion-restored L chains, suggesting that the secretion-restoring potential of the L chains may rest in their ability to allow complete assembly.

The L chain mutations that restore the H chain mutants are distal to the H/L interface; thus, the mechanism for restoration is most likely due to long range effects (see Fig. 4D). Although the exchange at position 17 does alter the charge (Lys to Glu), a single mutation at position 16 (from Ser to Gly) can at least partially restore secretion of the mutant H chains (data not shown); thus, a change in surface charge at position 17 may not explain the restored secretion. We speculate that the changes in the β turn of T15L FW1 affect the structure of L chain CDR1 and its interaction with HCDR3. Deletion of four residues in the apex of HCDR3 restored secretion of these H chain mutants when expressed with the wtT15L chain (21). The deleted residues in HCDR3 make multiple contacts with L chain CDR1 (see Fig. 4D). Because the secretion defect may be in part due to inefficient assembly with L chain (8), the interaction of these regions may be critical. The CDR1 of T15L is quite long, being 17 aa; shortening the 17-residue CDR1 in the McPC603 L chain by 6 aa resulted in a considerable increase in stability (22), although the sequence was altered as well as the length. Thus, it is possible that the FW1 changes stabilize CDR1 and promote the assembly with H chain. Furthermore, others have shown that mutations within L chain FW1 can increase the assembly and secretion of Fv proteins (22, 23) and whole Ab molecules (24).

Examination of L chains carrying mutations that prevented secretion led to the hypothesis that a secretion signal was encoded within the VL domain by conserved amino acids in FW1 and FW3 (12). These amino acids (residues Gly, Pro, Arg, and Phe) are distal from the H/L interface, and it was suggested they may be important for secretion of free L chains as well as complexed Ig (12). Our data are partially consistent with this hypothesis, as the secretion-competent double-mutant T15L chains that restore the secretion of the mutant H chains preserve all of these residues, while the secretion-impaired wtT15L has the conservative substitution at G16S. However, other L chains that preserve these residues (Gly, Pro, Arg, and Phe) such as D16L and J558L, do not permit secretion of our mutant H chains (21), while the secretion-restored K17E single mutant that has Ser was able to restore secretion. Thus, the pairing of T15H with T15L most likely allows both the VH and VL domains to fold more efficiently than other H/L pairings. This was also suggested by Wall and Pluckthun (23), using VH1 and VL fragments expressed in E. coli. Although initial folding of H and L chains occurs cotranslationally, there is considerable evidence that H/L assembly allows for additional folding to complete the H2L2 complex (25–29). Additionally, cooperative folding of isolated VH and VL domains has also been observed (23, 30).

The endoplasmic reticulum resident chaperones BiP and GRP94 associate with both secreted and nonsecreted Ig proteins (16, 31–33). We have shown that the low secretor H chains have an increased association with these chaperones as compared with the secretion-competent wtT15H chain when coexpressed with the wtT15L chain (8). Preliminary evidence suggests the levels of BiP and GRP94 associated with the H chains are similar in the secretion-restored mutants and their secretion-impaired counterparts (data not shown); thus, the mechanism for secretion impairment and restoration is not due directly to the degree of chaperone association. However, as L chain displaces BiP on the H chain during Ig assembly (28, 29), it is possible that displacement is more efficient by the secretion-restored L chains, thus promoting the association of H/L over the H chain/BiP interaction.

Others have shown that mutation of L chain residues can increase the secretion and folding of Ig fragments in E. coli (22, 34). VhFW1 residues have frequently been implicated in the stability of Fv proteins (23, 35, 36). Wall and Pluckthun (23) showed that mutation of four FW1 residues in T15L, including S16G, increased folding and secretion of the T15Fv. These studies have shed considerable light on the residues important for folding, secretion, and stability of Ig fragments expressed in E. coli. However, these Igs are secreted efficiently when expressed as whole Abs in mammalian cells. Thus, our studies add new information to the growing body of work on Ig folding and secretion.

Our data indicate that deleterious H chain mutations can be overcome by compensatory L chain mutations. This may be a mechanism whereby B cells in the germinal center are not wasted due to secretion-impairing H chain mutations. We have evidence suggesting that secretion-impairing H chain mutations occur in vivo during an immune response (G. D. Wiens, M. Brown, and M. B. Rittenberg, manuscript in preparation). Whether restorative mutations are also observed is currently under investigation. Additionally, it will be of interest to see whether the secretion-restored L chains can also restore secretion of impaired FW2 T15H chain mutants previously described (2). Dul and Argon (12) reported that mutation of an L chain V region could impair secretion of assembled Ab. To our knowledge, our results represent the first example of L chain mutations that can rescue secretion-defective Abs.

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**References**


