Extreme Elevation of Transcobalamin II
Levels in Multiple Myeloma
and Other Disorders

By Ralph Carmel and Daniel Hollander

The source of transcobalamin (TC) II in man and the factors controlling its serum level are unknown. We studied 98 patients with extreme elevation of this protein (>2000 ng/liter, a level approximately twice normal mean), grouped by predominant diagnosis in the categories of liver disease (primarily alcoholic cirrhosis) (17 patients), infection and other inflammatory disorders (21), cancer (17), lymphoproliferative disorders (9), and, most interestingly, multiple myeloma and related disorders (25); the remaining patients had miscellaneous disorders. Although many patients had persistent TC II elevation, in seven instances transitions between high and normal TC II levels were identified. These transitions most often appeared to parallel changes in acute inflammatory processes. Thus TC II had many of the characteristics of an acute-phase reactant. Of the possible cellular sources for TC II previously suggested, a myeloid origin was not supported by our data, and other evidence may be marshalled against a reticuloendothelial source; a hepatic source remains possible. However, our striking finding of TC II elevation in multiple myeloma and Waldenström macroglobulinemia and the frequent coexistence of hyperglobulinemia in the other patients suggests that plasma cells and B lymphocytes also merit consideration as sources.

TRANSCOBALAMIN (TC) II is the crucial transport protein for vitamin B₁₂ in the blood, yet little is known about its source or the factors affecting its production. We therefore focus here on one approach that might provide clues to that end, namely, identifying those patients whose serum TC II levels are extremely high. Similar identification in the case of TC I led to important insights into possible cellular sources for that protein.

We arbitrarily selected 2000 ng/liter as the minimum level of unsaturated TC II for inclusion of patients in our study, and we analyzed the patients’ clinical and laboratory data.

MATERIALS AND METHODS

Samples were obtained from sera submitted for vitamin assay requested by other physicians or obtained for various studies at the Grace Hospital, Detroit, and at Los Angeles County-USC Medical Center, Los Angeles. Sera with TC II levels >2000 ng/liter were selected for further study (normal 940 ± 228 ng/liter, mean ± 1 SD). In several cases serial blood samples were available. Since TC II is unaffected by clotting in vitro, plasma was not used. Serum vitamin B₁₂ binding proteins were fractionated by Sephadex G-200 gel chromatography in 0.1 M Tris-1 M NaCl buffer pH 8.6. The low molecular weight binder (approximately 35,000 daltons) was taken as TC II and quantitated. This was confirmed in three instances by demonstrating reaction with rabbit anti TC II (kindly provided by Dr. C. A. Hall, V.A. Hospital, Albany, N.Y.) or with

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Table 1. Data on Patients With Elevated TC II Levels

<table>
<thead>
<tr>
<th>Category*</th>
<th>Range of TC II Level (ng/liter)</th>
<th>High Unsaturated R Binder Level</th>
<th>Neutrophilia (&gt;7500/μl)</th>
<th>Fever</th>
<th>Documented Infection</th>
<th>Liver Abnormality§</th>
<th>Hyperglobulinemia (&gt;4 g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple myeloma†</td>
<td>2030–5000 (25)</td>
<td>8/25</td>
<td>1/13</td>
<td>10/18</td>
<td>7/13</td>
<td>0/20</td>
<td>25/25</td>
</tr>
<tr>
<td>Lymphoproliferative disease ‡ (9)</td>
<td>2090–9500</td>
<td>9/9</td>
<td>1/8</td>
<td>8/9</td>
<td>4/5</td>
<td>3/9</td>
<td>2/9</td>
</tr>
<tr>
<td>Infection and inflammation** (21)</td>
<td>2000–3240</td>
<td>16/21</td>
<td>13/19</td>
<td>15/19</td>
<td>18/18</td>
<td>8/20</td>
<td>9/20</td>
</tr>
<tr>
<td>Liver disease (17)</td>
<td>2000–5180</td>
<td>13/17</td>
<td>4/16</td>
<td>7/15</td>
<td>2/12</td>
<td>17/17</td>
<td>13/16</td>
</tr>
<tr>
<td>Miscellaneous†† (9)</td>
<td>2000–4220</td>
<td>3/9</td>
<td>4/6</td>
<td>5/8</td>
<td>1/3</td>
<td>1/9</td>
<td>3/7</td>
</tr>
<tr>
<td>Total (98)</td>
<td>2000–9500</td>
<td>60/98 (61%)</td>
<td>28/78 (36%)</td>
<td>53/80 (66%)</td>
<td>36/65 (55%)</td>
<td>38/91 (42%)</td>
<td>58/88 (66%)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate numbers of patients in that category.
†Normal TC II = 940 ± 228 ng/liter (mean ± 1 SD).
‡Normal R binder = 213 ± 171 ng/liter (mean ± 1 SD).
§Criteria: elevated serum bilirubin level in the absence of hemolysis; elevated SGPT; elevated serum alkaline phosphate shown to be either liver-derived or occurring with elevated SGOT or SGPT; or abnormal liver scan.
†Including three patients with Waldenström macroglobulinemia.
‡Three patients with chronic lymphocytic leukemia, the rest with various lymphomas.
**Including septicemia, pneumonia, endocarditis, tuberculosis, leprosy, ulcerative colitis, and pancreatitis.
††Including agammaglobulinemia, subacute myelomonocytic leukemia, acute undifferentiated leukemia, untreated pernicious anemia, two cases of renal failure, and three cases of lupus erythematosus.
human anti-TC II\(^6\) antisera. TC II of selected patients was also tested for its ability to facilitate uptake of \(^{57}\)Co-labeled vitamin B\(_2\) by reticulocytes.\(^7\) Approximately half the sera to be reported were also fractionated by precipitation in 2 M (NH\(_4\))\(_2\)SO\(_4\),\(^8\) the results agreeing closely with the Sephadex gel chromatography results. Since TC I and TC III cannot be distinguished by any of the above fractionation methods and since that distinction was unimportant for this study, we will refer to those two binders by the generic term of R binders.

The chief diagnosis was known to us for all patients, but in almost all cases hospital records were further reviewed for specific information relevant to the date the blood sample was obtained, particularly in those instances where a patient’s TC II level was high at one time and normal at another. Besides the clinical diagnoses, the specific information sought included sex, age, race, hemoglobin level, white blood cell, neutrophil, and monocyte counts, platelet count, serum creatinine, uric acid, albumin, globulin, bilirubin, GOT, GPT, LDH, alkaline phosphatase, and folate levels, presence of fever or infection, presence of alcoholism or diabetes, and medication administration. Since not every patient had all data available, some results to be presented will be provided for less than the full complement of 98 patients.

RESULTS

The 98 patients were assigned to several categories based on their primary diagnosis (Table 1). However, overlap existed among the categories. For example, 66% of evaluable patients were febrile and 55% had documented infection, although only 21 patients were assigned to the category of predominant infection. Similarly, several patients with liver disease were not assigned to the category of liver disease, since other diseases predominated clinically.

None of the laboratory parameters selected for evaluation were consistently abnormal. These included liver chemistry tests, which were abnormal in only 42% of cases (Table 1), renal function, and blood counts. Granulocyte counts were elevated in only 36% of patients (Table 1). However, 66% of all patients in whom it was assayed (52% if myeloma patients are excluded) had elevated serum globulin. Where electrophoresis was also done this hyperglobulinemia was predominantly of the gamma globulin fraction. There was also a preponderance of black patients (59%), almost double the proportion of blacks in our patient population base. Most of the patients with liver disease (15 of 17) were alcoholic; 35 of 60 patients overall were alcoholic. No particular type of infection or cancer predominated in their respective categories.

The highest TC II levels were found in three patients with lymphoma involving the liver (9500, 5960, 5210 ng/liter), two with chronic lymphocytic leukemia and infection (4860, 4970), five with myeloma, three of whom also had infection (5000, 4710, 4630, 4600, 3820), and one each with cirrhosis and convulsions (5180), lupus erythematosus and myelofibrosis (4220), and esophageal cancer and hypercalcemia (3690 ng/liter).

The seven patients who had normal and elevated TC II levels at different times were of particular interest. One patient with myeloma and hypercalcemia developed TC II elevation at the time he developed pneumonia; TC II rose from 1180 to 4630 ng/liter. However, other myeloma patients had high TC II levels without infection, the TC II levels of two patients remaining elevated over the 4-mo and 16-mo periods that followup samples were obtained. Five patients lost their TC II elevation: TC II fell from 3260 to 860 ng/liter in a patient with myeloma whose hyperviscosity syndrome and pleural effusion responded to chemotherapy, from 5180 to 1360 ng/liter in a patient with alcoholic liver disease and seizures whose liver function tests became normal (his high serum
vitamin B₁₂ and R binder levels also became normal), from 3530 to 990 ng/liter in a patient with subacute myelomonocytic leukemia, monoclonal gammopathy, and hypercalcemia who was undergoing successful chemotherapy, from 3070 to 1570 ng/liter in a patient with leprosy undergoing treatment, and from 2610 to 1050 ng/liter in a patient with hemoglobin C disease when her transient red cell aplastic crisis and fever remitted. In a seventh patient, whose TC II rose from 1230 to 2440 ng/liter, no clinical or laboratory change was identified. On the other hand, several patients with malignancies had persistent TC II elevation for the periods (up to 3 mo) that they were studied, and one patient with alcoholic liver disease had unchanged TC II levels for 2½ yr.

Identification of the elevated TC in our patients as TC II was supported by confirmatory ammonium sulfate precipitation results. Furthermore, the TC II also was shown to react with anti-TC II antiserum in all three cases tested and to enhance uptake of vitamin B₁₂ by reticulocytes in the two cases tested.

Serum vitamin B₁₂ level was high in 38 patients, but this incidence is somewhat misleading, since patients with high serum vitamin B₁₂ levels were more likely to be selected for TC fractionation studies in the first place and thus for high TC II to be discovered than were patients with normal vitamin B₁₂ levels. Nevertheless, two of the three patients with very high vitamin B₁₂ levels had the bulk of their endogenous vitamin attached to R binder, as is usual, rather than to their TC II. The sole exception was the only one of them who had cirrhosis.

Serum unsaturated R binder levels were elevated in 61% of the patients, suggesting that TC II is greatly elevated in conditions where R binders also become elevated. Whether or not a more direct link between levels of these two vitamin B₁₂-binding proteins exists is unknown. However, it should be noted that in our patients with myeloma, R binder levels were most often normal or low (Table 1). Furthermore, only one of the seven patients showing transition between normal and high TC II levels showed any change in R binder levels.

DISCUSSION

Past reports of TC II elevation have been fragmentary, and some of the early studies cannot be evaluated because other TC were not separated adequately from TC II. Nevertheless, high TC II has been found fairly consistently in Gaucher disease¹⁰ and in patients with antibody against their own TC II. Elevated levels have also been reported variously in monocytic leukemia,¹² lymphoma and acute leukemia,¹³ certain myeloproliferative disorders with increased myeloblast proliferation,¹⁵ some cancers,¹⁶ and in a father and daughter with ulcerative colitis in whom a familial TC II elevation was postulated.¹⁷ TC II levels in liver disease have been variable.¹₈ ²⁰

No single factor seemed to be associated with high serum TC II levels in our series, but TC II resembled an “acute-phase reactant,” being elevated in infection, inflammatory disorders, and cancer. Nevertheless, we have found other patients with the same diseases to have normal TC II levels (and, in fact, we cannot estimate how common TC II elevation is in these disorders). Thus other factors clearly seem to be required as well. Among such factors, a genetic one may be important. For example, as in other studies from this laboratory, blacks predominated among our subjects with TC II elevation.
Most surprising and intriguing was our finding of 25 patients with multiple myeloma and Waldenström macroglobulinemia with very high TC II levels. This is the only group for which we could also provide a denominator: the 25 were found among 60 unselected myeloma patients whose TC were fractionated. Among the remaining 35, 15 actually had TC levels more than 2 SD above normal. Furthermore, our myeloma patients appeared to be unique among the groups in our survey in generally not having concomitant serum R binder level elevation. Thus a specific tendency towards high TC II seems to exist in myeloma. Bloomfield et al. also found some patients with myeloma to have high TC II, although their technique of separating out TC II with uncoated charcoal remains unvalidated.

We could identify no clinical difference in our series between the myeloma patients having normal and high levels. The connection of TC II to plasma cell proliferation is suggestive, but the possibility of such cellular origin for the protein remains speculative, and it should be noted that among our miscellaneous group of patients with high TC II was a patient with agammaglobulinemia. Nevertheless, 52% of nonmyeloma patients in our series in whom such information was available also had elevated globulin levels; in many cases this elevation was shown to be of the gamma globulin fraction. Similarly, immunoglobulins are often increased in Gaucher disease, the one condition to date in which TC II has been found most consistently to be elevated. Furthermore, it is interesting that 9 of our patients with lymphoproliferative disease had high TC II, and Rachmilewitz et al. found 14 of 20 patients with lymphoma to have high TC II. In our series, in fact, the greatest TC II elevations occurred among patients with lymphoproliferative disease.

Thus we suggest that the possibility of a relation between plasma cell or B lymphocyte proliferation and TC II merits further exploration. The three candidates for the source of TC II thus far have been hepatic cells, reticuloendothelial cells, and myeloid cells.

The hepatic source was suggested by animal studies and remains a strong, although unsubstantiated, possibility in man. Liver disease, particularly due to alcohol, was common in our subjects, and occult hepatocellular disturbance may have been undetected in many others.

A reticuloendothelial source was suggested by observations in Gaucher disease. The high TC II levels in monocyctic leukemia here and in previous reports supports such a source, as possibly does the fall in TC II levels following chemotherapy in our patient with monocytic leukemia. However, several findings militate against reticuloendothelial cells: leukemic monocytes taken from a patient with high serum TC II levels did not contain TC II, nor did normal monocytes isolated in a 95% pure preparation (Carmel R, Larkin E: Unpublished data) or granuloma extracts from skin biopsies of patients with leprosy (Carmel R, Rea T: Unpublished data). As mentioned above, high TC II levels in Gaucher’s disease may be related to the cause of the frequently co-existing immunoglobulin abnormalities rather than to reticuloendothelial cell activity.

Myeloid cell proliferation, suggested as a possible cellular source for TC II, is not supported by our data, and that report by Zittoun et al. of TC II in early
myeloid cells remains unconfirmed. Most of our patients had no evidence of myeloid disturbances, and the common thread in patients with transition between high and normal serum TC II levels seemed to be changes in infectious or hepatic status. Only one such patient manifested significant change in neutrophil count.

Finally, of course, elevation of serum levels of TC II need not be due to increased production or release but may reflect impaired catabolism or clearance of the protein, perhaps related to cell receptor site abnormality in some cases.

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REFERENCES

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