SPECIFIC BINDING OF ZINC TRANSFERRIN TO HUMAN LYMPHOCYTES

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Summary. Radioiodinated zinc transferrin has been found to interact with human lymphocytes in a manner characterized by rapid binding, saturability, reversibility, and specificity. Each lymphocyte can bind a maximum of approximately 61,000 molecules of zinc transferrin with an association constant of 4×10^7 M^{-1}. Binding is strongly inhibited by cyanide and fluoride and only moderately inhibited by sulfhydryl reagents.

Introduction. Serum transferrin is a glycoprotein of M.W. 76,600 that is capable of binding stoichiometrically 2 atoms of various metals per molecule of protein (1). The best studied metal-transferrin complex is Fe(III)-transferrin, and the interaction of this complex with iron-requiring cells, such as reticulocytes, is well documented (see review, Ref. 2). Recently, however, Phillips and Azari (3) demonstrated that zinc transferrin enhanced nucleic acid synthesis in phytohemagglutinin-stimulated human lymphocytes. It was hypothesized that zinc transferrin exerted its effect by: 1) binding of zinc transferrin to specific receptors on the lymphocytes, followed by 2) uptake of the transferrin-bound zinc by the lymphocytes. The uptake of zinc by stimulated lymphocytes is the subject of a separate communication (J. L. Phillips, submitted for publication). In this paper data is presented indicating the existence of a lymphocyte receptor specific for transferrin.

Methods. Iodination. Human serum transferrin (Behring Diagnostics) was iodinated to a specific activity of 10-100mCi/μ mole as described previously (4), using I_2, [^{125}I]NaI (carrier-free, New England Nuclear), and cold NaI. That iodination did not alter the properties of transferrin was determined as described by Phillips (4).

Lymphocyte isolation and culture technique. Lymphocytes were isolated from human peripheral blood as described by Phillips and Azari (3). Cells were cultured in serum-free RPMI-1640 (HEPES buffered, Grand Island Biological Co.) at a cell density of 1.5 - 1.8 x 10^6 cells/ml using 12 x 75-mm polypropylene culture tubes (Falcon Plastics).

Preparation of Zinc Transferrin. Radioiodinated apotransferrin was dissolved in Hanks' balanced salt solution and sufficient 0.02M zinc acetate to form the 2 Zn:1 transferrin complex, denoted ^{125}I-ZnTf. Where appropriate, dilutions were prepared in Hanks' solution.

Binding assay. To assess binding of transferrin to human lymphocytes, an amount of ^{125}I-ZnTf (5-200µg in 25µl) was added to 1.5 x 10^6 lymphocytes in 1 ml serum-free RPMI-1640. Cells were incubated for 30 minutes at 37° for routine binding assays or for other time periods as indicated in the text. Incubations were terminated with the addition of 2 ml ice-cold Hanks' solution followed by centrifugation at 1000 rpm for 10 minutes. The pellet was resuspended in 2 ml ice-cold Hanks' solution and centrifuged again. This procedure was repeated one additional time. To assess radioactivity associated with the lymphocytes rather than the polypropylene culture tubes (4), the final washed cell pellets were collected by vacuum filtration on Whatman GF/A glass fiber filters. The filters were then placed in Beckman Bio-Vials and the radioactivity associated with cells assessed with a Beckman Bio-Gamma. It was found necessary to run two controls simultaneously. First, control experiments were performed in which no cells were present. Culture tubes containing only medium and ^{125}I-ZnTf were treated as described above for washing and filtration, thus allowing a correction for a small amount of ^{125}I-ZnTf which becomes non-specifically associated with the glass fiber filters. Second, to correct for "non-specific" binding to the lymphocytes, a 50-100 fold excess of unlabeled zinc transferrin was added to some cultures prior to the addition of ^{125}I-ZnTf. These cultures were also treated as described above for washing and filtration. To determine specific binding of zinc transferrin to the lymphocytes, the radioactivity counted for both controls was subtracted from corresponding cultures containing cells and ^{125}I-ZnTf only. All experiments were run in duplicate and replicated three times.

Materials. Reagents used and their sources are: bovine, equine, and rabbit transferrins, Miles Laboratories; human serum albumin, Behring Diagnostics; sodium fluoride and potassium cyanide, Fisher Chemical Co.; ouabain, N-ethylmaleimide, and p-chloromercuribenzene sulfonate, Sigma Chemical Co.; phytohemagglutinin (M-form), Difco Laboratories.

Results. Binding of Transferrin to Lymphocytes. Fig. 1 presents the specific binding of ^{125}I-ZnTf to human lymphocytes as a function of time. Binding is a saturable process and is maximal within 5-10 minutes after addition of the ^{125}I-ZnTf to lymphocyte cultures. Additionally, the binding of ^{125}I-ZnTf to lymphocytes is reversible. If lymphocytes are incubated with ^{125}I-ZnTf and then washed and resuspended in fresh medium, bound ^{125}I-ZnTf dissociates from the cells within 30 minutes after reincubation at 37° and is detected in the medium.

The amount of transferrin bound is dependent on both the concentration of transferrin in the medium and the presence of zinc, as seen in Fig. 2. These data were analyzed by the method of Scatchard (5), as seen in Fig. 3. For the binding of ^{125}I-ZnTf to lymphocytes, an association constant of 4 x 10^7 M^{-1} is

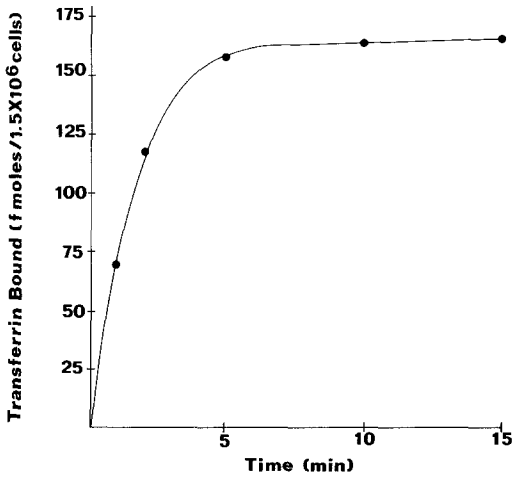

Fig. 1. Binding of ^{125}I-ZnTf to human lymphocytes as a function of time. Incubation and harvesting of cultures were performed as described under Methods. Each experiment was run in duplicate and replicated three times. Each data point represents specific binding of transferrin which was calculated as described in Methods.

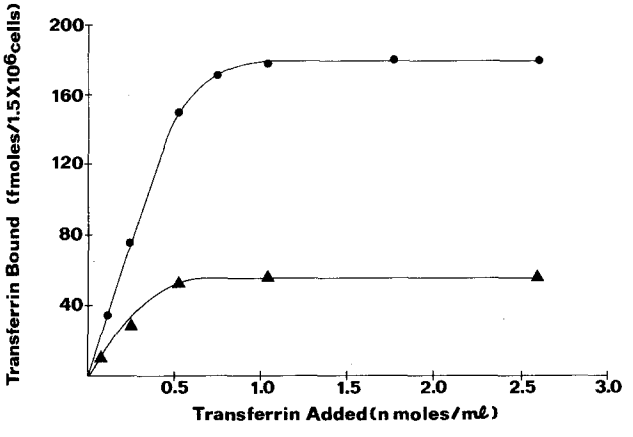

Fig. 2. Specific binding of ^{125}I-ZnTf as a function of ^{125}I-ZnTf concentration. Incubations were performed as described in Methods with an incubation time of 30 minutes. Each experiment was run in duplicate and replicated three times.

obtained. Additionally, extrapolation of the linear plot of Fig. 3 to the X-axis reveals that there are approximately 61,000 receptors for zinc transferrin per lymphocyte. Similar analysis of ^{125}I-apoTf binding to lymphocytes (not shown) revealed only approximately 24,000 molecules bound per

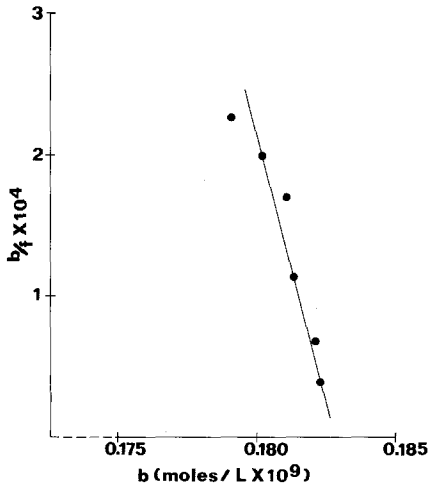

Fig. 3. Scatchard plot for the binding of ^{125}I-ZnTf to human lymphocytes. Values for bound (b) and free (f) transferrin were calculated from the data of Fig. 2.

TABLE I. Effect of Various Treatments on the Binding of Radioiodinated Zinc Transferrin to Human Lymphocytes.

Treatment[a]	Response (%)[b]
No additions	100
KCN (5×10^{-3}M)	0
NaF (5×10^{-3}M)	41
Incubate at 4°	35
Ouabain (5×10^{-3}M)	100
N-Ethylmaleimide (5×10^{-3}M)	67
p-Chloromercuribenzene sulfonate (1×10^{-3}M)	65
Unlabeled zinc transferrin (80µg)	10
Human Serum albumin (80µg)	100
Bovine zinc transferrin (80µg)	60
Equine zinc transferrin (80µg)	81
Rabbit zinc transferrin (80µg)	20

[a] Cultures contained 1.5×10^6 lymphocytes in a volume of 1 ml serum-free RPMI-1640. Additions were made in a volume of 10µl to give the quantity or final concentration indicated in parentheses. Cultures were incubated at 37° for 30 minutes with the additive prior to the addition of 40µg ^{125}I-ZnTf. After an additional 30 minutes at 37°, cultures were harvested as described in Methods.

[b] A response of 100% is assigned to cultures receiving no additions other than the ^{125}I-ZnTf. Various treatments were tested for their effect on transferrin binding relative to this value.

lymphocyte with an association constant of 2.5×10^7 M^{-1}.

Table I describes the effect of various compounds on the binding of ^{125}I-ZnTf to lymphocytes. For these experiments, lymphocytes were incubated for 30-60 minutes with the test compound prior to the addition of ^{125}I-ZnTf. Metabolic energy is apparently required for zinc transferrin binding as judged by the effect of KCN, NaF, and lowering the incubation temperature to 4°. Ouabain, an ATPase inhibitor, was without effect. Sulfhydryl reagents, such as N-ethylmaleimide and p-chloromercuribenzene sulfonate, inhibited zinc transferrin binding approximately 35%. Additionally, human serum albumin had no effect on binding, while other transferrins varied in their ability to compete with human transferrin for the lymphocyte receptors. Finally, it should be noted that treatment of lymphocytes with the mitogen, phytohemagglutinin, did not alter the binding of zinc transferrin to lymphocytes for periods of time up to 72 hours after addition of phytohemagglutinin.

Discussion. Data is presented indicating the existence of lymphocyte receptors specific for serum transferrin. That such receptors do exist is important in explaining the mechanism by which zinc transferrin enhances nucleic acid synthesis in mitogen-stimulated human lymphocytes (3). Indeed, it has been shown that transferrin-bound zinc is incorporated by lymphocytes (J. L. Phillips, submitted), this process occuring presumably after the interaction of zinc transferrin with specific lymphocyte receptors.

The transferrin-lymphocyte interaction described here differs in many respects from the transferrin-reticulocyte interaction. For instance, the interaction of iron transferrin with rabbit reticulocytes (6) was insensitive to NaCN, NaF, and oyabain, while the interaction of zinc transferrin with human lymphocytes was sensitive to KCN and NaF and insensitive to ouabain. Additionally, sulfhydryl reagents inhibit more strongly the binding of iron transferrin to reticulocytes (6, 7) than the binding of zinc transferrin to lymphocytes. Apparently, it is possible that the mechanism of binding for the transferrin-lymphocyte system and perhaps the lymphocyte receptor for

transferrin itself are different from binding in the transferrin-reticulocyte system and the reticulocyte receptor for transferrin.

Because previous reports detailing the number and affinity of reticulocyte receptors for transferrin (8) may be in error due to non-specific binding of radioiodinated transferrin to inert materials, such as culture tubes (4), no comparison of these parameters with the transferrin-lymphocyte system will be attempted.

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