

## CONCISE REPORT

# Synthesis of Abnormal Heavy Chains in Bence-Jones Plasma Cell Leukemia With Intracellular IgG

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**In a patient with plasma cell leukemia and a  $\kappa$  type Bence Jones protein in serum and urine, the immunofluorescence study of blood plasma cells showed intracellular  $\gamma$  and  $\kappa$  chain determinants. Biosynthesis experiments showed the production of abnormally short heavy chains (45,000 daltons) that assembled with normal sized light chains with a partial block. These abnormal heavy chains were secreted at a slow rate and were degraded after secretion.**

**B**ENCE-JONES (BJ) myeloma or leukemia are defined by the presence in the patients' urines (and/or serum) of monotypic free light chains without detectable whole monoclonal immunoglobulin (Ig) molecules and they account for about 20% of myeloma cases.<sup>1</sup> Immunocytochemical study of plasma cells showed apparently unreleased intracellular heavy chains in 1 of 7<sup>2</sup> and 10 of 36<sup>3</sup> cases of BJ myeloma studied and in an additional patient.<sup>4</sup> The mechanism of the apparent nonsecretion of heavy chain containing molecules is still unclear. In a study of Ig biosynthesis by plasma cells from BJ myeloma patients, the secretion of small amounts of assembled IgG molecules was found in one case.<sup>5</sup> However, this study provided no information on the heavy chain size nor any kinetic data on its secretion and possible degradation. We report here biosynthesis experiments in one patient with BJ plasma cell leukemia with intracellular IgG. These IgG had abnormally short heavy chains that assembled with light chains with a partial block. They accumulated in the cytoplasm and some of these defective IgG molecules were secreted at a slow rate and were degraded after secretion.

## MATERIALS AND METHODS

### Patient

The patient studied was a 30-yr-old woman who was referred for a rapidly progressive deterioration of general status with disseminated lymphadenopathies and hepatosplenomegaly. She had anemia ( $2.8 \times 10^6$  red cells per cu mm, hemoglobin 8.8 g) and hyperleucocytosis (27,000 to 40,000 WBC per cu mm with 50% small plasma cells). Study of lymph node puncture and biopsy showed a massive infiltration by small plasma cells. The bone marrow contained 50% plasma cells. There was a hypercalcemia (3.45 mmole), but skeletal x-ray survey was normal. Electrophoretic study of serum proteins

showed hypogammaglobulinemia without monoclonal band. However, the immunoelectrophoretic analysis showed the presence of a  $\kappa$  type BJ protein (Fig. 1). Proteinuria was 4.7 g/day with a single  $\beta_2$  peak on electrophoresis that was identified as free  $\kappa$  chains by immunoelectrophoresis. The patient was therefore affected with plasma cell leukemia with serum and urinary BJ protein.

### Immunofluorescence Study of Blood Cells

Mononucleated cells were isolated from the defibrinated blood by Ficoll centrifugation and studied for surface and cytoplasmic Ig by immunofluorescence using rhodamine-conjugated F(ab')<sub>2</sub> fragments of rabbit IgG monospecific for  $\mu$ ,  $\gamma$ ,  $\delta$ ,  $\alpha$ ,  $\kappa$ , and  $\lambda$  chains. The preparation, characteristics and specificity controls of these reagents have been described in detail before, as well as the immunofluorescence procedure.<sup>6,7</sup>

### Biosynthesis Experiments

Study of Ig biosynthesis by the same blood cells was performed as previously described.<sup>8-10</sup> Briefly, the cells were continuously labeled with <sup>14</sup>C valine, threonine, and leucine in depleted medium for 1, 2, and 3 hr. Supernatants (secretions) and Nonidet-P40 cell lysates were indirectly precipitated by a polyvalent rabbit anti-human Ig serum and a goat anti-rabbit IgG serum. The resulting immunoprecipitates were washed, dissolved in sodium dodecyl sulfate (SDS), and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) either unreduced or after reduction and alkylation. Internal markers in these gels were <sup>3</sup>H-labeled IgG (150,000 daltons), heavy (55,000 daltons), and light (24,000 daltons) chains obtained by internal labeling of the murine plasmacytoma MPC 11. The amounts of heavy and light chains produced and secreted by the patient's cells were estimated by the sum of the counts in the corresponding peaks on reduced gels (after background subtraction) and calculated for each whole samples containing the same number of cells at different incorporation times.

## RESULTS

The cell suspension obtained by Ficoll centrifugation of blood cells contained 82% plasma cells that all strongly stained with anti- $\gamma$  and anti- $\kappa$  sera (and not with the other conjugates) by cytoplasmic immunofluorescence. Membrane staining showed that only 5% of these plasma cells dimly stained for  $\gamma$  and  $\kappa$  chains. In addition, the sample contained 9% T lymphocytes (as shown by E rosette formation) and a few normal B lymphocytes (figures for surface Ig staining of lymphocytes were  $\mu$ :4%;  $\delta$ :4%,  $\alpha$  and  $\gamma$  <1%,  $\kappa$ :4% and  $\lambda$ :1.5%).

SDS-PAGE of immune precipitates obtained from the cell lysates showed similar patterns in 1 hr, 2 hr,

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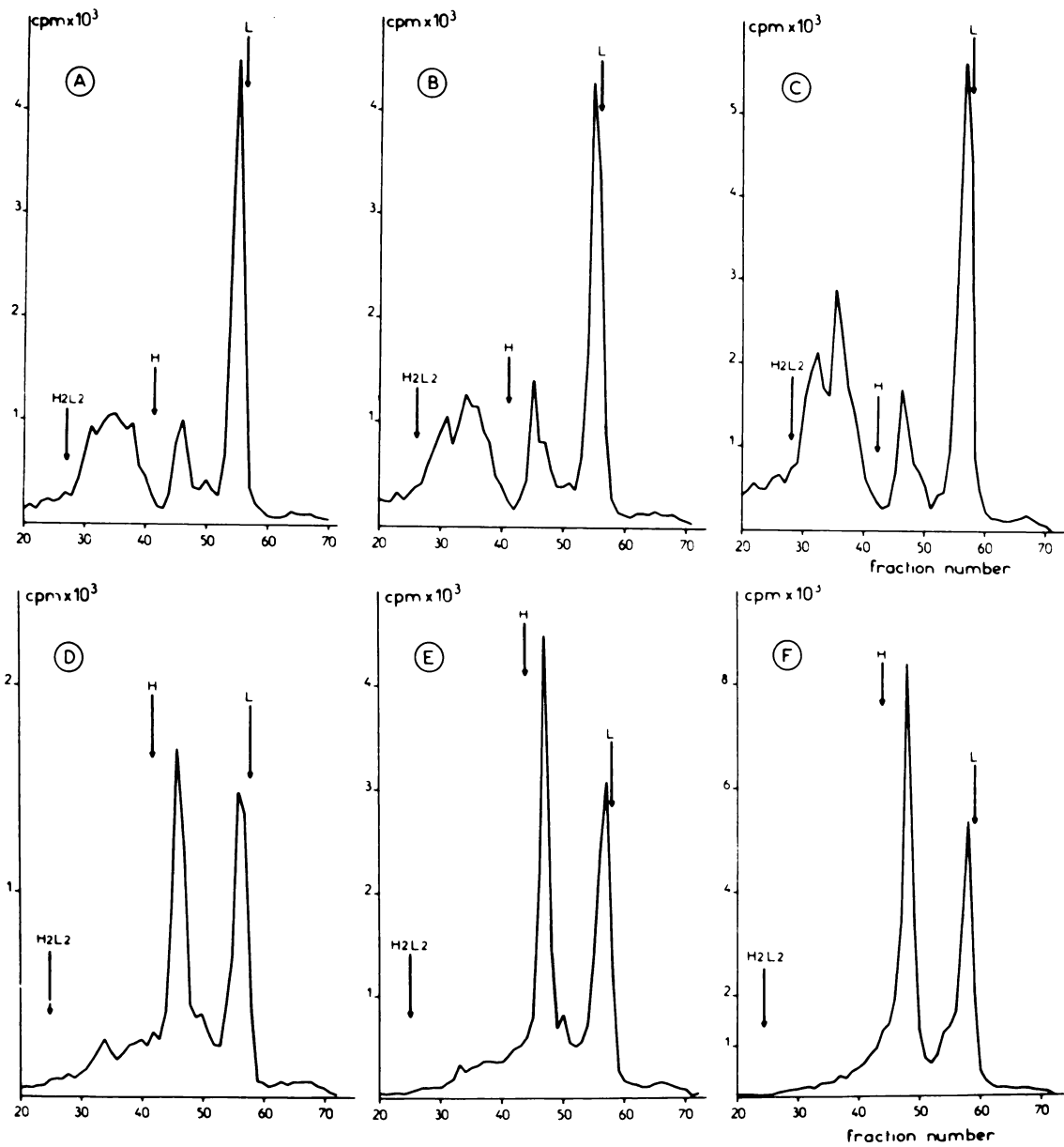
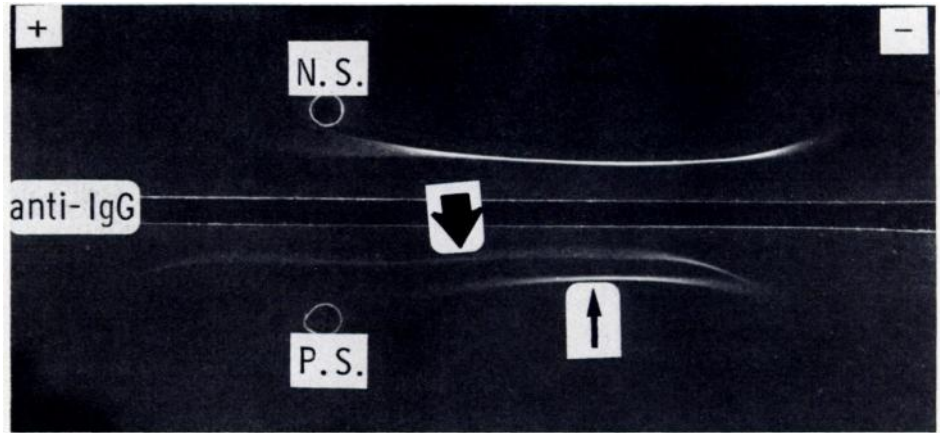
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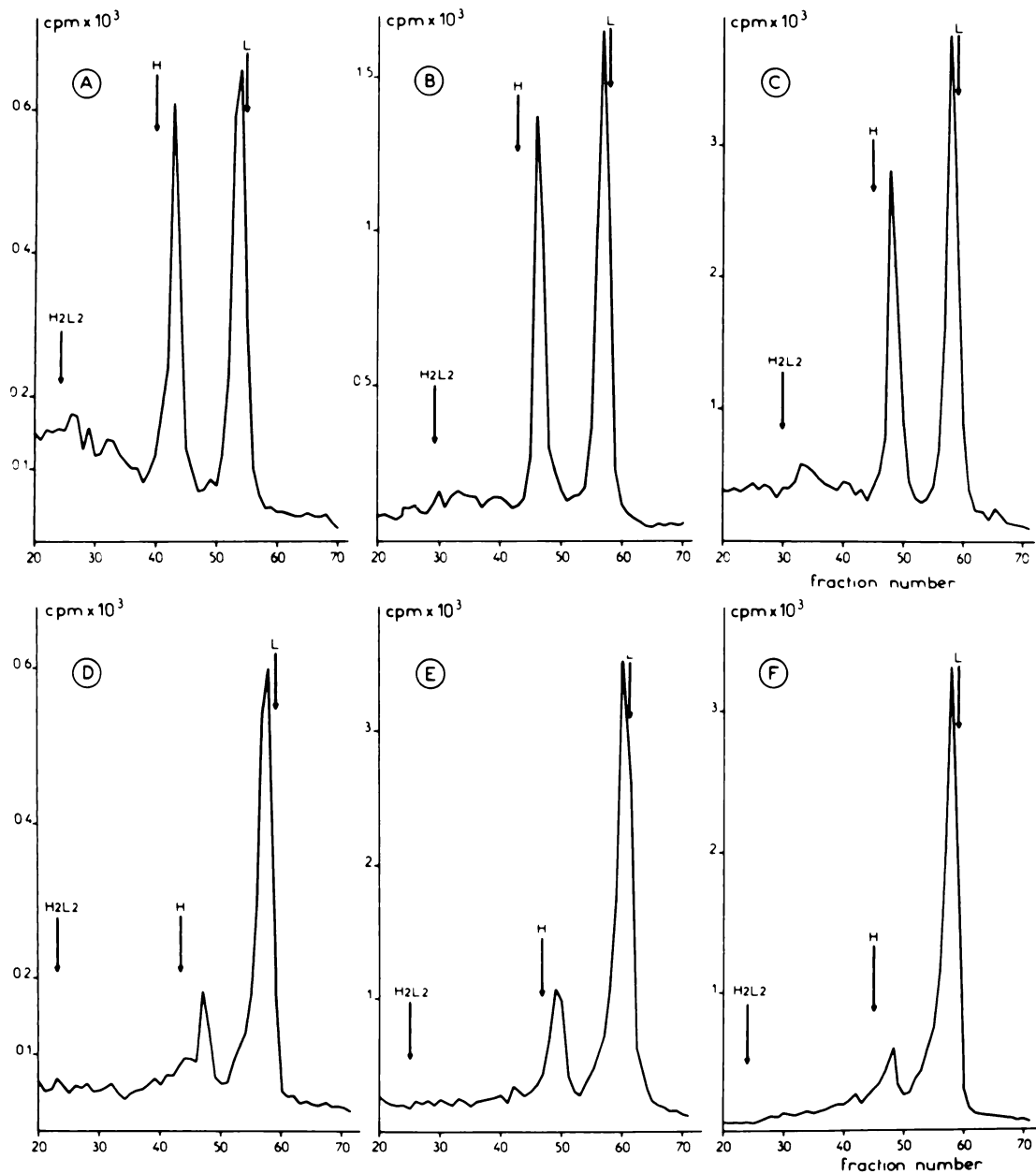
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**Fig. 1.** Immunoelectrophoretic analysis of the patient's serum (PS) compared to normal human serum (NS) using an anti-IgG serum (containing antibodies to  $\gamma$ ,  $\kappa$ , and  $\lambda$  chains). Note the decrease of the IgG line (small arrow) and the heterogeneous external precipitation line of the BJ protein (large arrow). This line was revealed by anti- $\kappa$  serum but not by antisera to  $\lambda$  chains and to any heavy chains. Its anodic part corresponded to the well-known interaction with  $\alpha$ 1-antitrypsine (data not shown).



**Fig. 2.** SDS-PAGE analysis of immunoprecipitates from the cell lysates obtained after 1 hr (A and D), 2 hr (B and E) and 3 hr (C and F) labeling. A, B, and C: unreduced samples; D, E, and F: reduced and alkylated precipitates. Arrows indicate the position of the marker proteins (see Materials and Methods).



**Fig. 3.** SDS-PAGE analysis of immunoprecipitates from 1 hr (A and D), 2 hr (B and E) and 3 hr (C and F) secretions. A, B, and C: unreduced samples; D, E, and F: reduced and alkylated samples.

and 3 hr labeling samples (Fig. 2). On reduced gels, there were a light chain peak of 25,000 daltons and a heavy chain peak of abnormally small size (45,000 daltons) (Fig. 2D, E, and F). The analysis of unreduced samples showed a free light chain peak, a small peak of the size of the short heavy chains, and broad peaks of larger molecular weights that necessarily corresponded to assembled molecules (Fig. 2A, B, and C). These assembled molecules had apparent molecular weights of 70,000, 90,000, and 115,000 daltons and therefore probably corresponded to HL half molecules, H<sub>2</sub> heavy chain dimers (the predominant assem-

bled molecules) and H<sub>2</sub>L. The patient's cells therefore produced abnormally short heavy chains that assembled with light chains with an assembly block.

Study of unreduced samples from secretion showed light chains and light chain dimers with virtually no molecules of higher molecular weight except for a barely detectable 115,000 dalton peak (Fig. 3A, B, and C). Reduction yielded light chains and a small heavy chain peak (Fig. 3D, E, and F).

The amount of heavy and light chains produced and secreted (estimated from the counts in the corresponding peaks on reduced gels) is shown in Table 1 and Fig.

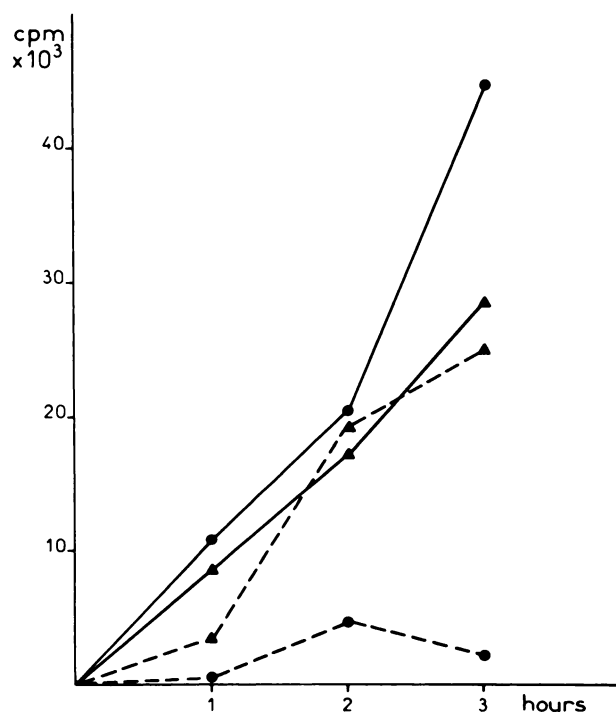


Fig. 4. Radioactivity incorporated in cytoplasmic and secreted heavy (circles) and light (triangles) chains (estimated from reduced gels). Solid lines: cytoplasmic extracts; dotted lines: secretions.

4. During the 3-hr experiment, the amount of light chains in both cytoplasmic extracts and secretions showed a roughly linear increase. A fair amount of heavy chains was synthesized and the intracellular content of these chains appeared to increase exponentially, suggesting some accumulation. As a result, the molar ratio of heavy over light chains increased from about 0.7 after 1 hr and 2 hr incorporation to 0.85 at 3 hr. Comparatively to that of light chains, heavy chain secretion was delayed and minimal (maximum H/L ratio 0.13). Moreover, we found much less heavy chains in 3 hr than in 2 hr samples from secretion, which indicates that a part of heavy chains were degraded after secretion.

Table 1. Quantitative Data on Heavy and Light Chain Synthesis

Sample	cpm (x 10 <sup>3</sup> ) in		H/L Ratio	
	H Chain Peak	L Chain Peak	cpm	Molar*
<b>Cytoplasm</b>				
1 hr	10.8	8.6	1.26	0.70
2 hr	20.5	17.1	1.20	0.66
3 hr	44.7	29.3	1.53	0.85
<b>Secretions</b>				
1 hr	0.5	3.5	0.14	0.08
2 hr	4.7	19.5	0.24	0.13
3 hr	2.1	25.0	0.08	0.04

\*The molar ratio was calculated assuming a similar labeling of heavy and light chains.

DISCUSSION

The patient herein reported was a young woman affected with plasma cell leukemia with a serum and urinary  $\kappa$  type BJ protein without whole monoclonal Ig, a relatively common situation in this form of plasma cell proliferation.<sup>11,12</sup> In contrast, immunofluorescence study of plasma cells showed both  $\gamma$  and  $\kappa$  chain determinants. Such a finding is not rare in BJ myeloma.<sup>2-5</sup> This result was confirmed by biosynthesis experiments that showed that heavy chains were produced with a heavy/light chain ratio of about 0.7 similar to that commonly found in myeloma.<sup>5</sup> These heavy chains had an abnormal size (45,000 daltons) and their assembly with light chains was blocked. This is not surprising since an assembly block was usually found in the case of mouse myeloma mutants producing short heavy chains.<sup>8,13,14</sup> Whereas the light chains were rapidly secreted as free light chains and light chain dimers, heavy chain containing molecules were secreted more slowly and at a low rate (maximum H/L ratio of 0.13 in secreted Ig). Moreover, these heavy chains were degraded after secretion. The degradation is probably related to the structural abnormality since a rapid intra- or extra-cellular proteolysis featured several murine or human myeloma producing deleted heavy chains.<sup>3,8,15-18</sup> These findings explain the lack of detectable monoclonal IgG in our patient's serum.

Low rate secretion with extra-cellular degradation is probably not the only possibility to explain BJ myelomas with intracellular whole monoclonal Ig. Indeed, in a similar mouse myeloma, only light chains were secreted, whereas abnormally short heavy chains were degraded intracellularly.<sup>15</sup> The situation is likely similar to the so-called nonsecretory myeloma that corresponds to several mechanisms. In a series of 30 patients with myeloma and no monoclonal Ig in serum and urine, we found several possibilities<sup>3</sup> (and unpublished results): (1) synthesis of structurally abnormal Ig with intracellular degradation and no secretion, (2) synthesis and nonsecretion of apparently normal Ig (true nonsecretors, 1 single case in our series), and (3) synthesis and secretion of abnormal Ig with probable or ascertained postsecretory proteolysis (the most frequent situation in our experience). It must be pointed out that secretion of structurally abnormal Ig that are not detectable in serum and urine (or only as trace amounts) is not rare and may result in a severe systemic disease due to the deposition of these abnormal Ig in various tissues.<sup>10,18</sup>

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