Adherence of L1210 Murine Leukemia Cells to Sephacryl-Aminopropylcobalamin Beads Treated With Transcobalamin-II

By Donald W. Jacobsen, Yolanda D. Montejano, Karin S. Vitols, and F.M. Huennekens

Sephacryl beads containing an immobilized aminopropylcobalamin–transcobalamin-II complex serve as foci for the adherence of L1210 murine leukemia cells. Bead-cell interaction does not occur when (A) nonderivatized beads are used; (B) transcobalamin-II is omitted or presaturated with cyanocobalamin in the preparation of the bead complex; (C) intrinsic factor replaces transcobalamin-II; and (D) the complex is removed from beads by photolysis. These observations suggest that adherence results from the ability of transcobalamin-II to form a bridge between immobilized cobalamin on the bead and receptors in the plasma membrane of the cell.

Transport of cobalamins into mammalian cells proceeds according to the following sequence: (A) capture of the cobalamin by the serum protein, transcobalamin-II (TC-II); (B) binding of the cobalamin–TC-II complex to a specific receptor on the plasma membrane; and (C) internalization of the complex via endocytosis. Membrane receptors involved in this process can be estimated via the binding of radiolabeled cobalamin–TC-II (under conditions where internalization is suppressed), and a cobalamin–TC-II receptor protein has been solubilized and purified from human placenta. The present communication describes a novel procedure for detecting cobalamin–TC-II receptors on individual cells. Receptor-containing L1210 murine leukemia cells, used to illustrate this procedure, adhere to Sephacryl-aminopropylcobalamin beads charged with TC-II.

Materials and Methods

Cell culture. L1210 cells were propagated in RPMI 1640 medium containing 5% fetal bovine serum penicillin (100 U/ml) and streptomycin (100 µg/ml).

S-AP-Cbl beads. Aminopropylcobalamin, prepared from cobalamin and chloropropylamine, was attached covalently to Sephacryl S-200 (Pharmacia, Uppsala, Sweden) that had been activated with cyanogen bromide by the procedure of March et al. The resultant S-AP-Cbl beads contained 0.9 µmole cobalamin/ml (settled volume), which corresponds to approximately 10^11 molecules of cobalamin per bead. Further characterization of S-AP-Cbl and its use in the purification of cobalamin-binding proteins by affinity chromatography has been described elsewhere.

Treatment of S-AP-Cbl beads with serum TC-II. S-AP-Cbl beads (1.0 ml) were added to 1 liter of rabbit serum (clarified by filtration through gauze and Whatman 4 paper and by centrifugation at 27,000 g for 30 min at 2°C), and the mixture was stirred in the dark for 3 hr at room temperature. Uptake of the apo-form of TC-II onto the beads was verified using the method of Gottlieb et al. The beads were collected by centrifugation, washed successively with 0.5 M NaCl-0.05 M potassium phosphate (pH 7.0) and phosphate-buffered saline (PBS), suspended in 7 vol of PBS, and stored at 4°C at the dark. The final preparation contained approximately 10 µmole TC-II/ml, which corresponds to approximately 10^9 molecules TC-II/bead. Beads containing intrinsic factor were prepared similarly using neutralized human gastric juice as a source of the apo-binder.

Treatment of S-AP-Cbl beads with partially purified TC-II. Clarified rabbit serum (25 ml) was labeled to 1% of the total cobalamin-binding capacity using 6.5 µg of 3^7-cyanocobalamin (220 µCi/µg; Amersham-Searle) and applied to a 5 x 55 cm column of Sephacryl S-200 that had been equilibrated previously with 0.5 M NaCl-0.05 M potassium phosphate, pH 7.0. The same buffer was used for elution, and 3-ml fractions were collected. Holo-TC-II (detected by its radioactivity) and apo-TC-II (assayed via cobalamin-binding capacity) appeared as nearly confluent peaks (fractions 208-232) that were well resolved from other cobalamin binders. S-AP-Cbl beads (0.2 ml) were treated with the pooled TC-II fractions (75 ml) and then processed as described above.

Visualization of bead-cell interaction. TC-II-treated S-AP-Cbl beads (0.15 ml suspended in 0.35 ml of PBS) were added under dim red light to a suspension of L1210 cells (1.5 x 10^5 in 10 ml of the culture medium described above), and the mixture was incubated at 37°C. After 5 hr, 2.0 ml of the suspension was layered carefully over 2.0 ml of Ficoll-Paque (Pharmacia) and centrifuged (65 g for 2 min at room temperature). Free cells at the liquid–liquid interface were removed by aspiration, and the pelleted beads were washed twice with PBS. The bead–cell preparation was fixed with a neutral isotonic paraformaldehyde-glutaraldehyde solution and examined by light microscopy. For scanning electron microscopy, the fixed preparations were mounted on polysine-coated cover slips and processed according to Kelley et al.

Results and Discussion

Cultured L1210 cells adhere tightly to S-AP-Cbl beads that have been treated with rabbit serum TC-II. (This serum was selected for these experiments because it is particularly rich in the apo-form of TC-II.) Over a period of several hours, the beads...
Fig. 1. Photomicrographs of (A) L1210 cells bound to S-AP-Cbl beads charged with TC-II; and (B) Absence of L1210 cell binding to photolyzed beads. Experimental details are given in Materials and Methods (× 270).
Fig. 2 Scanning electron micrographs of L1210 cells bound to S-AP-Cbl beads charged with TC-II. Experimental details are given in Materials and Methods. (A) x 700; (B) x 3500.
accumulate increasing numbers of adherent cells. At any stage, beads containing bound cells can be separated from free cells by centrifuging the preparation through Ficoll-Paque. Bound cells are not released when the beads are washed with minimal mechanical agitation. The bead–cell interaction is also unaffected by exposure to light, despite the fact that a considerable amount of cobalamin (presumably from unpopulated areas of the beads) is released under these conditions. Thus, the photolabile carbon-cobalt bond linking cobalamin–TC-II to the bead\textsuperscript{a} appears to be stabilized when the complex is also bound to a cell receptor.

A representative example of bead–cell interaction is shown in Fig. 1A. In some instances, individual beads (average diameter, 70 \textmu m) are almost completely covered with cells (diameter, 10 \textmu m). Trypan blue exclusion demonstrates that attached cells have not lost viability. Scanning electron microscopy (Fig. 2A and B) reveals further details of the cell–bead interaction. Extensive contact between the bead surface and cell microvilli suggests that cobalamin–TC-II receptors may be concentrated in the latter structures.

Adherence of L1210 cells to S-AP-Cbl beads depends on the presence of TC-II to serve as the bridge between immobilized cobalamin molecules and receptors in the cell membrane. This conclusion is supported by various control experiments. Cells do not adhere to beads when the standard procedure for treatment of S-AP-Cbl beads with serum TC-II (see Materials and Methods) is modified in any of the following ways: (A) nonderivatized beads are used; (B) TC-II is omitted or presaturated with cyanocobalamin in preparation of the bead complex; (C) intrinsic factor replaces TC-II; and (D) the complex is removed from beads by photolysis. These controls rule out the involvement of serum constituents other than a cobalamin-binding protein in cell adherence. Although TC-II comprises >80% of the total protein absorbed from rabbit serum by S-AP-Cbl,\textsuperscript{a} consideration had to be given to the possibility that transcobalamin-I or other cobalamin binders might also contribute to the process. However, the ability of partially purified TC-II (see Materials and Methods) to promote cell adherence confirms that the effects observed with whole serum are not due to these other binders.

The present method for visualizing TC-II receptors on L1210 cells should be applicable to other eukaryotic cells that possess a TC-II-mediated cobalamin transport system. Membrane receptors for other cobalamin–binder complexes (e.g., cobalamin–intrinsic factor or cobalamin–transcobalamin-III) might also be detected by the adherence technique. Use of radiolabeled components will permit quantitation of the average number of bound cells per bead or the minimum numbers of receptors required to maintain cell adherence.

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REFERENCES

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