Metabolism of Radio-iodinated Human Serum Albumin in Patients With Monoclonal Gammopathy

By J. Vivian Wells and H. Hugh Fudenberg

(means HSA level 3.3 g/100 ml, normal with $^{125}$I-labeled human serum albumin (HSA) in five patients with monoclonal gammopathy; three with Waldenström's macroglobulinemia (one also had a $\gamma$C monoclonal protein) and one patient each with $\gamma$G multiple myeloma and $\gamma$G benign gammopathy. Several abnormalities in HSA metabolism occurred in different combinations in different patients and no single consistent pattern was seen. The abnormalities included hypoalbuminemia (means HSA level 3.3 g/100, normal controls 4.7 g/100 ml); hypervolemia due to an expanded plasma volume (mean plasma volume 54.4 ml/kg, normal controls 41.0 ml/kg); decreased plasma and total body albumin pools; increased plasma localization of albumin; shortened plasma $T_1/2$; reduced fractional turnover rate; and reduced albumin synthesis rate. The most marked changes were noted in two patients with Waldenström's macroglobulinemia who required plasma-pheresis for the hyperviscosity syndrome.

Hypervolemia due to an expanded plasma value (PV) is observed in patients with monoclonal gammopathy. The most marked increases in PV are seen in Waldenström's macroglobulinemia (WM) associated with $\gamma$M monoclonal proteins, and such patients frequently display the clinical stigmata of the hyperviscosity syndrome—namely, central nervous system abnormalities, bleeding from mucous membranes, gastrointestinal bleeding, blurred vision, and dilated retinal veins and hemorrhages. The hyperviscosity syndrome occurs rarely with $\gamma$G monoclonal proteins.

Hypoalbuminemia is frequently found in these patients with hypervolemia and monoclonal gammopathy, but its etiology is unknown. Albumin is the single most important plasma protein in the maintenance of the plasma colloid oncotic pressure at a normal level in health and disease, and increased plasma oncotic pressure has been reported in WM. The relationship between increased PV and serum viscosity has been studied in detail for both $\gamma$M and $\gamma$G monoclonal proteins, but there are few studies of albumin metabolism and distribution in such patients. Metabolic turnover studies were therefore performed with homologous $^{125}$I-HSA in five patients with monoclonal serum

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Table 1.—Summary of Clinical Data for Five Patients With Monoclonal Gammopathy

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>Diagnosis</th>
<th>Enlargement of</th>
<th>Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver Spleen Lymph Nodes</td>
<td></td>
</tr>
<tr>
<td>1BT</td>
<td>F</td>
<td>47</td>
<td>56</td>
<td>Waldenström's macro-globulinemia</td>
<td>+ + +</td>
<td>Hyperviscosity Hemolytic anemia</td>
</tr>
<tr>
<td>2LC</td>
<td>F</td>
<td>84</td>
<td>56</td>
<td>Waldenström's macro-globulinemia</td>
<td>+ - +</td>
<td>Osteoporosis</td>
</tr>
<tr>
<td>3SF</td>
<td>M</td>
<td>73</td>
<td>60</td>
<td>Waldenström's macro-globulinemia</td>
<td>+ - +</td>
<td>Hyperviscosity</td>
</tr>
<tr>
<td>4JH</td>
<td>M</td>
<td>55</td>
<td>75</td>
<td>Multiple myeloma</td>
<td>- - -</td>
<td>Respiratory infections</td>
</tr>
<tr>
<td>5WM</td>
<td>M</td>
<td>72</td>
<td>52</td>
<td>Benign monoclonal gammopathy</td>
<td>- - +</td>
<td>Polycythemia</td>
</tr>
</tbody>
</table>

proteins: three with WM (one also had a γG monoclonal protein, that is, biclonal gammopathy) and one each with γG multiple myeloma and γG benign gammopathy.

**Materials and Methods**

**Patients**

The clinical data are summarized in Table 1. The diagnosis was established from the clinical features, characterization of the abnormal serum immunoglobulin with specific antisera, and examination of bone marrow aspirate. The most marked anemia and hyperviscosity were seen in patient 3SF; both this patient and patient 1BT required plasmapheresis at some stage of their management. Recurrent episodes of Coombs-negative hemolytic anemia occurred in 1BT. All patients in the study fully understood the nature of the experiments and were true volunteers.

**Serum Protein Studies**

Measurements were performed in an Auto-Analyzer (Technicon) with Biuret and brom cresol green methods. Serum levels of IgG, IgA, and IgM were measured by radial immunoprecipitation in antibody-agar plates. Serum electrophoresis was run on cellulose acetate at pH 8.6 by standard methods.

**125I-HSA**

Homologous 125I-HSA was prepared by the Bhabha Atomic Research Centre, Bombay, from Behringwerke albumin by the iodine monochloride method and was used within 10 days of preparation. Studies in normal subjects have indicated it is suitable for metabolic studies, with no significant protein denaturation and less than 2% free 125I.

**Metabolic Turnover Study**

The methods have been described in detail. A known dose of 125I-HSA (5–7 μCi) was given by i.v. injection and 24-hr urine specimens collected. Plasma samples were obtained daily for 1 wk and then on alternate days for 2 wk. All were taken without venous stasis after 15 min lying prone. The 125I-gamma-ray activities of 5-ml aliquots of plasma, urine, and injection standard were measured in an automatic scintillation counter (Packard Instruments). Zero plasma activity (Q₀) was estimated from extrapolation of values in plasma at 6 and 12 min on a semilog graph. The curve for residual plasma activity was plotted on 2-cycle semilog graph paper and the resultant values used in a series of equa-
Table 2.—Summary of Laboratory Data for Five Patients With Monoclonal Gammopathy

<table>
<thead>
<tr>
<th>Subject</th>
<th>Serum Proteins (g/100 ml)</th>
<th>Serum Immunoglobulins (mg/100 ml)</th>
<th>Monoclonal Protein (M-band)</th>
<th>Position of M-band in EPG</th>
<th>Bone Marrow*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Albumin Globulin</td>
<td>IgG 24</td>
<td>IgA 1120</td>
<td>γM γ1 80% L</td>
<td></td>
</tr>
<tr>
<td>1BT</td>
<td>7.1 3.8 3.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2LC</td>
<td>8.0 2.8 5.4</td>
<td>620</td>
<td>28 3240</td>
<td>γM γ2 60% L</td>
<td></td>
</tr>
<tr>
<td>3SF</td>
<td>11.0 2.6 8.4</td>
<td>3300</td>
<td>316 2600</td>
<td>γM γ1 85% L</td>
<td></td>
</tr>
<tr>
<td>4JH</td>
<td>9.8 3.8 6.0</td>
<td>3720</td>
<td>155 46</td>
<td>γG γ1 75% P</td>
<td></td>
</tr>
<tr>
<td>5WM</td>
<td>7.1 3.5 3.6</td>
<td>1640</td>
<td>130 90</td>
<td>γG β2 Erythroid hyperplasia</td>
<td></td>
</tr>
</tbody>
</table>

Normal 6.0–8.0: range 3.8–5.5 20–25 100–150

* L, lymphocytes; P, plasma cells.

Turnover Parameters

Plasma volume (PV) (ml/kg): activity of standard × injected volume × 100/PV.

Plasma albumin pool (g/kg): PV × serum albumin level (g/100 ml)/100

Urinary excretion (Q_{U,1,2}, %): urinary free 125I activity in initial 2 days/total injected activity.

Fractional turnover rate (FTR, %/day): the percentage of the plasma pool catabolized and cleared into the urine per day.

Distribution ratio (DR): the fraction of total body albumin located in the plasma pool; that is, DR = plasma albumin pool/total body albumin pool.

Albumin catabolic rate (mg/kg/day): FTR × plasma albumin pool. If the patient is in a metabolic steady state as indicated by steady body weight and serum albumin levels during the period of the study, the synthesis rate is equal to the catabolic rate. The values for normal controls in Table 3 are from studies in five normal subjects and from published studies using comparable methods and calculations.

Results

Serum Proteins

The laboratory data are summarized in Table 2. Total serum protein concentration ranged from 7.1 to 11.0 g/100 ml, the highest value occurring in biclonal gammopathy. The serum albumin level was significantly reduced in three patients and at the lower limit of normal in the other two (3.8 g/100 ml). The mean serum albumin level of 3.3 g/100 ml for the patients was significantly lower than the normal mean of 4.7 g/100 ml.

PV and Albumin Pool Sizes

The results are summarized in Table 3. The PV was increased in three patients, the highest value occurring in biclonal gammopathy (3SF, 7.7 ml/kg). The mean PV of 54.4 ml/kg for the patients was significantly higher than the normal mean of 41.0 ml/kg. The plasma albumin pool was reduced in two
### Table 3.—Results of Albumin Turnover Studies in Five Patients With Monoclonal Gammopathy *

<table>
<thead>
<tr>
<th>Subject</th>
<th>Serum Albumin (g/100 ml)</th>
<th>PV (ml/kg)</th>
<th>Plasma Albumin Pool (g/kg)</th>
<th>DR</th>
<th>Total Body Albumin Pool (g/kg)</th>
<th>Q_{\text{cl}1/2} (Per Cent)</th>
<th>Plasma T1/2 (days)</th>
<th>FTR (Per Cent/day)</th>
<th>Synthesis Rate (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1BT</td>
<td>3.8</td>
<td>52.6</td>
<td>2.00</td>
<td>0.60</td>
<td>3.33</td>
<td>12.0</td>
<td>11.3</td>
<td>10.3</td>
<td>11.51</td>
</tr>
<tr>
<td>2LC</td>
<td>2.6</td>
<td>44.2</td>
<td>1.15</td>
<td>0.47</td>
<td>2.45</td>
<td>7.1</td>
<td>14.4</td>
<td>10.2</td>
<td>6.55</td>
</tr>
<tr>
<td>3SF</td>
<td>2.6</td>
<td>78.7</td>
<td>2.05</td>
<td>0.53</td>
<td>3.85</td>
<td>4.0</td>
<td>23.0</td>
<td>5.6</td>
<td>6.90</td>
</tr>
<tr>
<td>4JH</td>
<td>3.8</td>
<td>39.2</td>
<td>1.49</td>
<td>0.33</td>
<td>4.52</td>
<td>3.9</td>
<td>20.0</td>
<td>10.4</td>
<td>11.61</td>
</tr>
<tr>
<td>5WM</td>
<td>3.5</td>
<td>57.2</td>
<td>2.00</td>
<td>0.40</td>
<td>5.00</td>
<td>3.5</td>
<td>15.5</td>
<td>12.3</td>
<td>12.80</td>
</tr>
<tr>
<td>Mean</td>
<td>3.3</td>
<td>54.4</td>
<td>1.74</td>
<td>0.47</td>
<td>3.83</td>
<td>6.1</td>
<td>16.8</td>
<td>9.8</td>
<td>168</td>
</tr>
<tr>
<td>Mean values in 5 controls</td>
<td>4.8</td>
<td>40.0</td>
<td>1.92</td>
<td>0.41</td>
<td>4.68</td>
<td>&lt; 12.0</td>
<td>18.0</td>
<td>10.4</td>
<td>—</td>
</tr>
<tr>
<td>Normal range †</td>
<td>3.8–5.5</td>
<td>33.0–50.0</td>
<td>1.60–2.40</td>
<td>0.32–0.50</td>
<td>3.50–6.00</td>
<td>14.0–23.0</td>
<td>8.5–13.0</td>
<td>—</td>
<td>120–250</td>
</tr>
<tr>
<td>Normal mean †</td>
<td>4.7</td>
<td>41.0</td>
<td>1.90</td>
<td>0.40</td>
<td>4.60</td>
<td>10.5</td>
<td>185</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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patients (2LC and 4JH) and the total body albumin pool reduced in two patients (2LC and 1BT). The distribution of albumin between plasma and extravascular pools was abnormal in two patients with WM (1BT and 3SF), with a significant increase in plasma localization.

Metabolic Parameters

Initial isotope excretion was normal in all five patients, excluding significant protein denaturation. The plasma T½ was shortened in 1BT (11.3 days) and at the upper limit of the normal range in 3SF (23.0 days). The FTR was reduced significantly in 3SF with a turnover of only 5.6% of the plasma pool per day. The values for these parameters were otherwise normal in the patients. The albumin synthesis rate was decreased in 2LC and 3SF, at the upper limit of normal in 5WM, and normal in 1BT and 4JH.

DISCUSSION

Several abnormalities in albumin metabolism were observed; these included hypoalbuminemia, increased PV, decreased plasma and total body albumin pools, increased plasma localization, shortened plasma T½, reduced FTR, and reduced albumin synthesis rate. However, no consistent pattern was obvious. Similar findings (but not hypervolemia) were noted by Steinfeld,28 who studied albumin metabolism in 12 patients with advanced malignancy, including one patient with multiple myeloma. Interpretation of data in the latter study was complicated by evidence of denaturation in the injected preparation with rapid excretion of radioisotope in the initial 24 hr. Steinfeld stated that the abnormalities were consistent with a primary defect in albumin synthesis and a reduced FTR. Reduction in the FTR was also observed by Waldmann29 in studies in patients with lymphoma, whereas Rossing30 found an increased FTR to be a common observation in his studies of 16 patients with neoplastic diseases (including three with multiple myeloma). Rossing30 agreed that reduced albumin synthesis rate was a common finding and suggested that the differences in the studies28,30 were due to a combination of patient selection, denaturation of the injected preparation, and variations in the method of analysis in the turnover data. The present studies were performed with a preparation that was not denatured and the data analyzed by a method that expressed FTR as a percentage of intravascular albumin catabolized per day (as did Rossing30) and not as a percentage of total body albumin (as did Steinfeld28 and Waldmann29). Nevertheless, an increased FTR was not a feature of the present studies in patients with monoclonal proteins.

The presence of monoclonal macroglobulins in increased concentration in plasma is a key factor in the frequent finding of increased serum viscosity and hyperviscosity syndrome.9 Both 1BT and 3SF required plasmapheresis as a therapeutic measure; both had a significantly increased PV and were the only patients with clearly significant increases in plasma localization of albumin (high DR). One possible explanation for the latter is the formation of complexes between the macroglobulin and albumin.31 There was no evidence of loss of labeled protein via the gastrointestinal or urinary tracts in any of the
present subjects during their turnover studies that might account for these abnormalities.

Although both 1BT and 3SF had a normal plasma albumin pool mass, they showed significant differences in albumin metabolism; 1BT had a shortened plasma T ¾, with normal FTR and normal synthesis rate, while 3SF had a plasma T ¾ at the upper limit of normal with a decreased FTR and decreased synthesis rate. It is possible that the presence of a second monoclonal protein (IgG) in 3SF may have accounted for or contributed to these differences, but this seems unlikely since a similar pattern was not seen in either of the two patients with a single γG protein (4JH and 5WM).

It is difficult to compare the present data with data from albumin turnover studies in patients having polyclonal increases in one or more serum immunoglobulins. Studies in hepatic cirrhosis are influenced by the hepatic parenchymal cell necrosis, ascites, and therapy with corticosteroids, which can increase plasma localization and increase albumin catabolism.7,17,23,24 Studies in the nephrotic syndrome with polyclonal increase in serum IgM are complicated by a marked deficiency of IgG and albumin and therapy with steroids.17,24 Crane32 studied albumin metabolism in New Guinea natives with tropical splenomegaly syndrome; the syndrome is characterized by marked splenomegaly, hepatomegaly, anemia, hypervolemia, hypoalbuminemia, and polyclonal increases in serum IgG and especially IgM.32-34 Increased immunoglobulin synthesis was postulated as the primary event causing an increase in plasma colloid oncotic pressure which was corrected by expansion of the PV. The latter would produce a fall in serum albumin concentration and then increased albumin synthesis as a compensatory mechanism.32 He found the mean albumin synthesis rate in natives with the syndrome to be 247 mg/kg/day (range 189–313 mg/kg/day). However, in the present study, the highest albumin synthesis rate of 247 mg/kg/day was found in 5WM, who had no signs of multiple myeloma or hyperviscosity syndrome with his γG protein, and a serum IgM level of only 90 mg/100 ml.

Further studies are indicated to examine the relationship between abnormalities in albumin metabolism and the findings of hypoalbuminemia, hypervolemia, increased serum viscosity, and hyperviscosity syndrome in patients with monoclonal proteins. It is not clear whether the abnormalities noted in the present study are related primarily to the presence of a neoplastic disorder of the plasma cell series, or more specifically to the serum concentration of the monoclonal protein, its immunological type, or its existence in an abnormal form, e.g., as an aggregate.

REFERENCES


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