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Specific binding of placental acidic isoferritin to cells of the T-cell line HD-MAR

A.M. Konijn, E.G. Meyron-Holtz, R. Levy, H. Ben-Bassat* and Y. Matzner+

*Departments of Nutrition and *Experimental Surgery, The Hebrew University-Hadassah Medical School* and *+Hematology Unit, Hadassah University Hospital Mount Scopus, Jerusalem, Israel*

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Acidic placental isoferritin inhibited the blastogenic response of peripheral human lymphocytes to T-cell activating lectins. We measured specific binding of radioiodinated placental isoferritin to cells of the T-cell line HD-MAR and found specific high-affinity binding. Binding was faster and more ferritin was bound at 37°C than at 4°C. Displacement experiments indicated that most of the binding occurred at the cell surface. Acidic placental ferritin and isolated H-type ferritin subunits but not isolated L-type subunits, competed for the binding. Scatchard plot analysis showed characteristics of a single binding species with a dissociation constant (K_d) of 1.3–4.4 × 10^{-11} M. The results suggest the presence of receptors for acidic isoferritin on T-lymphocytes and thus, a regulatory role for the acidic ferritin H-type subunit in T-cell function.

T-cell; Ferritin; Isoferritin; Receptor

1. INTRODUCTION

Ferritin is an intracellular iron storage protein with an average molecular mass of 480 kDa. It is composed of at least two different types of subunits; an acidic, heavy (H) subunit and a more basic, light (L) subunit. These subunits assemble into a shell of 24 subunits [1] and their ratio in the ferritin molecule determines the surface charge of the molecule. Thus, ferritin rich in H-type will be more acidic than ferritin rich in L-type subunits [2]. Small amounts of ferritin are found in the serum of normal individuals while increased concentrations have been noted in the serum of patients with various proliferative disorders associated with impaired cell mediated immunity [3,4]. We have previously reported that ferritin inhibits the blastogenic response of peripheral human lymphocytes to T-cell activating lectins [3]. Using isoferritins isolated from human term placenta revealed that only the acidic isoferritin caused a marked suppression of phytohemagglutinin-induced blastogenesis [5]. We also found that peripheral lymphocytes were able to bind iodinated ferritin [3]. These findings as well as the presence of ferritin on the surface of peripheral T-lymphocytes from patients with various malignant diseases [6–8] led us to the assumption that specific binding sites exist for acidic isoferritin on T-lymphocytes. In this paper we describe the specific, high-affinity binding of placental acidic isoferritin to the T-cell line HD-MAR.

Correspondence address: A.M. Konijn, Department of Nutrition, The Hebrew University-Hadassah Medical School, PO Box 1172, Jerusalem 91010, Israel

2. MATERIALS AND METHODS

2.1. *Cell culture*

The HD-MAR T-cell line is a continuous T-cell line established from a patient with Hodgkin's lymphoma. Its T-cell characteristics have been previously defined [9]. Cells were cultured at 37°C in 5% CO_2 in air at a relative humidity above 90% in RPMI 1640 (Bio-Lab, Jerusalem, Israel) supplemented with 25% FCS (Gibco Labs, Grand Island, NY, USA), 100 U penicillin and 100 μg streptomycin (Biological Industries, Beth Haemek, Israel) per ml. The cells were harvested in the log phase of growth, generally at day five and washed free of FCS with 3 changes of RPMI 1640 containing 1% BSA and 25 mM Hepes, pH 7.4. Cell number was determined with a hemocytometer.

2.2. *Ferritin*

Ferritin was isolated from human term placenta and fractionated into isoferritins as previously described [10]. The acid ferritin used for the binding experiments was the 'Acidic I' fraction and the basic ferritin the 'Basic I' fraction.

2.3. *Ferritin subunit preparation*

This was carried out by simultaneous reduction and subunit dissociation of human intermediate placental isoferritin with acetic acid and thioglycolic acid and gel chromatographic exchange into 0.1% trifluoroacetic acid. The H- and L-ferritin subunits were separated by reverse-phase high performance liquid chromatography (HPLC) on a Spectra Physics liquid chromatograph using a C_{18} reversed-phase column with a pore size of 300-A (Synchropak RP-P, 25 cm × 4.1 mm i.d., SynChrom, USA) as described by Collawn et al. [11].

2.4. *Radioiodination procedure*

'Acidic I' placental isoferritin was radioiodinated by solid-phase enzymatic radioiodination with Enzymobead (Bio-Rad Laboratories, Richmond, CA, USA) by the procedure advised by the manufacturer. This reagent consists of immobilized preparations of lactoperoxidase and glucose oxidase. Following iodination, and again before an experiment was done, ^{125}I-ferritin was separated from free iodine on a 5 ml Biogel P-6 DG column (Bio-Rad). Using this method, the $t_{1/2}$ of

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the iodinated 'Acidic I' isoferritin was 142 days as determined by fluorogenic ELISA [12]. Before each experiment ^{125}I-acidic isoferritin concentration was measured by fluorogenic ELISA [13]. Free iodine was estimated by TCA precipitation and was generally less than 5%.

2.5. *Binding studies*

These were done in RPMI 1640 containing 25 mM Hepes, pH 7.4 and 10 mg/ml BSA. Following three washes with the above medium, cells (1.5×10^6/ml) were incubated with $0.1–8.0 \times 10^{-11}$ M ^{125}I-'Acidic I' isoferritin for 90 min (unless otherwise designated), in sterile polypropylene tubes. Binding was measured at 37°C and/or 4°C. The cells were washed with ice-cold incubation medium not containing ferritin, transferred to new polystyrene tubes and the ^{125}I-acidic isoferritin bound was counted in a gamma-counter. Specific binding was measured by deducting the binding of ^{125}I-acidic isoferritin to the cells in the presence of a 500-fold unlabeled acidic isoferritin concentration.

2.6. *Competition studies*

Cells (10^6) were incubated in triplicates for 90 min at 37°C or 4°C in 0.5 ml incubation medium containing 4×10^{-11} M ^{125}I-acidic isoferritin and increasing noniodinated concentrations of either 'Acidic I' or 'Basic I' placental isoferritin, or HPLC-isolated H-type or L-type placental ferritin subunits. The cells were then washed and counted as described above.

2.7. *Displacement experiments*

Cells (10^6) were incubated with 16×10^{-11} M ^{125}I-acidic isoferritin at 37°C for 90 min, thereafter unlabeled acidic isoferritin was added and the cells were incubated for additional 70 min. Cells were then washed and counted as described above.

3. RESULTS

^{125}I-Placental acidic isoferritin bound specifically to the HD-MAR T-cell line at 37°C as well as at 4°C. Nonspecific binding was high and amounted in some experiments to 50% or more of the total binding (see Fig. 2). Nevertheless the specific binding was consistently and significantly elevated above the nonspecific binding. High levels of nonspecific binding of ferritin (up to 60% of the total binding) were also observed to erythroleukemia K562 cells [14] and rat hepatocytes [15]. In further experiments the concentration of albumin added to the medium in binding experiments was increased from 0.2 to 1%, resulting in decreased nonspecific binding without affecting the specific binding. Consequently, specific binding was more than 50% of the total binding (see Fig. 5). Steady-state binding was reached after 60 min at 37°C and after 90 min at 4°C (Fig. 1). Binding at 4°C is considered to be binding to the cell membrane, and binding at 37°C is thought to reflect both binding and internalization of ferritin [16]. In the described experiments, however, there was probably very little internalization at 37°C. As can be seen in Fig. 2, when cells were incubated with ^{125}I-acidic isoferritin for 90 min and then for 70 min with a 500-fold excess of unlabeled ferritin, more than 80% of the specifically bound ^{125}I-placental acidic isoferritin was replaced. Competition experiments with 'Acidic I' and 'Basic I' placental isoferritins, as well as with the HPLC-isolated acidic H-type

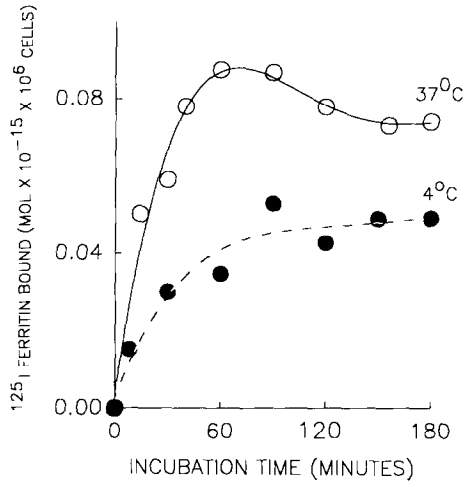

Fig. 1. Specific binding of ^{125}I-placental 'Acidic I' isoferritin to HD-MAR cells. Cells (2×10^6/ml) were incubated for the designated time period at 37°C or 4°C with 1.8×10^{-11} M radioiodinated isoferritin.

and basic L-type ferritin subunits, at 37°C and at 4°C indicated that the ferritin binding sites on the HD-MAR cell line were more specific for acidic than for basic isoferritins (Fig. 3). The isolated L-type subunit did not compete significantly with the radioiodinated acidic isoferritin for binding sites on the cell membrane. One could argue that the ferritin subunits isolated by HPLC were denatured, thereby resulting in nonspecific binding to the cells and masking of the competition experiments. This explanation is rather unlikely because the isolated subunits reassembled into intact apoferritin shells as assessed by gel electrophoretic analysis in 5% (T) polyacrylamide gels under non-reducing conditions (Fig. 4). In addition only the H-type subunit competed for acidic ferritin binding to HD-MAR cells while the L-type did not, accordingly the competition of the acidic H-type

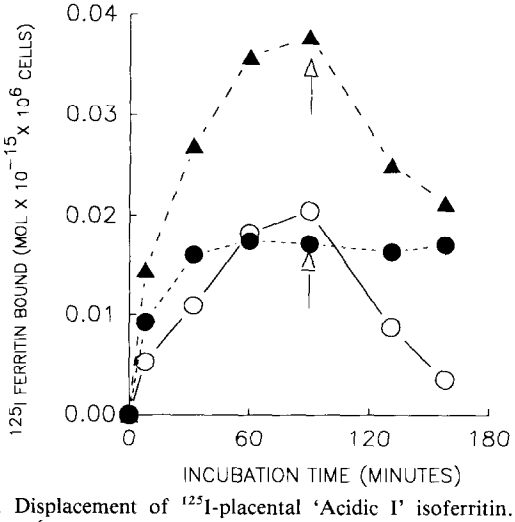

Fig. 2. Displacement of ^{125}I-placental 'Acidic I' isoferritin. Cells (1.5×10^6/ml) were incubated for 90 min with 2×10^{-11} M radioiodinated ferritin followed by 10^{-8} M nonradioactive 'Acidic I'. Specific binding (○), nonspecific binding (●), total binding (▲).

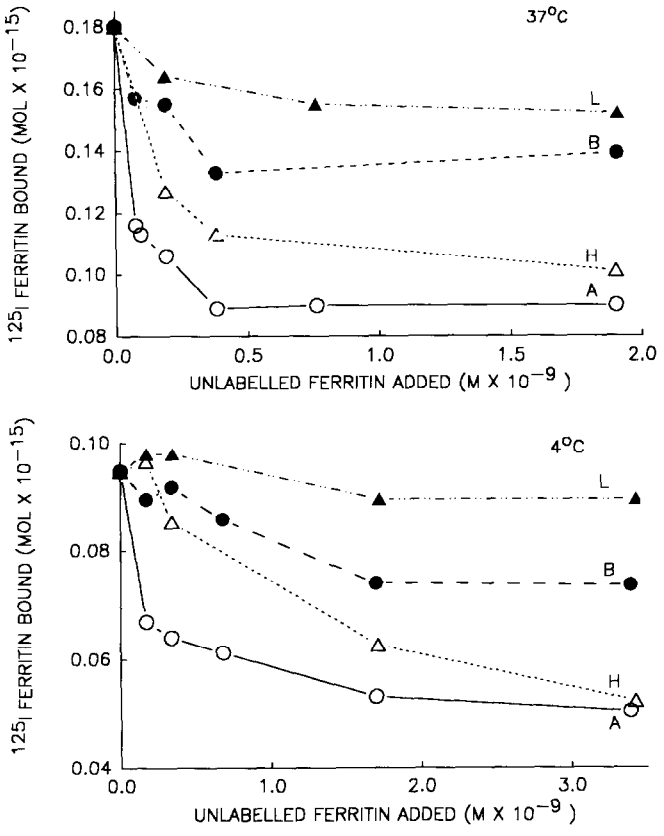

Fig. 3. Competition of placental isoferritins and ferritin subunits for ferritin binding sites on HD-MAR cells. Cells (10^6) were incubated in 0.5 ml medium with 4×10^{-11} M radioiodinated 'Acidic I' isoferritin and designated concentrations of unlabeled ferritin fractions, at 37°C or 4°C. A, 'Acidic I' placental isoferritin; B, 'Basic I' placental isoferritin; H, apoferritin of HPLC-isolated H-type ferritin subunits; L, apoferritin of HPLC-isolated L-type ferritin subunits.

subunit for the binding of ^{125}I-acidic isoferritin does not seem to be an artifact caused by denaturation of the subunits. Regardless, some denaturation of the HPLC-isolated H-type subunit was conceivable because the

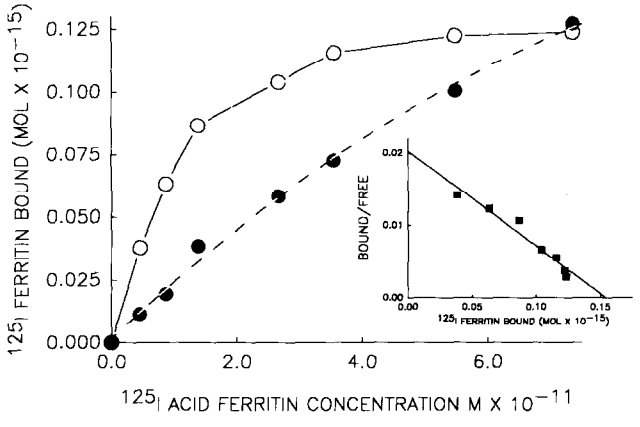

Fig. 5. Specific placental 'Acidic I' isoferritin binding to HD-MAR cells as a factor of isoferritin concentration. Cells (10^6) were incubated in 0.6 ml medium at 37°C for 90 min. Specific binding (○), nonspecific binding (●). Insert, Scatchard plot.

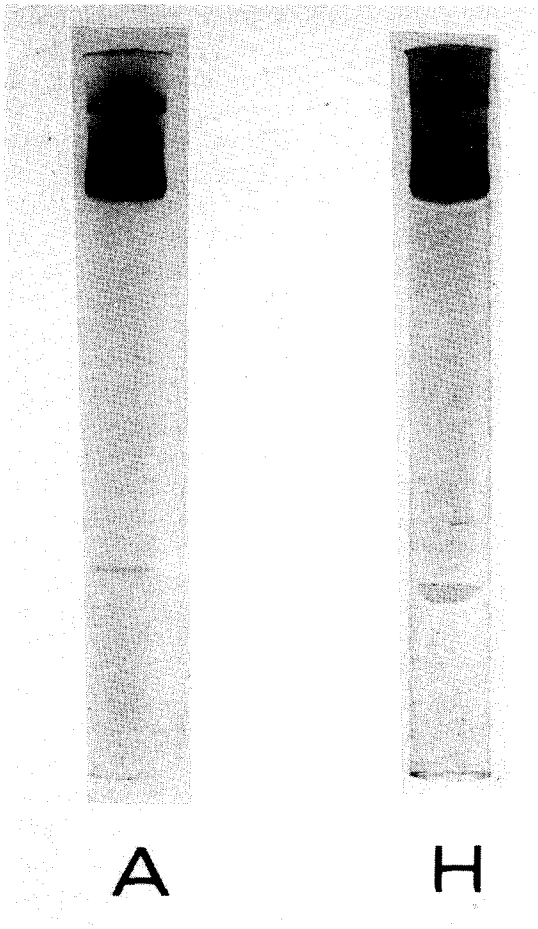

Fig. 4. Analysis of 'Acidic I' placental isoferritin (A) and HPLC-isolated H-type subunits of ferritin (H), by electrophoresis on 5% (T) polyacrylamide gels under nonreducing conditions, showing reassembly of the subunits.

isolated subunit did not compete as effectively as the 'Acidic I' fraction for binding with tracer 'Acidic I' isoferritin. It has to be mentioned that the 'Basic I' placental isoferritin contained some H-type subunits [10] and was therefore partially able to compete with H-type subunit binding sites on the cell. As shown in Fig. 5, increasing amounts of tracer acidic isoferritin added to the cells at 37°C led to saturable binding. The number of binding sites per cell in various experiments was calculated to be 98–460 per cell. By Scatchard plot analysis only a single binding species was found with a dissociation constant (K_d) of 1.3–4.4 × 10^{-11} M (association constant (K_a) = 2.3–7.7 × 10^{10} M).

4. DISCUSSION

Previous studies described by our group have shown a specific inhibitory effect of placental 'Acidic I' isoferritin on phytohemagglutinin-induced blastogenesis [5] and binding of ferritin to peripheral lymphocytes [3]. Placental ferritin has shown to be immunosuppressive in Hodgkin's disease and in HIV infection [7,17]. A

high number of T-lymphocytes in cancer patients were found to have surface-bound ferritin [8] which was identified by a specific monoclonal antibody and it seemed to resemble placental isoferritin [18]. The ferritin associated with those lymphocytes could be removed only with the immunopotentiating reagent levamisol [6]. These results led us to assume that there are specific binding sites for acidic placental ferritin on T-lymphocytes. To prove this assumption we have used a continuous T-cell line, HD-MAR (established from a patient with Hodgkin's lymphoma [9]) as a model system. It seems that placental 'Acidic I' isoferritin is attached to the surface of the cell at binding sites specific for the acidic isoferritin, most probably for the H-type subunit. The L-type subunit was not able to compete for these binding sites. The basic isoferritin does contain some H-type subunits [10] and therefore was able to compete to some extent. The Scatchard plot analysis indicated that only one receptor species exists with a very high affinity for placental 'Acidic I' isoferritin. The low receptor number (98–460 per cell) and the high affinity (K_d = 1.3–4.4 × 10^{-11} M) resemble receptors for other regulatory peptides such as the human interleukin-6 receptor [19]. Thus, it is possible that the H-type subunit of ferritin has a general regulatory effect on cell growth and proliferation. Accordingly, H-type ferritin has an inhibitory effect on the proliferation of precursors of granulocytes and monocytes in cultured cells [20]. Consequently, the acidic heart ferritin but not the basic liver ferritin binds specifically to the promyelocytic cell line HL60 [21]. The inhibition of BFU-E colony formation by H-type ferritin and the specific binding of H-type ferritin to the human erythroleukemia cell line K562 have also been described [14,22].

The effect of temperature on ferritin binding seems to be controversial. While some authors reported binding of ferritin to HL60 [21], K562 [14] and guinea pig reticulocytes [23] only at 37°C, others found ferritin binding to K562 [22], HeLa cells [24] and rat hepatocytes [16] at 37°C as well as at 4°C. Our results indicate significant acidic isoferritin binding to HD-MAR cells at 37°C and to a lesser extent at 4°C. The evidence presented here suggests the occurrence on T-cells of specific, high-affinity receptors for H-subunit-rich placental isoferritin. It further points towards a regulatory role [5,17,25] of the ferritin H-type subunit in cellular immunity.

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