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QUIESCENT LYMPHOCYTES EXPRESS INTRACELLULAR TRANSFERRIN RECEPTORS [1]

James E. Weiel and Thomas A. Hamilton

Department of Pathology
Duke University Medical Center
Durham, North Carolina 27710

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Both quiescent and concanavalin A stimulated murine splenic lymphocytes were examined for the expression of surface and intracellular binding sites for the serum glycoprotein transferrin. Transferrin binding activity was observed on the surface of mitogen stimulated cells only. When soluble detergent extracts of both populations were studied, quiescent lymphocytes were shown to contain a significant pool of non-surface exposed, intracellular receptors which was approximately 20% of the total receptor complement of proliferating cells. Because the ratio of surface to intracellular binding sites was dramatically increased following mitogen stimulation, the regulation of transferrin receptor expression during this process may involve a substantial alteration in its subcellular distribution in addition to the well documented increase in number of binding sites.

Expression of cell surface receptors for the serum glycoprotein transferrin has been associated with the initiation of cell proliferation (1,2) and the significance of this relationship is supported by studies documenting a functional role for transferrin and its specific receptor in this process (3,4). In previous reports, we and others have observed a stringent regulation of cell surface transferrin receptor expression in normal lymphocytes undergoing mitogen induced proliferative responses (5,6). Thus, expression of this receptor on normal lymphocyte cell surfaces appears to be restricted to cells actively involved in DNA synthesis and cell division. Recently, studies from several laboratories have documented the existance of a substantial pool of intracellular transferrin receptors (7,8) representing nearly 80% of the total complement of cellular binding sites. In the present communication we examined both quiescent and concanavalin A stimulated murine splenic lymphocytes with respect to the existance and size of

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Abbreviations: PBS, Dulbecco's phosphate buffered saline; Con A, Concanavalin A.

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a pool of intracellular transferrin binding sites. These studies confirmed the earlier observations regarding the stringency of cell surface receptor expression and the existance of an intracellular pool in mitogen stimulated populations. However, quiescent lymphocytes, which exhibited no detectable surface receptors, also contained a significant pool of non-surface exposed, intracellular receptors.

MATERIALS AND METHODS

Mice

Inbred C57B1/6 mice (6-8 weeks of age) were purchased from the Trudeau Institute (Saranac Lake, NY) and were used in all experiments. The animals were maintained in the Duke University Animal and Laboratory Isolation Facility and fed mouse chow and water ad libitum.

Preparation of splenic lymphocyte cultures

Mice were sacrificed by CO_2 exposure and spleens were aseptically removed. Splenic cells were prepared by teasing the spleens apart and pressing them gently through a No. 40 mesh wire screen (EC Corp., St. Petersburg, FL) into Dulbecco's phosphate buffered saline (PBS) (Gibco, Grand Island, NY) using a sterile glass pestle. The resultant suspension was centrifuged at 250x g for 5 min at 4°C. The cell pellet was resuspended in an excess volume of 0.15M ammonium chloride 0.01M potassium bicarbonate (pH 7.4) and kept on ice for 5 min to selectively lyse the erythrocytes. The cell suspension consisting of lymphocytes was centrifuged as before and washed twice with PBS. Lymphocytes were resuspended in culture medium (RPMI 1640 supplemented with glutamine, penicillin-streptomycin, and 10% fetal calf serum) and stimulated with concanvalin A (Con A) as described previously (5). Stimulated cells were incubated for 48 hrs at 37°C in 5% CO_2. Prior to use in transferrin binding assays, fresh lymphocytes were treated with Con A and then both cell groups (fresh and 48 hr Con A stimulated) were washed 3x in PBS. Lymphocytes to be used in the 4°C intact binding assay were incubated at 4°C in PBS containing 0.02% sodium azide for an additional 30 min prior to assay.

[^{3}H]-Thymidine incorporation

Cell proliferation was determined in lymphocyte preparations by measuring the incorporation of [^{3}H]-thymidine into trichloroacetic acid insoluble material as described previously (5).

Transferrin binding assays

Human transferrin (90% iron free, Sigma Chemical Co., St. Louis, MO) was radiolabeled with Na [^{125}I] (Amersham, Arlington Heights, IL) using lactoperoxidase catalyzed iodination as described previously (5). Specific activities of approximately 2 x 10^6 cpm/pmole were routinely obtained. Transferrin binding assays on both soluble cell extracts and intact lymphocytes were performed as reported previously (5,7).

RESULTS AND DISCUSSION

The effect of a proliferative stimulus on cell surface expression of the transferrin receptor in murine splenic lymphocytes was evaluated by measuring cell surface binding of transferrin at 4°C and cellular uptake at 37°C (fig.1). Both quiescent lymphocytes and 48 hour Con A stimulated lymphoblasts were examined. While quiescent cells showed neither detectable surface binding nor uptake of

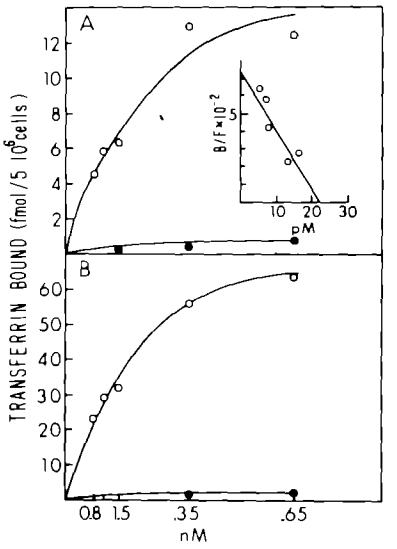

Figure 1A Specific binding of [^{125}I]-Transferrin to intact cells at 4°C. Equilibrium binding of [^{125}I]-Transferrin (1 x 10^5 cpm, 0.05 pmole) was measured at 4°C using 5 x 10^6 fresh or 48 hr Con A stimulated lymphocytes per sample as described in Materials and Methods (●——●, fresh; ○——○, 48 hr Con A stimulated). Insert: Scatchard analysis of data. Fresh lymphocytes had no detectable transferrin binding sites, 48 hr Con A stimulated cells had 2700 sites/cell, Kd = 3.0 x 10^{-10}.

1B Specific cellular uptake of [^{125}I]-Transferrin by cells at 37°C. Uptake of [^{125}I]-transferrin (1 x 10^5 cpm, 0.05 pmole) was measured at 37°C using 5 x 10^6 fresh or 48 hr Con A stimulated lymphocytes per sample as described in Materials and Methods (●——●, fresh; ○——○, 48 hr Con A stimulated).

radiolabeled transferrin, mitogen stimulated cells undergoing rapid DNA synthesis (Table 1) had readily measurable surface binding activity (approximately 3000 sites/cell).

When quiescent or Con A stimulated lymphocytes were examined for the presence of intracellular binding sites (in non-ionic detergent extracts) a pattern distinct from that seen for surface binding emerged. In this case, quiescent cells exhibited a readily detectable amount of transferrin binding activity which represented approximately 20% of that seen in extracts of Con A stimulated cells (fig.2). Comparison of the number of binding sites on intact mitogen stimulated cells (- 3000) versus detergent extracts (~ 14,000) indicated that approximately 80% of total cellular transferrin receptors are localized in an intracellular locus.

Because binding to intact cells was performed at 4°C while the method for soluble binding called for incubation at 37°C we compared binding to soluble

TABLE 1. Uptake of [^{3}H]-Thymidine by Fresh and Con A Stimulated Lymphocytes

Experiment #	Cell Type	[^{3}H]-Thymidine Incorporated (cpm ± S.E.M.)
1	Fresh	427 ± 69
	48 hr Con A stimulated	7326 ± 182
2	Fresh	254 ± 38
	48 hr Con A stimulated	7457 ± 116

1 x 10^6 fresh or 48 hr Con A stimulated lymphoyctes were incubated for 1 hr with 1 uCi [^{3}H]-Thymidine in a total volume of 1 ml and the trichloroacetic acid insoluble radioactivity was measured as described (5). Results given represent cpm [^{3}H]-Thymidine incorporated/1 x 10^6 cells for two representative experiments.

extracts of quiescent lymphocytes at 4°C (4 hr incubation) versus 37°C (1 hr incubation period). This experiment established that under both conditions detergent extracts of quiescent lymphocytes contained an equivalent number of binding sites (data not shown). Thus, the difference between binding to intact cells and detergent extracts was not due to differences in assay incubation temperatures.

The results presented above confirm previous findings demonstrating that quiescent lymphocytes have no detectable surface oriented receptors, while

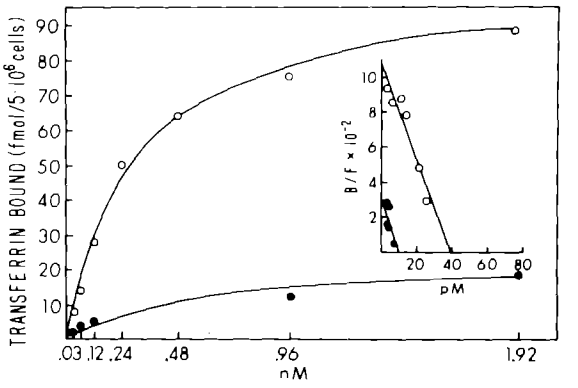

Figure 2 Specific binding of [^{125}I]-Transferrin to non-ionic detergent extracts. Equilibrium binding of [^{125}I]-Transferrin (1 x 10^5 cpm, 0.05 pmole) was measured at 37°C using the detergent extracts of 2.5 x 10^6 fresh or 1.67 x 10^6 48 hr Con A stimulated lymphocytes per sample as described in Materials and Methods. Values presented are normalized to a constant cell number of 5 x 10^6 (●——●, fresh; ○——○, 48 hr Con A stimulated). Insert: Scatchard analysis of the un-normalized data from A. Fresh lymphocytes had 2300 sites/cell, Kd = 3.3 x 10^{-10}, 48 hr Con A stimulated cells had 14000 sites/cell, Kd = 3.5 x 10^{-10}.

induction of cell cycle traverse is accompanied by a dramatic increase in surface transferrin binding activity (5,6). These findings have been interpreted to suggest that the transferrin receptor provides a useful and selective marker of lymphocyte activation. The observation that quiescent lymphocytes possess substantial transferrin binding activity sequestered in an apparently intracellular location argues that the expression of this activity in lymphocytes is not soley restricted to cells responding to a proliferative stimulus. Instead, quiescent lymphocytes appear to have a basal level of expression which can be enhanced approximately 5 fold by appropriate proliferative signals.

Our examination of the intracellular versus surface expression of transferrin receptors in quiescent and proliferating lymphocytes illustrates a dramatic difference between the two cell types. Specifically, the ratio of surface to intracellular binding activity in proliferating cells was about 1 to 4. Because quiescent cells exhibit no detectable surface binding a maximum estimate of the ratio of surface to intracellular receptor in non-proliferating cells would be 1 to 20 (this estimate is based upon a sensitivity limit of 200 sites/cell in our binding assay). These calculations suggest that the molecular signal(s) which induce lymphocytes to enter cell cycle traverse and elevate expression of transferrin binding activity may further result in a substantial shift in the subcellular location of receptors. Whether this change is limited to a particular subset of proteins or is reflected in all plasma membrane proteins, perhaps as a consequence of an increase in the cell surface area during blast transformation, cannot be determined on the basis of the available data and remains the subject of further research.

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