CONTROL OF IMMUNOGLOBULIN SECRETION IN THE MURINE PLASMACYTOMA LINE MOPC 315*

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Most proteins made for export are synthesized on polysomes associated with the membrane of the rough endoplasmic reticulum (RER). The nascent polypeptides enter the RER and move through the membranes into smooth endoplasmic reticulum. Later they can be found in the Golgi apparatus from which they travel to the cell surface where they are released into the surrounding medium (1). As might be expected, secretory proteins can often be detected on the cell surface (2).

Most secreted polypeptides are now known to be synthesized in precursor form with 10–30 extra amino acid residues on their amino terminal ends. The extra sequences are rich in hydrophobic amino acids (3–6). Blobel and Dobberstein proposed that during the process of translation these signal sequences serve to anchor the polysomes in the membrane and direct the entry of the nascent chain into the RER (7). Specific proteolysis removes the signal sequences, probably before translation of the polypeptide is completed. The polypeptide is then processed and assembled (1, 7–11).

Immunoglobulin production in myeloma cells has been a model system used extensively in elucidating the pathway outlined above. Mouse myeloma MOPC 315 cells synthesize and secrete an immunoglobulin (Ig) of the IgA class. Both the light (L) and heavy (H) chains are made on membrane-bound polysomes (12). The heavy chain is modified by stepwise addition of sugar residues (13); during assembly it forms a dimer (H₂) before combining with light chain (14). Just before secretion, J chain joins the complex and polymeric IgA is formed (15, 16). Little is known about the regulation of this process. By analyzing variant cell lines blocked at different stages in Ig maturation we hope to learn what controls secretion. In this paper we report studies on the synthesis and secretion of an altered α-heavy chain in the MOPC 315 variant line, 315LV-1 which has lost the ability to make any λ light chain. Cells of 315LV-1

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1. Abbreviations used in this paper: DMEM, Dulbecco's modified Eagle's medium; H, heavy; L, light; PBS, phosphate-buffered saline; RER, rough endoplasmic reticulum.
synthesize H chain but then degrade most of it rather than secrete it. Secretion of the α-chain is restored by fusion of 315LV-1 cells with cells of κ-light chain producing myeloma variant of the MPC 11 cell line (4T001.01). This phenomenon is reminiscent of L chain secretion in the MOPC 21 cell line (17).

Materials and Methods

Cell Lines and Culture Media. Mouse myeloma MOPC 315 parent cell line 315LK produces both λ light chain and α-heavy chain. A tumor line, NP1, was isolated as a nonsecreting tumor by Lynch and co-workers by using techniques described previously (18); an isolate of it was adapted to tissue culture and then a single cloned isolate 315LV-1 was obtained with standard procedures. Mouse myeloma MPC 11 lines, kindly supplied by M. Scharff (Einstein University) are 4T001, a parent line producing κ-light and γ-heavy chains and a variant line 4T001.01, which only synthesizes and secretes κ chain. The 4T001 cell lines are 6-thioguanine resistant and ouabain resistant. A hybrid line, X-2, was constructed between 315LV-1 and 4T001.01 by using polyethylene glycol as a fusing agent (19).

The cells were grown and maintained in Dulbecco's modified Eagles' medium (DMEM) supplemented with 20% fetal calf serum. For amino acid cell labeling experiments, medium lacking the appropriate amino acids and containing dialyzed fetal calf serum was used. Radioactive amino acids threonine, leucine, valine, or methionine were used as indicated.

Radioimmunoassay. Radioimmunoassays specific for α- and γ-heavy chains and κ- and λ-light chains were performed as described elsewhere.

Samples of either cell cytoplasmic lysates (i.e., post nuclear supernates of Nonidet P40 lysed cells), cell secretions (i.e., medium) or unlysed cells (washed twice in DMEM supplemented with fetal calf serum) were used as indicated.

Preparation of Labeled Cell Cytoplasmic Ig Proteins. After the appropriate incubation and labeling treatments, cell suspensions were chilled and centrifuged for 5 min at 1,500 rpm in an International centrifuge (International Equipment Co., Boston, Mass.). The cells were washed with 50 mM Tris-HCl (pH 7.6) + 50 mM NaCl (TN) buffer + 1% Nonidet P40 (Gallard-Schlesinger Chemicals Mfg. Corp., Carle Place, N. Y.). Debris and nuclei were removed from the cytoplasmic proteins by centrifugation at 1,000 g for 10 min. The Ig proteins were purified by either of two methods. Staphylococcus A precipitation was performed as described, but by using two times the amount of fixed bacteria for each sample. Indirect precipitation from the soluble proteins was carried out as follows: the supernate of a 100,000 g (30 min) centrifugation was incubated in phosphate-buffered saline (PBS) with rabbit anti-315 protein serum for 2 h at room temperature. Goat anti-rabbit Ig serum was added and the incubation continued for an equal time followed by an overnight incubation at 4°C. Precipitates were centrifuged through a 0.3-mi cushion of 1 M sucrose in PBS containing detergents (PBS, 1% Triton X-100, 1% deoxycholate) for 10 min at 9,000 rpm in a Sorvall centrifuge (Ivan Sorvall, Inc., Newtown, Conn.). The pellets were washed several times in PBS-detergents, then in TN buffer, and finally resuspended in 50 mM Tris-HCl (pH 7.6) containing 2% sodium dodecyl sulfate and dissolved by heating for 1 min in boiling water.

RNA Translation in a Wheat Germ Cell-Free System. To isolate polysomes, cells were chilled quickly, washed with a 0.85% saline solution, and swollen hypotonically in 10 mM Tris-HCl (pH 7.6), 10 mM KCl, 1 mM MgCl₂. For a total polysome preparation, cells were lysed with 1% Nonidet P40, nuclei removed by centrifugation, and polysomes purified by precipitation with 30 mM MgCl₂ (20). When membrane bound and free polysomes were to be separated, swollen suspensions were adjusted to 10 mM Tris-HCl (pH 7.6), 100 mM KCl, 1 mM MgCl₂, 0.88 M sucrose, and the cells lysed by Dounce homogenization. The polysome fractionation and total RNA extraction proceeded as described previously (21). A wheat germ cell-free system was prepared and 25-μl reactions incubated with 0.25 OD₅₀₀ total RNA and 25 μCi [³⁵S]methionine as described by Roberts and Paterson (22). After 3 h at room temperature, reactions were centrifuged at 100,000 g for 30 min and the light- and heavy-chain polypeptides purified by a direct immune precipitation. In this case it was important to allow only a short incubation period (no more than 2 h at room tempera-
ture) to keep the background of nonspecific protein precipitation low. The precipitates were washed and dissolved as described above.

**Polyacrylamide Gel Electrophoresis.** Samples were adjusted to 50 mM Tris-HCl (pH 7.6), 2% sodium dodecyl sulfate, 75 mM β-mercaptoethanol, 10% glycerol, then heated in boiling water for 1 min, and subjected to electrophoresis on gradient polyacrylamide-sodium dodecyl sulfate slab gels (with a 3% stacking region) as described previously (23). After staining with Coomassie Blue and destaining, gels were either prepared for fluorography (24, 25) or dried down directly.

**Reagents.** Rabbit anti-mouse myeloma α-460 protein (IgA, κ) antiserum was obtained from Gateway Immunosera Co., St. Louis, Mo. Preparation of immunoglobulin proteins and other antisera were as described.

**Results**

The 315LV-1 tumor selected as a nonsecreting myeloma, was found to have low intracellular levels of immunoglobulin heavy chain (H. Eisen, personal communication). Its growth was adapted to tissue culture and a single clonal derivative was isolated. The level of H and L chain present in the cytoplasm was tested by radioimmunoassay. Cells of the 315LV-1 line were found to contain approximately 1–3% of the level of intracellular H chain found in 315LK cells (Table I). No λ chain could be detected in 315LV-1 (Table I). When a hybrid line (X-2) was made by fusing 315LV-1 cells with a light chain (κ) producing MPC 11 clone (4T001.01), the intracellular α-chain level increased to about the level found in the wild type 315LK line. No induction of λ chain light chain or increase in the very low steady-state level of γ2b was observed. Thus the hybrid cell X-2 does not show complementation of either of these nonexpressed chains (footnote II, Table I). Synthesis of κ-light chain is continued as in the 4T001.01 parent. Neither of the defects resulting in failure to produce a given chain is a dominant phenotype since the hybrid cell continued to express chains of both of its parental lines (315LV-1 and 4T001.01) (Table I).

**Loss of Secretion of the α-Heavy Chain in 315LV-1.** To compare quantitatively the level of secretion of H chain in 315LK and 315LV-1 cells, exponentially growing cultures were diluted to the same density and grown for 72 h. After counting the cells, they were removed by centrifugation and the level of H chain in the culture medium was measured by radioimmunoassay. The average number of cells was calculated by integration of the initial and final cell counts. The 315LK cells were found to secrete an average of 2.6 × 10^{-7} μg/cell per h (Table II). For the 315LV-1, the maximum amount of medium that could be added gave a response that was barely above background. We estimate a value of 4 × 10^{-11} μg/cell per h for this line (Table II). From the intracellular concentration of α-chain in 315LV-1 one would expect a level of secretion equal to 1% of the level seen in the parent 315LK line if secretion were not also defective in this mutant.

**Recovery of Heavy-Chain Secretion in the Hybrid X-2.** The X-2 hybrid cell line not only has high intracellular levels of H chains but also secreted the H chain it synthesized. Thus in an Ouchterlony double-diffusion plate test, the culture fluids of 315LK and X-2 cells both gave positive responses when challenged with rabbit antiserum directed against the myeloma α-chain protein of MOPC 460 whereas 315LV-1 culture fluids gave no response (data not shown). When tested by radioimmunoassay, the medium from X-2 cells was found to secrete H chain at about the same rate as the parental 315LK cells (Table II).
TABLE I
Steady-State Intracellular Immunoglobulin Chain Levels

| Cell line | Cell proteina | Cell proteinb | γh | κc |
|-----------|---------------|---------------|----|----|
| 315LK     | 1.4           | 1.8           | *§ | *  |
| 315LV-1   | 0.01          | *             | *  | *  |
| 4T001     | *             | *             | 3.1| 6.3|
| 4T001.01  | *             | *             | 0.02| 1.0|
| X-2       | 1.1           | *             | 0.02| 3.1|

a Error in determination ± 30% of listed value.
† If parental H2Lκ can be formed, free light chain is underestimated. This is also true for the X-2 cell line. Otherwise, total light chain can be determined.
§ Star (*) signifies less than 10^-4 of positive control.
|| The level of γh antigen detected is significantly above background. Preliminary experiments suggest that it represents a low level of complete γh H chain being synthesized in 4T001.01 and X-2.

TABLE II
Secreted and Cell Surface α-Heavy Chain

| Cell line | μg α-Chaina | Molecule/cell surface |
|-----------|-------------|-----------------------|
|           | cell/h      |                       |
| 315LK     | 2.6 × 10^-7 | 5.0 × 10^6            |
| 315LV-1   | <4 × 10^-11 | <2 × 10^0             |
| X-2       | 2.2 × 10^-7 | 3.6 × 10^3            |

* Error in determination ± 50% of listed value.
† Assay actually measures α-chain molecule equivalents. Error in determination ± 15% of listed value.

Synthesis Rate of the Ig Proteins. To measure the rates of synthesis of immunoglobulin chains, cells were labeled with [35S]methionine for 20 min and the L and H chains were purified by immune precipitation of cytoplasmic proteins as described in Materials and Methods. The immunoglobulin chains in the precipitates were reduced with mercaptoethanol and subjected to electrophoresis in polyacrylamide-sodium dodecyl sulfate gels. Labeled bands were visualized by fluorography. As shown in Fig. 1, the 315LV-1 cells synthesized α-heavy chain at about the same rate as did 315LK cells. Thus the low intracellular level of H chain is not due to a failure of the cell to synthesize H chain in 315LV-1. The possibility that intracellular degradation is responsible for lack of secretion and the low steady-state level was explored next.

Turnover of Immunoglobulin Protein. Since the heavy chain does not
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appear to be secreted or accumulate within the cell, one can postulate that H chain is degraded intracellularly in 315LV-1. To test this hypothesis, cell suspensions of 315LK, 315LV-1, and X-2 were incubated with 10 μCi/ml [35S]methionine; after 20 min a 130-fold excess of cold methionine was added to prevent further incorporation of label. Samples removed after various times of incubation were lysed as described above and the H and L chains purified by immune precipitation with Staphylococcus A and subjected to gel electrophoresis (Fig. 2). The incorporation of [35S]methionine in 315LV-1 cells was somewhat lower than in 315LK or X-2. The gels from this and a similar experiment, where the amounts of incorporation were equal, were scanned and the amount of label in H chain determined. The label in the α-chain of 315LK and X-2 was essentially stable over a 60-min period. In contrast, the label in 315LV-1 H chain began to decline immediately and by 60 min had nearly disappeared. Thus it appears that turnover of α-chain in 315LV-1 cells is much more rapid than in either 315LK or X-2 cells. Presumably this turnover is due to intracellular degradation. We do not find any evidence either for precipitated H chain associated with the nuclear pellet or for extracellular or surface antigens cross-reacting with H chain. (Data not shown.)

Nature of the Heavy Chain in 315LV-1 and X-2. It can be seen in Figs. 1 and 2 that the H chain synthesized in 315LV-1 migrates slightly faster in acrylamide gel electrophoresis than does the H chain from 315LK cells. The hybrid X-2 synthesized an H chain similar in migration to 315LV-1 α-chain. To determine whether the faster migration was due to a size difference or to aberrant α-chain processing in 315LV-1 and X-2 cells, total polysomal RNA was extracted from the three lines and used to direct translation in vitro in a wheat germ cell-free system. Ig proteins among the products of translation were collected by immune precipitation and analyzed on polyacrylamide gradient gels as described above (Fig. 3). The amount of precipitable H chain synthesized is slightly greater with RNA extracted from 315LK cells than from the other
FIG. 3. Electrophoretic analyses of translation products obtained with either total or fractionated RNA preparations. RNA, extracted either from total polysomes or from fractionated membrane-bound and free polysomes, was used to direct a wheat germ cell-free system. After incubation for 3 h at 23°C, reactions were centrifuged at 100,000 g for 30 min. The supernate, i.e. released proteins, were immune precipitated and electrophoresed as described above. Products were obtained either with: total, total RNA; A, free polysomal RNA; B, membrane-bound polysomal RNA.

The RNA from both 315LV-1 and X-2 cells directed synthesis of heavy chain with greater electrophoretic mobility than did RNA from 315LK cells. It is unlikely that any processing such as clipping of a precursor or carbohydrate addition occurred in vitro in the wheat germ lysate. From a similar gel in which marker proteins were included one can estimate that the α-chain synthesized in 315LV-1 and X-2 cells is approximately 3,000 daltons smaller than the one made in 315LK cells. Consequently we refer to it as H' chain. The nature of the alteration is presently unknown. Regardless of the alteration in the H chain, it is unlikely that it affects the phenotype we have observed in 315LV-1 (i.e., lack of secretion and high turnover rate) since the same H' chain is made in both
315LV-1 and X-2. The hybrid cell line does not show the phenotype of 315LV-1.

**Site of Ig Synthesis and Processing.** Since the H chain synthesized in 315LV-1 cells was not secreted, it was of interest to know whether its mRNA was bound to membranes. Therefore, polysomes were separated into membrane-bound and free fractions and the extracted RNA used to program a wheat germ cell-free system. The free and membrane-bound populations were equally active in the synthesis of proteins in vitro. For all three cell lines, however, only the membrane-associated polysomal RNA directed the synthesis of the immunoglobulin chains (Fig. 3). Thus the mRNA active in the synthesis of H' chain in 315LV-1 cells is associated with membranes.

To test whether any H' chain in fact reaches the cell surface of 315LV-1, cells were grown for two generations, collected and washed twice with fresh medium and samples tested by radioimmunoassay. The results shown in Table II indicate that the relative amount of α-chain on the surface of 315LV-1 cells is no more than that found intracellularly (the analysis is not sensitive enough to detect much less than 1% of the parental amount). In addition, 315LK, 315LV-1, and 4T001 cells were tested for cell surface α-H chain on the fluorescence activated cell sorter (courtesy S. Schlossman). The 315LV-1 cells were indistinguishable from the negative control 4T001, whereas 315LK showed bright fluorescence (G. R. Siebert, unpublished observations).

**Discussion**

Initial selection of the NP1 tumor was based on loss of Ig secretion. The tissue culture line, derived from the tumor (315LV-1) contains low levels of an H (H') chain of altered electrophoretic mobility and no detectable amount of L chain. It secretes essentially none of the H' protein and very little can be detected on the cell surface. Surprisingly we find that the rate of the H' chain synthesis in 315LV-1 is similar to that of the H chain in 315LK control cells. Lack of appearance of extracellular H' chain seems to be at least partially due to rapid turnover in 315LV-1 cells. The lesion(s) responsible for altered H chain processing, resulting in nonsecretion and rapid degradation, can be complemented by cell fusion with an MPC 11 κ-producing variant clone. The hybrid cell synthesizes the same H' chain in the same amount as the 315LV-1 parent but it secretes it in a complete Ig molecule with the κ-L chain. On the other hand the alteration(s) in the 315LV-1 H chain was not corrected by fusion with 4T001.01. This lesion, however, is not likely to be responsible for the observed phenotype of 315LV-1 (i.e., lack of secretion and turnover) since it is not exhibited by X-2 which also synthesizes the altered H chain.

It is noteworthy that in these experiments, as well as in those previously reported (17, 26, 27), cell fusion has not resulted in complementation of the mutation which originally led to loss of synthesis of an H or L chain. X-2 produces neither the 315 L chain nor the MPC 11 H chain in wild type amounts. Negative complementation is also not observed. X-2 continues to produce the α-H chain and κ-L chain synthesized in the parents. The only complementation we have observed is the stabilization and secretion of the altered H chain in X-2.

The phenomenon of recovery of secretion may be similar to that reported by Kohler and co-workers (17) with a variant line of P3 cells (MOPC 21, IgG1, κ),
which synthesizes only light chains but does not secrete them. The variant line was fused with the P1 myeloma line, which synthesized and secreted excess free light chains in addition to H\textsubscript{2}L\textsubscript{2}. In the hybrid, the P3 \( \kappa \)-chain was secreted, but only in a covalent structure with the P1 heavy chain and not as free L chain. Thus the lack of secretion of L chain in the parent was recovered in the hybrid. Furthermore these authors isolated variants of the hybrid which had lost P1 heavy chain production and all of the resultant segregants had also lost secretion of P3 light chain even though they still contained the protein intracellularly. (They continued to secrete the free P1 light chain.) Thus the P3 \( \kappa \)-chain seems to require a heavy chain for secretion. The rates of synthesis and degradation of the nonsecreted L chain were not examined in that study.

Morrison and Scharff (28) have isolated heavy-chain producing variant lines of P3. In those lines, although the H chain is not secreted, its intracellular concentration is like that of the parent. The authors conclude that a decreased rate of synthesis rather than turnover, is responsible for the level of H chain found.

A major difference between the P3 case and that of 315LV-1 is the rapid degradation of the nonsecreted \( \alpha \)-H' chain in 315LV-1. An explanation of this phenomenon may be that in the absence of light chain, a free \( \alpha \)-chain may not assume the appropriate secondary structure and may therefore be susceptible to proteolysis. Such a model has been proposed by Schubert and Cohn (29) to explain their observation with a P1 myeloma variant line. In this line an altered H chain was synthesized which could not combine with the L chain. They found, as in the P3 variants discussed above, that the free H chain did not accumulate nor was it secreted. In this case, it was degraded intracellularly, presumably at a rate equivalent to its synthetic rate. The increased degradation of the H' chain in 315LV-1 could be due solely to its unassociated state, although alternative explanations are possible (see below).

Experiments of Hickman et al. (30) suggest that glycosylation is required for \( \alpha \)-chain secretion in MOPC 315. When they added the glycosylation inhibitor tunicamycin to cell cultures, secretion of H chain was blocked; whereas in a MOPC 315 L-chain producing variant line, the unglycosylated \( \lambda \) chain was secreted normally. Although tunicamycin may have had pleiotropic effects, these experiments seem to indicate that glycosylation is required for secretion in MOPC 315. Possibly in 315LV-1 the H' chain cannot be glycosylated correctly. One would propose that the MPC 11 \( \kappa \)-chain producing cell is able to complement the lesion in the glycosylation system. We are currently examining the pattern of carbohydrate addition to H' in 315LV-1 and in X-2. It should be noted that whereas normal MOPC 315 \( \alpha \)-chain was not degraded when secretion was inhibited with tunicamycin, it is possible that unglycosylated H' chain might be susceptible to proteolysis. To test all these possibilities we are presently trying to isolate revertants of 315LV-1 which have recovered the ability to secrete the H' chain. In addition we are attempting to isolate more H chain producing variants of 315LK to examine \( \alpha \)-chain secretion in these lines.

The MOPC 315 H chain has recently been shown to be synthesized in a precursor form (6), as has been shown for L chain and other secretory proteins (3–6). It would be interesting to determine whether this is true for the altered 315LV-1 H' chain as well.
Mosmann and Baumal (31) have looked at MOPC 315 variant cells which do not synthesize detectable amounts of H chain, but do produce and secrete L chains. These variants synthesize but no longer secrete J chain, which normally is covalently attached to the Fc portion of the H chain (16). Since the addition of J chain is a very late step in maturation of 315 protein, the J chain presumably must enter the membrane itself. However without the H chain it remains intracellular. This suggests yet another form of regulation of secretion. We hope that continued study of nonsecreting variants of these myeloma cell lines will further our understanding of these different levels of regulation involved in the control of secretion of immunoglobulin molecules.

The results presented in this paper and in Siebert et al.² suggest a mechanism that may be involved in the selection of functional H,L pairs. A developing cell may successively produce different H and L chains. If those heavy chains unable to combine with a light chain are degraded intracellularly, they will fail to reach the cell surface. Thus the synthesis of functional H,L pairs could be stabilized by a feedback mechanism which recognizes complete immunoglobulin on the cell surface.

Summary

Cells of the 315LV-1 (derived from NP1) variant line of MOPC 315 contain approximately 1% the normal intracellular level of the heavy (α) chain of IgA and no detectable light (λ1) chain. The synthesis rate of α-chain in the variant, however, is similar to that in cells of the parent line. Moreover the relative amount of translatable α-chain mRNA that can be extracted from 315LV-1 cells is about the same as for parental cells. No light-chain synthesis can be detected either in vivo or in vitro in a wheat germ cell-free system. The 315LV-1 heavy chain synthesized in vivo or in vitro has slightly greater electrophoretic mobility than normal H chain and turns over rapidly intracellularly. The variant fails to secrete any of its heavy chain, despite the fact that its H chain mRNA is bound to membranes, as one would expect for a secretory protein message. Fusion of 315LV-1 cells with cells of a κ-producing MPC 11 variant line leads to stabilization of the intracellular H chain and also to full recovery of secretion of the H chain as an H2L2 molecule.

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