CONCISE REPORT

Bone Marrow Participates in the Biosynthesis of Human Transcobalamin II

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Studies concerning the site of synthesis of the vitamin B₁₂ binding serum protein, transcobalamin II, have been done in various mammalian animals. The actual site of biosynthesis in man has not yet been defined. The finding that bone marrow derived cells release apo-transcobalamin II in the mouse led us to examine the genetic patterns of transcobalamin II in man, both before and after marrow transplantation. A gradual but incomplete transformation of the recipient's transcobalamin II type into donor's type, corresponding to 75% or less of the total activity, was registered in 4 cases. Surprisingly, persistent host-type TC II, in spite of different donor type, was observed in 4 further marrow recipients. We conclude that hematopoietic cells transferred with the transplanted marrow participate in the biosynthesis of human transcobalamin II.

TRANSCOBALAMIN II (TC II) is an essential trace protein in plasma that binds vitamin B₁₂ (Cobalamin, Cbl) and delivers it to cells and tissues. TC II is immunologically and functionally distinct from the other Cbl-binding proteins in human blood, called TC I and III or R-binders. Studies concerning the site of synthesis of TC II have been done in mammalian animals: there is evidence for release of, or proof of synthesis by the liver and other organs, in particular by hepatocytes, by ileal mucosa cultured in vitro, by fibroblasts, and by mononuclear phagocytes. Human intestine releases TC-II-Cbl in vivo, however, no data on the biosynthesis of TC II in man have been reported. Extrapolation of animal data to man is not self-evident because most species differ from man in the sense that they do not produce TC-I-Cbl.

Polymorphic TC II variants in human serum express a genetically determined isoprotein system. The finding that mononuclear phagocytes derived from murine marrow release apo-TC II, led us to study the genetic variants of TC II in human serum, both before and after bone marrow transplantation (BMT).

MATERIALS AND METHODS

Patients

Marrow recipients were conditioned for BMT according to diagnosis: 7 patients with severe aplastic anemia (AA) were pretreated...
Isoprotein patterns of marrow recipients before and at various intervals after BMT were compared to those of their respective donors. Of the 17 cases, characterized by unequivocal engraftment, 9 had TC-II-identical donors, and as anticipated, no changes of TC II genotypes were observed. Eight patients had TC-II-nonidentical donors. In 4 of these 8 patients host-type TC II persisted after BMT (group I, Table I), but in the remaining 4 patients, it was significantly transformed (group II, Table I). Two marrow recipients (A.D. and C.R. in group II) had donors with completely different, homozygous TC II types, and their TC II exhibited gradual, but incomplete transformation into the donor types (C.R. in Figs. 1 and 2). At 4 and 11 mo, respectively, after BMT, their TC II isoprotein patterns corresponded to that of the heterozygous TC II type 1–3, indicating that at least half of

**RESULTS AND DISCUSSION**

Isoprotein patterns of marrow recipients before and at various intervals after BMT were compared to those of their respective donors. Of the 17 cases, characterized by unequivocal engraftment, 9 had TC-II-identical donors, and as anticipated, no changes of TC II genotypes were observed. Eight patients had TC-II-nonidentical donors. In 4 of these 8 patients host-type TC II persisted after BMT (group I, Table I), but in the remaining 4 patients, it was significantly transformed (group II, Table I). Two marrow recipients (A.D. and C.R. in group II) had donors with completely different, homozygous TC II types, and their TC II exhibited gradual, but incomplete transformation into the donor types (C.R. in Figs. 1 and 2). At 4 and 11 mo, respectively, after BMT, their TC II isoprotein patterns corresponded to that of the heterozygous TC II type 1–3, indicating that at least half of...
their apo-TC II had been synthesized by marrow-derived cells. Patient C.R. even showed a transient further increase of the donor-type TC II, accounting for approximately 75% of the total at 3.5 mo after BMT (Figs. 1 and 2). Two marrow recipients in group II (S.R. and G.K.) had partially different heterozygous donors. A slow, but incomplete transformation and the appearance of a new TC II band (Fig. 3) showed again in both cases that donor-type TC II was synthesized after BMT.

Thus, our observations of four TC II genotype transformations after BMT (group II) provide proof of marrow involvement during de novo synthesis of apo-TC II. The simultaneous observance of “complete” chimerism (i.e., donor cell engraftment as documented by established cell markers, Table I) and partial transformation of TC II persisting in one case (C.R.) for 11 mo after BMT can be explained by assuming that at least two different cell types, one of them derived from the transplanted marrow, produce TC II in man. If bone marrow cells were the only source of TC II, and considering that the plasma half-life of TC II is extremely short, one would expect reduced TC II levels in patients with AA, a condition characterized by reduction or lack of myeloid and erythroid stem cells in human marrow. Findings of normal and even elevated TC II levels during AA in this study and in the literature also support a multiple origin of human TC II.

Prevailing host-type TC II after marrow engraftment in spite of different donor type was observed in the first group. A possible explanation is provided by assuming that only specific stimulation of grafted donor cells induces expression of donor-type TC II, e.g., as demonstrated by K.R. (group I). This patient temporarily exhibited donor TC II bands in his serum. Similarly, permanent TC-II-type transformations evidenced in group II could reflect persistent activity of stimulated cells. The striking differences in the occurrence of graft-versus-host disease (GVHD) (Table I) observed in group I (25%) and group II (100%) also seems to support this assumption. A recent report describing low levels of intracellular apo-TC II in human peripheral mononuclear cells may be compatible with our observations. However, only future studies, including careful long-term follow-up of patients with transplanted marrow and equivalent studies in an animal model, will aid the decision as to how the production of marrow-derived TC II is regulated.

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REFERENCES

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