Brief Definitive Report

FRACTIONATION OF HUMAN T LYMPHOCYTES ON WHEAT GERM AGGLUTININ-SEPHAROSE*

By U. HELLSTRÖM, M-L. DILLNER, S. HAMMARSTRÖM, AND P. PERLMANN

(From the Department of Immunology, University of Stockholm, Fack, S-104 05 Stockholm, Sweden)

Thymus-derived lymphocytes (T cells) are known to be functionally heterogenous comprising cells with helper, suppressor, and memory functions; mitogen-responsive cells; and cytolytic effector cells. Recent studies in the mouse have indicated that several of these cell types differ in their expression of various surface markers such as the Ly antigens (1, 2). Much less is known about differences in surface structures of human T cells. Most or all human T cells have receptors for sheep erythrocytes (3) and, after neuraminidase treatment, for the A hemagglutinin from *Helix pomatia* (HP) (4). A large fraction of the human T cells also has receptors for IgM (5).

To find surface markers suitable for further characterization and fractionation of subpopulations of human T cells we have recently directed our attention to wheat-germ agglutinin (WGA). WGA is a carbohydrate-binding protein with specificity for a sequence of three β-(1→4)-linked *N*-acetyl-D-glucosamine (D-GNAc) units (6, 7). Similar to concanavalin A (Con A) and phytohemagglutinin (PHA), WGA binds to all lymphocytes. However, in contrast to the former, WGA is nonmitogenic (references 8 and 9, and Hellström et al., unpublished observation).

In this study we demonstrate that T cells from human peripheral blood can be divided into two discrete subpopulations distinct in regard to the strength with which they interact with WGA. The two subpopulations could be separated from each other on WGA-Sepharose columns. They differed in their mitogenic response to the lectins leukoagglutinin (La) from *Phaseolus vulgaris* and Con A.

Materials and Methods

Human peripheral blood lymphocytes were purified from defibrinated venous blood by gelatin sedimentation, treatment with carbonyl iron, and Ficoll-Isopaque centrifugation (≥98% lymphocytes) (10). In some experiments contaminating erythrocytes (5–10%) were lysed with Tris-buffered isotonic NH_4Cl, pH 7.2. T lymphocytes were obtained by passage of peripheral blood lymphocytes over a human gamma globulin (HGG)/anti-HGG complex column (11).

Highly purified WGA was obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden. It was purified by affinity chromatography using D-GNAc coupled to Epoxy-activated Sepharose.

For fractionation of cells WGA was coupled to cyanogen bromide-activated Sepharose 6MB (5 mg WGA/ml gel, Pharmacia Fine Chemicals). 15–50 × 10^6 purified T cells in Tris-buffered Hanks' solution (TH) plus 0.2% human serum albumin (HSA) were fractionated on columns (K9/15, Pharmacia Fine Chemicals, supplied with an 85-μm nylon net) containing 1.5–2 ml of extensively washed WGA-Sepharose at a flow rate of 6–10 ml/min. The lymphocytes were incubated on the

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column for 3 min. Nonretained cells were washed off with 100–150 ml buffer and retained cells were thereafter eluted with 100 ml of buffer containing 25 mg D-GNAc/ml. During elution with the sugar the flow was stopped for 5 min in order to increase the efficiency of dissociation. Cells were washed in buffer, counted, and tested for viability.

For binding studies the WGA was labeled with Na[^{125}I] (sp act >14 mCi/μg I; Radiochemical Centre, Amersham, England) using the chloramine-T procedure. In the binding experiments 1×10^6 lymphocytes were incubated with increasing amounts of ^{125}I-WGA in a total vol of 2.7 ml TH plus 0.2% HSA plus 0.02% NaN_3. The cells were kept in slow vertical rotation for 2 h at 20°C. The binding data were treated according to Scatchard's derivation of the law of mass action.

WGA was labeled with fluorescein isothiocyanate (FITC; Baltimore Biological Laboratories, Baltimore, Md.) (12). The fluorescein/protein molar ratio was 15. 0.5×10^6 lymphocytes were incubated with increasing amounts of FITC-WGA in 50 μl of TH plus 0.02% NaN_3 for 30 min at 4°C.

Incorporation of [^{14}C]thymidine into DNA was determined after exposure of the cells to different doses of La and Con A for 72 h. The cells were cultured in V-shaped microplates (Linbro Chemical Co., New Haven, Conn.) at a concentration of 10^6 lymphocytes/ml in HEPES-buffered RPMI 1640 (Biocult Laboratories, Paisley, Scotland) plus 0.4% HSA. The cells were pulsed for 16 h with [^{14}C]thymidine [0.2 μCi/10^6 lymphocytes/ml, sp act 51 mCi/mmol (Radiochemical Centre)].

Results and Discussion

Immunofluorescence studies with FITC-WGA demonstrated that all human peripheral blood lymphocytes bind WGA. The titration curves were, however, found to be biphasic with only a fraction of the cells stained at low lectin concentrations. A biphasic binding curve was also obtained when increasing amounts of ^{125}I-labeled lectin was reacted with a constant number of lymphocytes. Fig. 1*a* and *b* show that this heterogeneity with respect to WGA binding also could be demonstrated for purified T lymphocytes [<1% SIg^+ cells (13), >90% E^+ and HP^+ cells (4, 13)].

The biphasic binding curve (Fig. 1*a*) gave $K_{1app} = 7 \times 10^7\ M^{-1}$, $n_1 = 6 \times 10^6$; $K_{2app} = 5 \times 10^6\ M^{-1}$, $n_2 = 15 \times 10^6$. The difference in apparent binding constants was approximately 14-fold. That the difference in affinities of the lectin-lymphocyte receptor interactions was at least partly due to strong and weak binding receptors distributed on different cells rather than on the same cells is shown in Fig. 1*b*. As can be seen low concentrations of fluorescein-labeled WGA only stained a fraction of the cells (~20%), whereas all cells were stained at higher concentrations. The chemical nature of the WGA receptors on the cell surface is unknown. However, it may be assumed that the chemical structures responsible for strong and weak WGA binding are different since the binding curve is biphasic and the difference between the two apparent binding constants was relatively small.

In order to separate T cells with strong and weak binding WGA receptors from each other, purified T cells were applied to a column containing WGA covalently bound to Sepharose. Loosely bound cells (passed cells) were eluted with buffer and more firmly bound cells (eluted cells) with buffer containing D-GNAc. 43 ± 15% (mean ± SD) of the cells passed the column and 22 ± 11% were eluted with the sugar (11 experiments). The total cell recovery was 65 ± 19%. The cells in both fractions were fully viable and were not stimulated by passage over the column. Before use in further tests the passed cells were washed once with D-GNAc (25 mg/ml) in order to remove traces of WGA that might have leaked from the column.

When tested by immunofluorescence the titration curves for either the passed

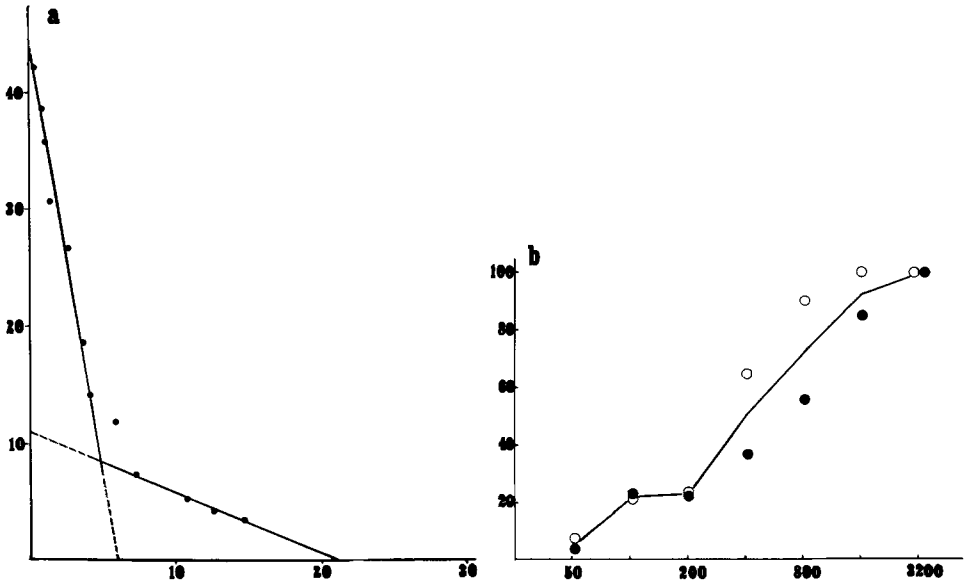

FIG. 1. (*a*) Binding of ^{125}I-labeled WGA to human T lymphocytes obtained by passage through a column charged with HGG/anti-HGG. 1×10^6 lymphocytes were used per tube. Data plotted according to Scatchard's derivation of the law of mass action. Ordinate: $r/A \times 10^{-13}$. Abscissa: $r \times 10^{-6}$. (*b*) Immunofluorescent staining of human T lymphocytes from two donors (○, ●) with FITC-conjugated WGA (0.5×10^6 lymphocytes, 50 μl WGA-FITC, 30 min, 4°C). Ordinate: percent of cells showing positive membrane fluorescence. Abscissa: nanograms of WGA-FITC/0.5×10^6 lymphocytes.

or the eluted cells were found to be continuous with no plateau level similar to that seen with unfractionated cells (Fig. 1*b*). The eluted cells were furthermore stained at slightly lower FITC-WGA concentrations than the passed cells. The data indicate that weakly binding cells were enriched in the passed fraction and vice versa.

To investigate whether the two subpopulations of T cells were functionally different their responsiveness to the mitogenic lectins, La and Con A, was investigated. Fig. 2*a* and *b* show a typical experiment in which the mitogenic response to La of the cell fractions was measured. As can be seen the passed cells responded only marginally to La, while the eluted cells showed a 2- to 2.5-fold increase in DNA synthesis as compared to the T cells before fractionation or to the unfractionated lymphocytes (Fig. 2*a*). The unfractionated cells showed a broader dose-response curve than the T cells, in that they also reacted at low doses of La. This may indicate that certain responder cells or cells cooperating in the La response have been removed on the HGG/anti-HGG column.

When passed and eluted cells were mixed in different proportions and assayed for their ability to incorporate thymidine into DNA at an optimal La dose it was found that the response decreased linearly with the dilution of the eluted cells by the passed cells (Fig. 2*b*). Thus, the cells responding to La were enriched in the eluted fraction and depleted from the passed fraction. The results indicate that the increased responsiveness of the eluted cells was not due to removal of a suppressor cell. Conversely, the lack of La response found in the passed fraction does not reflect removal of an accessory helper cell present in the eluted fraction.

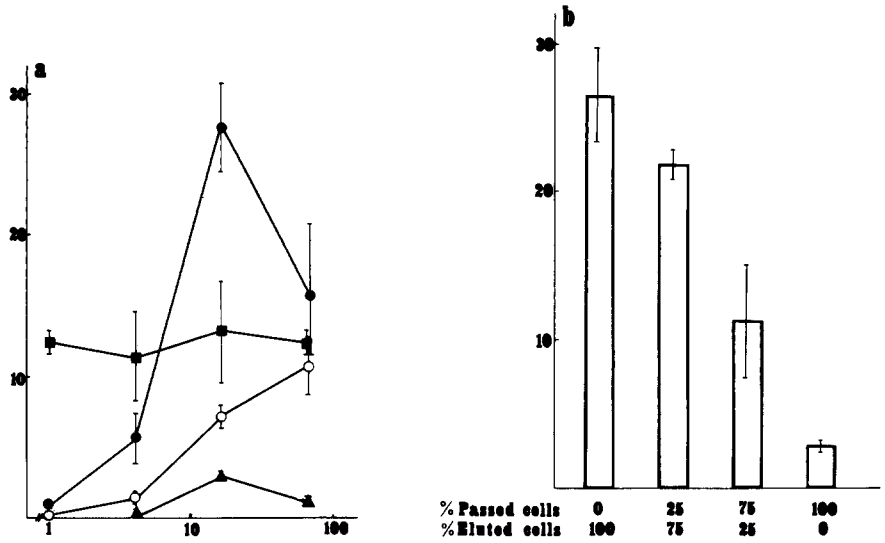

FIG. 2. (*a*) Dose-response curves for stimulation of DNA synthesis with La of unfractionated human blood lymphocytes, T cells, and T cells fractionated on WGA-Sepharose, respectively. Ordinate: (cpm/0.2 × 10^6 lymphocytes) × 10^{-3}. Abscissa: micrograms La/milliliter and 1 × 10^6 lymphocytes. (■), unfractionated lymphocytes; (○), T lymphocytes; (▲), passed T-lymphocyte fraction from the WGA-Sepharose column; (●), eluted T-lymphocyte fraction from the WGA-Sepharose column. Cells were activated for 72 h and thereafter pulsed for 16 h with [^{14}C]thymidine. The vertical bars show mean ± SD of triplicates. (*b*) Stimulation by La of DNA synthesis in passed or eluted T lymphocytes obtained by fractionation on a WGA-Sepharose column and subsequently mixed in different proportions. La was added at an optimal dose (16 μg/ml and 1 × 10^6 lymphocytes). Ordinate: (cpm/0.2 × 10^6 lymphocytes) × 10^{-3}. Abscissa: percent of passed and eluted cells in mixtures.

Studies performed with Con A showed that the cells stimulated by this lectin were also enriched in the eluted fraction and depleted from the passed fraction. However, the T-cell fraction generally gave a lower response than the unfractionated lymphocytes, suggesting that a fraction of the responsive cells may have been removed by passage over the HGG/anti-HGG column. Alternatively, cells cooperating in the Con A response may have been removed. It is known that the HGG/anti-HGG column also removes T cells with Fc receptors, which in the mouse have been shown to play an important role in the mitogenic response to Con A (14). In any case, our results show that the two subfractions of T cells obtained by fractionation on WGA-Sepharose are functionally different.

In preliminary experiments the passed and eluted cells were tested for their ability to act as responder cells in mixed leukocyte culture (MLC). In some experiments both passed and eluted cells responded in MLC to the same extent as the original cell population or as the T cells. However, in other experiments the responder cells were enriched in the eluted fraction and depleted from the passed fraction. Further experiments are needed to establish whether or not the proliferating cells and the cytotoxic effector cells in a MLC-cell-mediated lympholysis system differ in their WGA-binding capacity.

Summary

T cells from human peripheral blood were purified by fractionation on col-

umns charged with human immunoglobulin and rabbit anti-human immunoglobulin. When assayed with ^{125}I- or fluorescein isothiocyanate-labeled wheat-germ agglutinin (WGA), a weakly binding and a strongly binding subpopulation could be distinguished. These T-cell subpopulations were fractionated on columns charged with WGA, covalently bound to Sepharose 6MB. The cells responding to the mitogens leukoagglutinin from *Phaseolus vulgaris* and concanavalin A were enriched in the strongly binding subpopulation (~20% of the T cells) while they were depleted from the weakly binding subpopulation.

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