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Identification of the protein HC receptor

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In the present study, we demonstrate for the first time the presence of a specific receptor for protein HC on the surface of human cells using the human histiocytic lymphoma cell line U937. Cells treated for 4 days with the maturation inducer phorbol 12-myristate 13-acetate, were found to increase both the number of cells binding protein HC (76% higher than for untreated cells) and the expression of protein HC receptors. Protein HC bound to these cells in a specific and saturable manner. Scatchard analysis at 4°C, using radioiodinated protein HC, indicated a single class of low-affinity receptor ($K_a = 2–5 \times 10^7$ M^{-1}) and 20000–30000 receptors per cell. Monoclonal antibodies against protein HC abrogated specific binding of this protein to U937. In contrast, monoclonal antibodies that did not react with protein HC (anti-LFA-1α, anti-MO1α) were without effect on the binding reaction.

Protein HC; Receptor; Phorbol 12-myristate 13-acetate; (Cell line U937)

1. INTRODUCTION

Protein HC, also called α_1-microglobulin, is a 181-amino acid single-chain glycoprotein originally isolated from normal human urine [1,2]. The protein is associated with a brown chromophore and is present in different human body fluids both free and complexed to IgA [3–6]. This complex is composed of protein HC covalently linked to the Fc fragment of IgA in a 1:1 stoichiometric ratio [7]. Although protein HC was recently shown to be synthesized by hepatoma cell lines and isolated hepatocytes of human liver [8], there is still controversy concerning its tissue origin [9,10].

Several attempts have been made to define the biological role of protein HC and recently it has been described to inhibit the chemotactic response of neutrophils to endotoxin-activated serum [11] and to exert mitogenic effects on lymphocytes in the presence of accessory cells [12].

These observations prompted us to study the biochemical basis for protein HC-dependent cellular responses. Using radioiodinated protein HC we have established the presence of specific protein HC receptors in the human monocytic cell line U937.

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2. MATERIALS AND METHODS

2.1. *Radioactive ligand*

Purified protein HC was generously provided by Dr A. Grubb of Lund University (Lund, Sweden). ^{125}I-labeled protein HC (^{125}I-HC) was prepared by the chloramine-T method [13]. Protein-associated and free ^{125}I were separated by centrifugation through tubes containing Sephadex G-25 as described by Fishelson et al. [14]. Typical preparations were labeled to a specific activity of 3.8 μCi/μg.

2.2. *Fluoresceinated ligand*

Fluorescein isothiocyanate (FITC) labeling of protein HC (FITC-HC) was accomplished by incubation of 1 mg/ml protein with 2 mM FITC (Molecular Probes Inc., Plano, TX) in 10 mM borate buffer, pH 9.7, for 2 h at 4°C. Bound and free FITC were separated by gel filtration on a 10 ml Sephadex G-25 column. Protein concentration was determined by the method of Lowry et al. [15].

2.3. *Monoclonal antibodies*

Monoclonal antibody to protein HC was obtained from Dr

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A. Grubb (Lund University, Lund, Sweden). Monoclonal antibodies to LFA-1α and MO1α were generous gifts from Dr F. Sanchez-Madrid (Hospital de la Princesa, Madrid, Spain) and Dr J.E. De Vries (The Netherlands Cancer Institute, Amsterdam, The Netherlands), respectively.

2.4. *Cell culture*

The human histiocytic lymphoma cell line U937 was maintained in culture in RPMI-1640 (Flow laboratories, Inglewood, CA) supplemented with 10% heat-inactivated fetal calf serum (Gibco, Uxbridge, England), 2% L-glutamine, 1% sodium pyruvate (both from M.A. Bioproducts, Walkersville, MD), 50 U/ml penicillin and 50 μg/ml streptomycin (both from Eli Lilly and Co., Indianapolis, IN). Cells were incubated with phorbol 12-myristate 13-acetate (Sigma Chemical Co., St. Louis, MO) to a final concentration of 35 ng/ml for 1 to 4 days and the adherent population was analyzed for HC binding capacity.

2.5. *Binding assays*

Two million U937 cells were incubated with different concentrations of ^{125}I-HC in a total volume of 200 μl with RPMI-supplemented medium. After 2 h at 4°C, 180 μl was applied to 250 μl of an oil mixture consisting of six parts dioctylphthalate and four parts dibutylphthalate (Eastman Kodak Co., Rochester, NY) in 400 μl polyethylene microfuge tubes (Bio-Rad Lab., Richmond, CA) and microfuged at 4°C for 1 min in a Beckman microfuge B. Tubes were sectioned and the radioactivity of the pellet and supernatant counted. Specific binding was defined as the difference between total binding and the nonspecific binding occurring in the presence of a 1000-fold excess of unlabeled protein HC.

2.6. *Flow cytometry analysis*

Fluorescence analysis was performed with an EPICS-C cytofluorometer (Coulter Cientifica, Mostoles, Spain). Briefly, 400 000 cells were incubated with 1 μg FITC-HC for 80 min at 4°C. After three washes with cold PBS containing 1% BSA and 10 mM sodium azide, the cells were resuspended in 500 μl of the same buffer and analyzed by flow microfluorometry. Background fluorescence was determined by incubating cells with FITC-conjugated F(ab')$_2$ goat anti-human Ig (Behring Werke, Marburg, FRG). Data were displayed as profile histograms plotting fluorescence intensity vs cell number.

3. RESULTS

3.1. *Induction of protein HC receptor expression by PMA*

The ability to induce U937 maturation in vitro provided a mechanism to study the expression of the protein HC receptor at different stages of differentiation. In all experiments, differentiation was confirmed morphologically by light microscopy and by conversion of the cells to an adherent population. U937 cells were found to increase the expression of protein HC receptor after phorbol 12-myristate 13-acetate (PMA) treatment as shown by flow cytometry analysis (fig.1, right panel). Before incubation with PMA, 10.38% of the U937 cells expressed protein HC receptors. After one day induction with PMA, 35.50% of the adherent population expressed the receptor, without significant variation of fluorescence intensity. Interestingly, four day incubation with PMA increased both the percentage of cells binding protein HC (87.13%) and the fluorescence intensity of these cells. Background was determined by incubation of cells with FITC-conjugated F(ab')$_2$ fragments of goat anti-human Ig (fig.1, left panel). In all cases, less than 6% of the cells were positives.

3.2. *Characteristics of protein HC binding*

The protein HC receptor of U937 cells after differentiation by four day culture with PMA, was defined in ligand binding experiments. These studies used radioiodinated protein HC which retained the antigenic reactivity with monoclonal anti-protein HC.

Fig.2. shows that ^{125}I-HC binds to U937 cells in a specific and saturable manner at 4°C. In these experiments, more than 90% of the total binding was specific, since it was blocked in the presence of a 1000-fold excess of unlabeled protein HC. The cell population exhibited a single class of low-

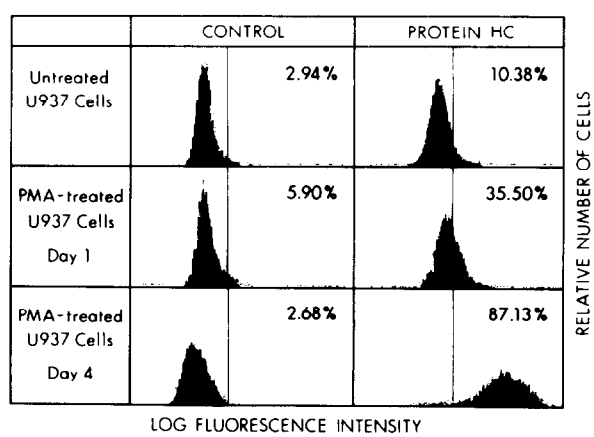

Fig.1. Induction of protein HC receptor expression by PMA. U937 cells were incubated with 36 ng/ml of PMA and the expression of protein HC receptor assessed by incubation with fluoresceinated protein HC (right). Background was established by incubating cells with FITC-F(ab')$_2$ goat anti-human Ig (left). Untreated U937 cells represented the negative control in this assay. Numbers indicate the percentage of positive cells.

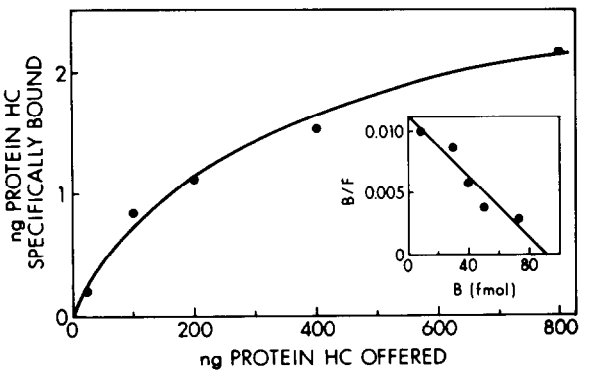

Fig.2. Quantitation of protein HC binding to U937. Cells were incubated at 4°C for 2 h with different amounts of ^{125}I-HC. The inset represents Scatchard analysis of the data. B, bound protein HC; F, free protein HC.

affinity binding sites, $K_a = 2\text{–}5 \times 10^7\ M^{-1}$. The number of binding sites per cell ranged from 20 000 to 30 000 for ^{125}I-HC.

3.3. *Specificity of protein HC binding*

As shown in fig.3, incubation of U937 cells with ^{125}I-HC in the presence of monoclonal antibodies against protein HC resulted in inhibition of more than 90% of the ^{125}I-HC binding to these cells (inputs of 100 ng ^{125}I-HC). On the other hand, monoclonal antibodies that did not react with protein

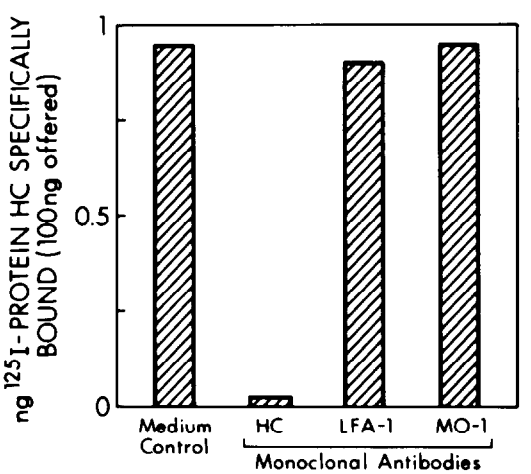

Fig.3. Specificity experiments for ^{125}I-HC binding to U937. Cells were incubated for 2 h at 4°C with ^{125}I-HC (100 ng) in the presence of different monoclonal antibodies. Binding assay was carried out as described in section 2.

HC (anti-LFA-1α, anti-MO1α) displayed no inhibitory activity on the binding reaction.

4. DISCUSSION

The data presented in this report establish the presence of specific protein HC receptors on the human histiocytic lymphoma cell line U937. Expression of protein HC receptors were shown to be influenced by cellular differentiation. After four day culture with PMA, the number of mature (adherent) U937 cells expressing the receptor was 76% higher than for untreated (non-adherent) cells (fig.1). In addition, these cells were found to increase the expression of protein HC receptor as shown by flow cytometry.

Binding of radioiodinated protein HC to mature U937 cells (PMA-treated for 4 days) at 4°C, was noncooperative and saturable. These cells expressed 20 000–30 000 receptors/cell and bound ligand with low affinity ($K_a = 2\text{–}5 \times 10^7\ M^{-1}$). The interaction was specific because uptake of radiolabeled ligand was inhibited only by unlabeled protein HC or by monoclonal anti-protein HC.

According to these results, protein HC receptor could be a component of the mature mononuclear phagocyte plasma membrane. This would relate the behaviour of the protein HC receptor to that of the Fc and C3b receptors, which are used as monocyte/macrophage differentiation markers, since they are expressed only on differentiated forms of mononuclear phagocytes [16,17].

Protein HC has been shown to induce lymphocyte proliferation in the presence of accessory cells [12]. On the basis of the studies presented here, protein HC could mediate lymphocyte activation through binding to specific receptors on mononuclear phagocytes. This interaction could induce the secretion of a second signal which would lead lymphocytes to proliferate. Protein HC did not induce the secretion of IL-1 when it was incubated with different monocytic cell lines (not shown). Thus, another mediator must be responsible for the postulated mechanism of lymphocyte activation.

It will be of interest to demonstrate the presence of protein HC receptors on other cells e.g. peripheral blood monocytes, macrophages, neutrophils, and to elucidate if there is a single type of protein HC receptor.

The identification of a protein HC receptor on U937 cells represents the first step toward understanding the mechanism of action of protein HC. Work is currently in progress to characterize the receptor and to determine its fate after receptor-ligand interaction.

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