CONCISE REPORT

A Technique for the Flow Cytometric Analysis of Lymphocytes Bearing Histamine Receptors

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Histamine receptors have been demonstrated on lymphocyte membranes by a variety of techniques. We now report a method that allows for the flow cytometric analysis of histamine receptors on human peripheral T cells. Histamine is conjugated to fluoresceinated human albumin by the coupling agent ECDI. This conjugated histamine compound (FHA-his) binds to approximately 45% of T cells. Fluoresceinated human albumin alone (FHA), not conjugated to histamine, does not bind to T cells. In addition, unconjugated histamine can inhibit completely the binding seen with FHA-his. We conclude that this technique demonstrates specific FHA-his binding to histamine receptors on T cells and can be used to determine the number of cells bearing such receptors. In addition, the reagent could be used with a cell sorter to isolate distinct histamine-receptor-bearing (HR⁺) cells for further immunologic study.

Surface membrane markers have been used to correlate various lymphocyte subpopulations and their functional roles. Generally, these markers have been defined by the presence of antigens or receptors of uncertain significance. One surface marker that appears to have physiologic significance is a receptor for histamine.

Lymphocyte membrane receptors for histamine have been identified previously by a variety of techniques. These have included the binding of lymphocytes to histamine-coated beads or red cells, and by the effect of histamine on intracellular cyclic adenosine monophosphate (AMP). More recently, we have reported the solubilization, separation, and partial characterization, by biochemical means, of specific histamine H₁ and H₂ membrane receptors on thymic lymphocytes. We describe now a new technique for the identification and analysis of histamine receptors on lymphocytes using histamine that has been conjugated to fluoresceinated human albumin. When cells are labeled with this reagent, they can be analyzed in a flow cytometer and the percentage of histamine-receptor-bearing cells (HR⁺) determined. This technique, when used in conjunction with a cell sorter, could separate distinct HR⁺ and HR⁻ populations for further study.

MATERIALS AND METHODS

Preparation of Lymphocytes

Lymphocytes were prepared by Ficoll-Hypaque fractionation of peripheral blood collected in preservative-free heparin. T cells were prepared by rosetting these cells with sheep erythrocytes.

Preparation of Fluorescein Human Albumin-Histamine Conjugate (FHA-his)

Fluoresceinated human albumin (FHA) was purchased from Cappel Laboratories (Cochranville, Pa.). Each molecule of this albumin is labeled with approximately 5 molecules of fluorescein. The FHA was dissolved in water at a concentration of 10 mg/ml. To 5 cc of this solution (50 mg albumin) was added 500 mg of histamine dihydrochloride (Sigma Chemical Co., St. Louis, Mo.) and 1 g of 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide hydrochloride (ECDI) (Sigma). This reaction was allowed to proceed for 2 hr at room temperature, in the dark, followed by dialysis × 2, against a 300-fold volume of water. A third and final dialysis was done against a 300-fold volume of normal saline. All dialyses were performed for 90 min, at 4°C, in the dark. Following the last dialysis, the solution was adjusted to a final albumin concentration of 3.3 mg/ml in normal saline. A [³H]-histamine (New England Nuclear, Boston, Mass.) tracer was added to each batch to monitor the amount of histamine conjugated to FHA. Approximately 70-90 molecules of histamine were bound per molecule of FHA. The control FHA that was used in the binding experiments described below was reacted with the ECDI in the absence of histamine and dialyzed in a manner identical to that used with the FHA-his.

Labeling of Cells With FHA-his

T cells were centrifuged and washed twice in phosphate-buffered saline (PBS). They were then centrifuged and resuspended in PBS containing 2 mmol CaCl₂ and 1 mmol MgSO₄ (PBS/Ca-Mg) at a concentration of 2 × 10⁶ cells/ml. Fifty microliters were placed in a plastic tube and 10 μl of FHA-his or FHA was added. In some experiments unconjugated histamine dihydrochloride was added to a final concentration of 5 × 10⁻⁴ M. In all experiments, the final volume of the reaction mixture was brought to 100 μl by the addition of PBS/Ca-Mg. The cells were incubated then for 30 min in a 37°C water bath. At that time the cells were removed from the

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Flow Cytometric Analysis

Following incubation, the cells were analyzed on a Bio/Physics (now Ortho Instruments, Westwood, Mass.) Cytoluxorgraf #4800A, using 10 mW excitation at 488 nm from an argon ion laser source. Green fluorescence was measured in the 510–590 nm wavelength range. Distributions of fluorescence from individual cells were accumulated in a Bio/Physics #2101 distribution analyzer. A minimum of 50,000 cells was analyzed for each sample. Laser power, photomultiplier tube gain, and pulse-height analyzer display settings were kept constant for all samples in an experiment.

RESULTS

Labeling With FHA-his

Analysis of T cells following FHA-his labeling demonstrated two distinct populations (Fig. 1, panel I), one of obviously higher fluorescence intensity than the other. Approximately 45% of the cells were found in the higher fluorescence peak. In order to see whether this peak represented specific binding, a similar incubation of cells was performed with FHA, i.e., fluoresceinated human albumin not conjugated to histamine.

Labeling With FHA

When FHA was used to label the cells there was a single peak that corresponded to the lower fluorescence peak in the FHA-his analysis (Fig. 1, panel II).

Labeling With FHA-his in the Presence of Unconjugated Histamine

Since the FHA alone did not produce a higher fluorescence peak as was seen with FHA-his, it seemed that the presence of histamine was necessary for albumin binding. To see whether this binding was biochemically similar to the binding of free histamine to membranes, we did the FHA-his incubation in the presence of a 1000-fold higher concentration of unconjugated histamine \((5 \times 10^{-3} M)\). When cells were incubated with FHA-his in the presence of unconjugated histamine, there again was only a single population with fluorescence comparable to the lower peak when the binding was done with FHA-his alone (Fig. 1, panel III).

DISCUSSION

We report that incubation of T cells with histamine conjugated to fluoresceinated human albumin (FHA-his) gives two distinct populations of differing fluorescence intensity. Conversely, incubation of cells with fluoresceinated human albumin (FHA) alone yields only one population, which is of low intensity fluorescence and corresponds to the lower peak seen in the FHA-his experiment. Our interpretation is that the FHA requires conjugation to histamine for any subsequent binding to the T cell to occur. Since it was possible that this effect of histamine was not due to specific histamine binding of FHA-his to the membrane but to some other cause, we studied the ability of unconjugated histamine to inhibit FHA-his binding. We found that a 1000-fold higher concentration of unconjugated histamine inhibits completely FHA-his binding, giving only a single peak of low intensity fluorescence. We believe, therefore, that FHA-his binds specifically to histamine receptors. Cells possessing such receptors are designated HR \(^*\) and represent approximately 45% of T cells. The remaining cells of low intensity fluorescence can be considered to not possess surface membrane histamine receptors and are designated HR \(^-\). Although we have done this study with T cells, the technique is applicable to any cell population that can be made into a single-cell suspension.

Histamine receptors on lymphocyte membranes have been identified by a variety of methods. This technique allows for the rapid determination of the number of lymphocytes bearing histamine receptors. Moreover, this methodology enables the isolation by a fluorescence-activated cell sorter of distinct HR \(^*\) (high intensity fluorescence) and HR \(^-\) (low intensity fluorescence) populations that then can be studied further.

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REFERENCES


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