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Species Specificity in the Immunologic Reactions and Biological Functions of Transcobalamin II (40668)[1]

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With the first studies of this topic it was observed that antibody made against human plasma transcobalamin (TC II), a plasma transport protein for cobalamin (Cbl), did not react with TC II from 4 mammals other than primates (1). The TC II from the non-reacting species failed to promote Cbl uptake by HeLa cells (1), an *in vitro* model used to measure the function of TC II (2). There has been only one other fully reported, comprehensive study of the subject with results which to a degree conflicted with those of our earlier study (3). The antibody for the more recent study (3) was made against rabbit TC II and did react with the TC II of 14 species of mammals but not with the TC II of other vertebrates. Isolated TC II of the rabbit promoted the uptake of Cbl by the reticulocytes of 4 species of mammals. Wright and Allen reported different degrees of immunoreactivity of 6 species of TC II with antibody from 2 species (4).

There are also conflicting data from limited studies of the function of TC II from a single species in cells of another. The earliest true study of the effect of a substance in human serum in the promotion of Cbl uptake by human cells, HeLa cells, observed species specificity (5). The uptake of monkey TC II-Cbl by human HeLa cells was 1/3–1/2 that of human TC II-Cbl (6). The uptake of rat TC II-Cbl by HeLa cells was 10% that of human TC II-Cbl (7). Murine fibroblasts took up human and murine TC II-Cbl equally well (8). Murine L121D leukemia cells took up human TC II by processes analogous to the entry of human TC II-Cbl into human cells (9), but the investigators did not compare the degree of uptake with that of murine TC II. Isolated rat liver mitochondria took up Cbl from rat serum to a greater degree than from human serum even without accounting for the holo TC II in the sera (10). In a somewhat different approach, the fates of doubly labeled human and rabbit TC II-Cbl were similar when infused back into the rabbit (11).

Resolution of the controversy outlined above is important if models used to study the function of TC II are to cross species lines. Certain technical aspects of the past studies have not been ideal especially in the cell-uptake experiments. No attention has been paid to the effect of native Cbl bound to TC II which is abundant in the sera of some animals and thereby diluting the uptake of any apo TC II labeled *in vitro*, the only component actually measured in the past. The present study is essentially a duplication of ours of a decade ago, but with considerably improved as well as different techniques.

Materials and methods. Materials. The sera were obtained either directly from the appropriate species or purchased and stored at −20° until use. The CN [^{57}Co]Cbl, nonradioactive Cbl, rabbit antibody against pure human TC II, and cultural cells have been described previously (2, 7, 8, 12–15).

Methods. A previously published method of radioimmunoassay (RIA) for human TC II (12) was used to determine the reactivity of each TC II against antihuman TC II. The standard curve was set up as described and each whole serum assayed was adjusted in volume to provide 100 pg of TC II-Cbl. The complete panel of sera was studied in duplicate on three occasions.

Prior to the measurement of the cell uptake of each TC II-Cbl, the holo and apo TC II were measured in each serum. Apo TC II was

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measured by saturation of the serum with CN [^{57}Co]Cbl followed by separation of the TC II by 1.96 *M* $(NH_4)_2SO_4$ (13). Total TC II was measured by saturation of the TC II of each serum with CN [^{57}Co]Cbl, precipitating the TC II with $(NH_4)_2SO_4$ and measuring the Cbl by bioassay with *Euglena gracilis* (14).

All cell uptake studies were performed with TC II-Cbl totally in the holo form but with part of the Cbl attached to the TC II *in vivo* through natural Cbl metabolism and part attached *in vitro*. The complex taken up by the cells was measured by counting the CN [^{57}Co]Cbl used in the *in vitro* conversion of the mixed apo and holo TC II to all holo TC II. The calculation of uptake, however, was in terms of the *total* TC II to which the cells were exposed and the amount of *total* TC II taken up. The TC II-Cbl of each serum was isolated by precipitation with $(NH_4)_2SO_4$ and the cells were exposed to this fraction of the serum only. The human TC II-Cbl studied for comparison was prepared in the same way or was partially purified as before (2).

Uptake by RPMI lymphocytes, HeLa cells or L-929 cells was measured as described (2, 8). For each experiment 3×10^6 lymphocytes, $2.5–3.0 \times 10^6$ HeLa cells, or $\pm 3 \times 10^6$ L-929 murine fibroblasts were exposed to 500 pg of TC II-Cbl for 3 hr at 37° in M-199 medium.

Results. The amounts of holo, apo, and total TC II in each serum are shown in Table I. The group values for normal human serum were derived from two studies (13, 14) and are included for comparison only. TC II in the animal sera was identified by physicochemical means. (With the exception of canine TC II, which has been studied in detail (16), the *function* of the TC II of other species has not been shown to be the equivalent of human TC II but is assumed to be.) The wide range in amounts of TC II in serum among species points out why it is necessary to account for this variability in planning studies of Cbl uptake, since presumably the Cbl attached to TC II naturally *in vivo* is functionally the equivalent of the CN [^{57}Co]Cbl attached to TC II *in vitro* as part of the determination. The "TC II" identified in chicken serum may not be TC II at all. The fractionation method used gives a small but known cross-contamination (13) and with the very large amount of non-TC II binding protein present in chicken serum, the apparent TC II in the $(NH_4)_2SO_4$ precipitate could well be a contaminant.

The reactivities of the different TC IIs in the RIA for human TC II are illustrated in Fig. 1. Only the TC II of the baboon showed definite reactivity with the antihuman TC II and the competition for antibody binding sites by baboon TC II was about a third that expected from equal amounts of human TC II.

TABLE I. COMPARTMENTS OF TC II IN DIFFERENT SPECIES AS NG CBL BOUND/ML SERUM

Species	Total UBBC[a]	Apo TC II	Holo TC II	Total TC II[b]
Man[c]	1.25 ± 0.24	1.16 ± 0.22	0.10 ± 0.05	0.92 ± 0.09
Baboon	1.56	0.48	0.84	1.32
Dog	1.34	0.85	0	0.85
Rabbit	10.98	8.66	35.80	44.46
Guinea pig	2.08	1.77	2.06	3.83
Goat	6.81	2.80	0	2.80
Horse	2.68	2.04	0.78	2.82
Pig	1.42	0.22	0	0.22
Chicken	297.50	6.19	0	6.19

[a] UBBC is unsaturated B_{12} (Cbl) binding capacity (13).

[b] Determined from bioassay of TC II fraction after conversion of all TC II to holo TC II (11).

[c] Values for 10 normal sera, means ± 1 SD, collected and studied as described (13, 14).

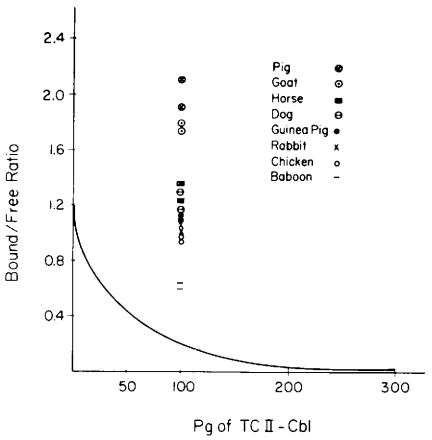

FIG. 1. RIA of the TC II from eight species by an assay for human TC II based on rabbit antibody against pure human TC II (12). The amount of material applied was adjusted to contain 100 pg of TC II. The two points shown for each TC II are the results of duplicate measurements. The line plot is the standard curve for the assay. The paired points for the standard curve were averaged for the plot.

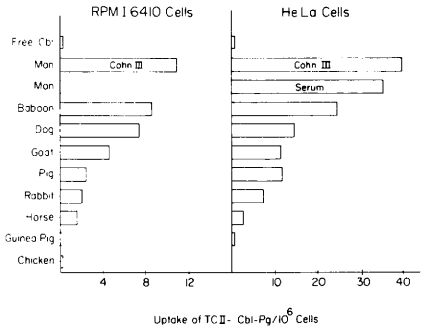

FIG. 2. The uptake of the TC II-Cbl from the sera of nine species by RPMI 6410 human lymphocytes and HeLa cells. The ratio of total TC II-Cbl to the number of cells was held constant at 500 pg per 2.5–3 × 10^6 cells. It should be noted that the measure of uptake was not of the *competitive* type as sometimes applied (2), but consisted of a *comparison* of the uptake of 500 pg of each TC II-Cbl with that 500 pg of human TC II-Cbl. The reference human TC II-Cbl was obtained from both serum and Cohn Fraction III for the HeLa cell study, but only from the latter for RPMI 6410 uptake. The values shown are means of duplicate experiments, and all forms of TC II-Cbl were examined in a single assay. Not illustrated because performed separately was the uptake of murine TC II-Cbl by HeLa cells which was 1/5 that of human TC II-Cbl but 15× that of free Cbl.

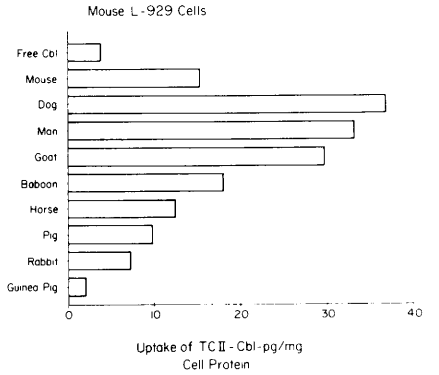

FIG. 3. The uptake of TC II-Cbl from the sera of 10 species by L-929 murine fibroblasts. The ratio of TC II-Cbl to cells was held constant at 500 pg per 3 × 10^6 cells. The values shown are means of duplicate experiments.

The uptakes of the various TC II-Cbls by RPMI 6410 human lymphocytes and HeLa cells are given in Fig. 2. As before (1), the TC II-Cbl of a primate, which most closely resembled that of man immunologically, was also closest in the ability to promote Cbl uptake by human cells.

The uptake of the same TC II-Cbls by murine fibroblasts are shown in Fig. 3. They were not equally potent in promoting Cbl uptake.

Discussion. The high degree of specificity of antihuman TC II in the reaction with TC II of species other than primates, as observed earlier, was confirmed by a completely different system of measurement. The apparent conflict between the two studies is not a serious one. Whereas by one technique (1) canine TC II did react, the reactivity was only partial (16). Neither are the present results in conflict with those of Tan and Blaisdell (3) who observed a low order of specificity between mammalian TC IIs and antibody raised against rabbit TC II. There may be specificity between two substances in one direction, as for guinea pig TC II and antibody against human TC II for example, but not necessarily in the reverse. The present study does show that the factor of immunologic specificity must be considered and measured in any studies where an attempt is made to identify TC II from one species by antibody made against TC II of another.

The varied potency of mammalian TC IIs as promoters of Cbl uptake by human cells was again demonstrated in the present study. However with the exception of the TC II of the baboon there was not the same close correlation between reaction with anti-TC II and function. The present uptake data should be considered more reliable than that of earlier studies because the uptake of the native TC II-Cbl, which varied from 0 to 35.8 ng/ml of serum, was included in the measurements. The present uptake studies were capable of showing different uptakes of an equal amount of the different TC II-Cbls. Perhaps the technique of showing immune responsiveness was incapable of detection of small gradations although in general we considered it superior to the earlier method (1). The possible relationships between immunoreactivity and function of promotion of Cbl uptake, or lack of them, may have bearing on the molecular sites of the respective activities. This is a most important question, but the present study was not designed specifically to answer it and it must be approached in another way.

Apparently murine cells are selective as

well, although the pattern of their response to TC II is different. The TC II of four species was more effective than murine TC II. This is not a new phenomenon, but is unexplained. In an earlier study (8) human TC II was possibly more effective than murine TC II in L-929 cells. Moreover, TC II from one source within an animal may be more effective than that from another (7, 8). For example, TC II-CN Cbl from dog kidney was more effective than TC II-CN Cbl from the serum of the same dog in promoting uptake by the dog liver. The form of Cbl bound to TC II which could vary in proportions among species does not seem to be a likely factor in the present study. Whereas most of the studies showing CN Cbl, OH Cbl, Me Cbl, and Ado Cbl to be equivalent in cell uptake have been conducted in microorganisms, there is one report of the equivalency of Me Cbl and CN Cbl in human systems (17).

It is difficult to compare the present studies with similar evaluations of species specificity incorporating the reticulocyte as the receiving cell (3). The originators of the reticulocyte model considered the uptake observed to consist of the primary phase only (18). Meaningful cellular uptake of Cbl requires a secondary phase as well (1) and there are circumstances where uptake begins but is never completed (2). However, species specificity may be observed at the primary level. Friedman *et al.* (19) found the affinity constant between the isolated receptor for rat TC II from human placenta to be less than 1/5 that of human TC II while bovine TC II did not bind at all. Youngdahl-Turner *et al.* (20) found rabbit TC II-Cbl to express only partial competition for the receptors of TC II-Cbl of human fibroblasts at 4°.

Summary. The reactivity between antihuman transcobalamin II (TC II) and the TC II from eight species of animals was evaluated by a radioimmunoassay for human TC II. None of the animal TC IIs reacted to the same degree as an equal amount of human TC II, although the TC II of the baboon expressed partial reactivity. The function of each TC II in promoting the uptake of Cbl was evaluated by exposure to cultured human HeLa cells and human lymphocytes. There was considerable variation in potency with some TC IIs having no effect, while others, such as that of the baboon, were almost as active as human TC II. The same species of TC II expressed varied potency for promoting uptake by murine fibroblasts as well. We have concluded that antibody against human TC II and the TC II mediated complete uptake of Cbl by human cells exhibit species specificity.

The TC II-Cbl from some species was less effective than murine TC II-Cbl or the murine L-929 system, but some forms of TC II-Cbl were more effective.

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