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Histamine-receptor leucocytes (HRL)

ORGAN AND LYMPHOID SUBPOPULATION DISTRIBUTION IN MAN

A. SAXON, V. DIANA MORLEDGE & B. BONAVIDA *Department of Microbiology and Immunology/ Immunobiology Group, UCLA School of Medicine, Los Angeles, California, U.S.A.*

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SUMMARY

The frequency of lymphoid cells with a membrane receptor for histamine was determined in various lymphoid organs in man using a histamine-rosette assay. Thymus had very low numbers of histamine-receptor cells while lymph node and peripheral blood had increasing percentages. Through a combination of cell separation techniques, we demonstrated that about one third (1/3) of periperal blood B lymphocytes and macrophages carry histamine receptors. Immature B cells or null cells (E-rosette and membrane-immunoglobulin-negative) do not have this receptor. Only 10% of peripheral blood T lymphocytes formed histamine rosettes. That these histamine receptor T lymphocytes are a subpopulation representing the differentiated suppressor/cytotoxic T cells is suggested by evidence showing complete removal of histamine receptor T lymphocytes on nylon wool adherence columns. Thus, the histamine receptor is expressed on differentiated B and T lymphocytes and may serve as a marker for developed suppressor/cytotoxic T cells in man.

INTRODUCTION

The surface of various lymphocyte subpopulations are characterized by the presence of receptors for immune molecules, immunoglobulin and complement (Samarut, Brochier & Renillard, 1976; Ross *et al.*, 1973), and hormones active in immune processes, beta adrenergic catacholamines, prostaglandins and histamine (Melmon *et al.*, 1972; Bourne *et al.*, 1974). The presence of these receptors may allow for the participation of the appropriate lymphocyte in various stages of immune processes.

The receptor for histamine has been shown to be found on several populations of mouse leucocytes (Kedar & Bonavida, 1974). Thymus contained few cells with this receptor while increasing numbers of histamine-receptor cells were found in lymph node, blood, bone marrow, spleen and peritoneal exudate.

These studies also suggested that some activated anti-tumor T lymphocytes may express a histamine receptor (Kedar & Bonavida, 1974). This correlates with the fact that cytotoxic thymus-derived (T) lymphocytes display more histamine binding units than do unsensitized cells (Plaut, Lichtenstein & Henney, 1973). Lymphoid-cell-bearing histamine receptors have also been implicated to play an important regulatory role in the humoral immune response. Removal of histamine receptor-bearing T lymphocytes caused a marked increase in antibody production in an adoptive transfer system (Shearer *et al.*, 1972). This evidence suggests that at least one subpopulation of mature T cells carries the histamine receptor. Since it has been shown that the T cells responsible for suppression of antibody production (suppressor cells) belong to the same subpopulation as cytotoxically active T lymphocytes, the histamine-receptor T cells may be the suppressor/cytotoxic lymphocytes. This suppressor/killer subpopulation is characterized by the presence of defined Ly antigens in mice (Cantor & Boyse, 1975), but this type of marker is not presently available in man.

Correspondence: Dr A. Saxon, Department of Microbiology and Immunology/Immunobiology Group, UCLA School of Medicine, Los Angeles, California 90024, U.S.A.

MATERIALS AND METHODS

Preparation and fractionation of lymphoid cells. All cell suspensions were prepared in minimal essential media (MEM) buffered to Ph 7·4 with 10 mM HEPES and washed 3 times before use. Peripheral blood leukocytes (PBL) were obtained from normal healthy volunteers or patients with lymphoproliferative or myeloproliferative malignancy using Ficoll–Hypaque (F–H) density separation of heparinized blood diluted 1:1 with 0·15 N saline (Böyum, 1968). Thymuses were obtained from children undergoing open-heart surgery for correction of congenital heart defects while cervical lymph nodes were obtained from patients undergoing reconstructive surgery or radial neck dissection for a primary head and neck tumor. Thymuses and lymph nodes were gently teased apart in a petri dish with two 18-gauge needles and the resultant suspension filtered through a nylon screen. Established lymphoid cells lines of characterized cell origin were kept in continuous culture and harvested on the day they were tested.

PBL were separated into T and non-T fractions by a modification of density separation of T lymphocyte SRBC rosettes on FH (Saxon, Feldhaus & Robbins, 1976), PBL T cells were also purified by incubation of the nylon wool columns in the presence of 10% FCS with subsequent slow elution (6 drops/min) of the non-adherent T cells (Greaves & Brown, 1974).

Analysis of histamine receptors. Preparation of histamine-rabbit serum albumin (H-R) conjugate. The procedure of Kedar & Bonavida (1974) for optimal conditions of H-R-SRBC rosette detection was used. One and four-tenths gram histamine dihydrochloride (H) (Sigma, St. Louis, Missouri), 200 mg rabbit serum albumin (RSA, Sigma) and 1·2 g of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (ECDI, Story Chemical Co., Ott. Div., Muskegon, Michigan) were dissolved in 20 ml of phosphate-buffered saline (PBS, 0·01 M phosphate buffer, pH 7·2, in 0·14 M NaCl). After incubation for 1 hr at room temperature with intermittent shaking, the solution was dialysed at 4°C against 2 l of PBS, changed three times over 48 hr. Approximately 30 mol of histamine per mol of RSA constitute the conjugate under these conditions. The H-R was either used fresh or after being frozen at −20°C for periods up to 1 month. The conjugate was centrifuged for 15 min at 10,000 *g* before coupling to erythrocytes in order to remove denatured protein.

Coupling of the H-R conjugate to SRBC was accomplished by mixing 0·25 ml of 50% SRBC suspension in PBS, 2·5 ml of dialysed H-R conjugate (contains approximately 20 mg of conjugated RSA and 40 mg of ECDI dissolved in 1 ml of PBS) and incubating for 45 min to 1 hr at room temperature with intermittent agitation. For controls, SRBC were coupled with 20 mg of RSA (R-SRBC) dissolved in 2·5 ml of PBS otherwise employing the same conditions as in the coupling of the H-R to the SRBC. The coated SRBC were washed three times in cold CA^+ and Mg^+ Dulbecco's PBS (Grand Island Biological Co.) containing 1% heat-inactivated foetal calf serum (absorbed with SRBC) and resuspended in the same medium at a concentration of $2{\cdot}5\times 10^8$/ml.

For rosette formation, lymphoid or tumour cells (0·1 ml of 5×10^6/ml) were mixed in a 10- ×70-mm test tube with an equal volume of R-SRBC or H-R-SRBC (all suspended at $2{\cdot}5\times 10^8$/ml), the tubes were centrifuged at 175 *g* for 7 min at 4°C, and placed in ice for 30 min. Immediately before counting, the supernatant was gently poured off and 0·1 ml of 0·5% crystal violet solution was added to each tube. The pelleted cells were gently resuspended by inverting the tube 20 times and then examined under the microscope (×400) in a haemocytometer. For each test 100 stained, nucleated cells were counted. A rosette was defined as a stained cell which bound three or more erythrocytes. Cell clumps were not scored.

Analysis of other cell markers. T cells were quantified by spontaneous rosette formation between lymphocytes and 1% suspension of treated SRBC in heat-inactivated SRBC-absorbed FCS (Jondal, Holm & Wigzell, 1967). Lymphocytes with a receptor for the Fc portion of IgG were measured by rosette formation with trypsinized SRBC at 10^8/ml coated with a subagglutinating dose of rabbit anti-SRBC IgG (Zighelboim *et al.*, 1974). Complement receptors (C′) were detected on lymphocytes by rosette formation with zymosan particles coated with complement (Huber & Wigzell, 1975). All rosettes were formed by mixing 0·05 mls of lymphocytes suspension at 5×10^6 cells/ml with an equal volume of rosette reagent. The tubes were incubated at 37°C for 5 min except for complement tubes which were left at room temperature. Subsequently, all tubes were centrifuged at 60 *g* for 5 min and then incubated at 4°C for at least 1 hr. The cells were resuspended gently by tipping the tubes. After addition of 0·05 ml of 0·5% crystal violet solution, the cell suspension was examined microscopically in a haemocytometer. Lymphocytes with three or more particles attached to their surface were scored as rosettes.

Membrane-bound immunoglobulin (MIg) was detected by direct immunofluorescence (Winchester *et al.*, 1975). After incubation at 37°C for 1 hr and washing to remove cytophilic Ig, 1×10^6 cells were incubated at 23°C for 20 min with a 1:10 dilution of fluorescent goat-polyvalent-antihuman immunoglobulin (Meloy) which had been ultracentrifuged just prior to use to remove aggregates of IgG which would bend to Fc receptors. Subsequently the cells were washed 3 times in cold MEM with 0·01% azide. The cells were placed on a microscope slide and viewed under epi-illumination using a Leitz Orthoplan fluorescent microscope.

Phagocytic cells were enumerated by their ability to injest 1μ latex beads (Dow Chemical) (Zucher-Franklin, 1974). 1×10^6 cells in 1 ml of MEM with 10% FCS were incubated with a 0·01 ml of a 1% solution of beads and subsequently washed three times. Cells that had ingested more than three beads were scored as positive.

RESULTS

Frequency of histamine-receptor cells in normal human thymus, lymph node and peripheral blood (Table 1)

Table 1 summarizes the results from experiments done over 6 months. Human thymocytes contained a very low percentage of cells with histamine receptors (range 0–3%). Greater than 90% of these cells

TABLE 1. Percentage HRL in peripheral blood, lymph node and thymus

	HRL	E	Fc	C′	MIg
Normal PBL: 17 samples					
Mean ± s.d.	23·5 ± 4·5	66·4 ± 6·4	23·7 ± 6·4	13·8 ± 5·1	12·1 ± 1·8
Range	16–30	55–76	16–36	7–30	11–15
Lymph node: 5 samples					
Mean ± s.d.	13·4 ± 3·8	67·4 ± 8·7	11·6 ± 4·7	8·8 ± 2·9	10·0 ± 2·7
Range	7–17	52–79	6–16	6–13	7–12
Thymus: 5 samples					
Mean ± s.d.	1·4 ± 1·1	92·8 ± 2·1	4·0 ± 1·9	3·2 ± 1·1	1
Range	1–3	91–96	3–6	2–5	0–1

formed spontaneous SRBC rosettes while lymphocytes with other receptors (MIg, C′, Fc) were very infrequently found.

Fresh human peripheral blood leucocytes obtained by F–H purification contained an average of 24% histamine-receptor cells (range 15–30%). The percentage of histamine-receptor cells in samples of PBL which had been frozen at −180°C in the gaseous phase of liquid nitrogen remained unchanged (23% mean).

Human lymph nodes consistently demonstrated a lower proportion of histamine-receptor cells than found in peripheral blood, 13% (range 7–17%). However, macrophages (phagocytic cells) which may exhibit histamine receptor are not found in our lymphocyte suspensions while they comprise 5–15% of our F-H purified PBL.

Subpopulations of PBL with histamine receptors

To determine what populations of cells among PBL carry the histamine receptor, several experiments were undertaken. The percentage of phagocytic cells in PBL which formed histamine rosettes was determined. This was accomplished by feeding the PBL latex beads prior to the histamine-rosette procedure. Approximately one of three cells which had ingested three or more latex beads formed histamine rosettes.

TABLE 2. Percentage HRL in T- and B-lymphocyte fractions

	HRL	E	Fc	C′	MIg
PBL					
T Fraction: 5 samples					
Mean ± s.d.	12·4 ± 1·5	91 ± 3·4	5·0 ± 2·2	2·6 ± 1·8	1·5
Range	11–14	87–96	2–7	1–5	1–2
PBL					
B Fraction: 4 samples					
Mean ± s.d.	35·0 ± 12·3	4·25 ± 1·5	57·0 ± 4·2	53·4 ± 5·7	60·0 ± 12·2
Range	19–48	3–6	54–63	47–54	52–74
PBL nylon eluted					
T Fraction: 5 samples					
Mean ± s.d.	1·2 ± 1·3	88·2 ± 3·1	9·5 ± 1·3	4·8 ± 3·1	1
Range	0–3	85–92	8–11	1–8	0–2
Lymph node					
T Fraction	5	92	6	4	3
B Fraction	23	4	47	50	49

T lymphocytes and non-T lymphocytes purified by density separation of SRBC lymphocyte rosettes were tested for the presence of HRL (Table 2).

To approach this problem of the role of histamine-receptor lymphocytes in man, we have determined the presence of this receptor on cells in different human lymphoid organs utilizing rosette formation between the lymphocytes and histamine-rabbit serum albumin conjugated covalently to sheep red blood cells. Furthermore, peripheral blood leucocyte subpopulations were obtained utilizing cell fractionation techniques and the presence of histamine-binding cells among the separated populations analysed. Finally, histamine receptors were investigated using monoclonally derived neoplastic cells derived from patients with chronic lymphocytic leukemia (CLL) or from established cell lines. These studies demonstrate that the histamine receptor on human lymphocytes is not found on immature T cells and is present on a small proportion of mature T cells. These T cells may represent a subpopulation similar to HRL to the mouse, i.e. suppressor/killer cells. A higher proportion of circulating B cells have histamine receptors while null (E-rosette and MIg-negative) lymphocytes do not express this surface marker. Nylon wool adherence columns completely remove all histamine receptors cells of both B- and T-cell type.

The T lymphocytes from peripheral blood contained approximately 10% cells which formed histamine rosettes while about 40% of the B-cell fraction were HRL.

Lymphocytes purified by nylon column elution were highly enriched for T cells with virtual absence of MIg-receptor cells or macrophages. These eluted T cells, however, were all histamine-receptor negative demonstrating that not only MIg cells (B cells) and macrophages were adhering to nylon columns but T lymphocytes which were HRL were also selectively removed.

Histamine receptors on lymph node T- and B-lymphocyte fractions

Lymph nodes contained less HRL than did PBL (Table 1). This could be partially accounted for by the absence of macrophages with histamine receptors from the lymph-node suspensions. However, selective depression in histamine-receptor lymphocytes among the T or B cells in lymph nodes might also account for this. Therefore, lymph-node cells were fractionated into T and B populations by density separation of SRBC-lymphocyte rosettes and the percentage of histamine-receptor cells determined for each fraction (Table 2). The lymph node B- and T-cell fractions both showed depression of histamine-receptor cells as compared to PBL fractions.

TABLE 3. Percentage HRL in moncclonally derived cells

Sample	HRL	E	Fc	C′	MIg
CLL_1	62	7	61	51	42
CLL_2	58	12	39	38	65
CLL_3	35	1	17	36	84
CLL_4	48	3	49	58	79
CML					
Blasts					
1	1	2	7	3	4
2	1	1	4	2	5
Monocytic Leukaemia	36	40	40	30	18
Cell type					
LA96	7	n.d.	n.d.	n.d.	n.d.
LA85	43	n.d.	n.d.	n.d.	n.d
LA109	20	n.d.	n.d.	n.d.	n.d.
LA249	11	n.d.	n.d.	n.d.	n.d.
LA237	2	n.d.	n.d.	n.d.	n.d.
MOLT-4	1	60	1	1	1

n.d. = Not done.

Histamine-receptors on clonally derived cells (Table 3)

The presence of histamine receptors on B cells was confirmed using PBL derived from patients with CLL, a known B-cell proliferation. These patients' lymphocytes were shown to be B lymphocytes by the presence of MIg on membrane immunofluorescence. Between 35 and 60% of their lymphocytes were positive for histamine receptors. Immature macrophages obtained from a patient with monocytic leukaemia which were positive for latex-bead phagocytosis and stained for esterase by the alpha-naphthylacetate method (Yam, Li & Crosby, 1971) expressed histamine receptors on 35% of the cells while myeloblasts from two patients with CML in blast crisis contained virtually no cells which formed histamine rosettes (0–1%).

Established cell lines were examined for histamine receptors. The number of histamine-receptor cells was quite variable (2–43%) for the B-cell lines derived from normals tested while the T-lymphocyte line MOLT 4 contained less than 1% cells which formed histamine rosettes.

DISCUSSION

The percentage of histamine receptor cells in human PBL (24%) is consistent with previous results obtained in the mouse using the same rosette assay (Kedar & Bonavida, 1974). Since about one of three of the phagocytic cells in PBL formed H rosettes and these cells comprised 10% of the PBL, approximately 21% of PBL lymphocytes had histamine receptors. Our results also are in relative agreement with those of Ballet *et al.* (Ballet & Merler, 1976), who found 30% histamine-receptor cells in human peripheral lymphocytes using an assay consisting of lymphocytes adhering to sepharose beads covalently bound to histamine. The difference seen may be due to the different assay systems used although those authors state that 'accurate quantification of histamine binding was difficult' using their method.

Histamine-receptor lymphocytes were found among both the B- and T-cell fractions of PBL. Again this is in agreement with Ballet *et al.*, who used zonal centrifugation to obtain T- and B-cell fractions, the absolute number of HRL in both their populations was approximately equal. Nylon column purification of T cells resulted in essentially complete removal of all histamine-receptor T cells as well as all B cells and macrophages. We have previously found nylon-column-purified lymphocytes contain not only a very high percentage of T cells, but are also enriched in non-T non-MIg bearing cells which can mediate antibody-dependent cellular cytotoxicity and phytohemagglutinin-dependent cellular cytotoxicity. Since nylon-purified cells did not bear the histamine receptor, the effect cells for these activities must not have this receptor also. These antibody-dependent cellular-cytotoxicity effector cells have been shown to be precursors of MIg-positive B cells (Chess *et al.*, 1975) and their lack of histamine receptors is consistent with the concept that this receptor is found only on more differentiated B cells. As nylon-purified T cells can provide helper function for B cells in immunoglobulin production (Seeger *et al.*, 1976), then helper T cells also need not carry histamine receptors. Besides retaining B lymphocytes and macrophages, nylon-column purifications may selectively remove preformed T-suppressor lymphocytes of Ig production (Siegal, Siegal & Good, 1976), but not the precursors of these suppressors (Feldman & Kontiainen, 1976, personal observations), suggesting that the differentiated suppressor T cells are among the HRL T cells.

The work by Ballet & Merler (1976), who demonstrated that histamine receptors were present on the human lymphocytes involved in cytotoxicity as well as proliferation to mitogens and allogeneic stimuli, further strengthens the hypothesis that the histamine receptor may be expressed on T cells in man which are the suppressor/killer subpopulation.

Our results showing almost complete absence of HRL in thymus is also consistent with the hypothesis that this receptor is expressed on differentiated T cells and probably almost exclusively those of suppressor/cytotoxic type. We cannot reconcile our data with that of Ballet & Merler (1976), who found 10–50% of thymocytes to bear histamine receptors. Certainly if as they also postulate histamine receptors are found on differentiated cells, this high a number of reactive thymocytes is unexpected. The explanation of their high results may be that only 50% of their thymocytes formed spontaneous SRBC rosettes

as opposed to greater than 90% of our thymocytes. The 50% of non-SRBC rosetting cells may have contained the HRL.

In summary, differentiated T lymphocytes, differentiated B lymphocytes and macrophages may express a receptor for histamine. The exact nature of the T cell carrying histamine receptors is unknown but the data from the mouse and man suggest that it is the suppressor/cytotoxic T-cell subpopulation which analogous to the LY 2, 3 T lymphocyte subpopulation in mice.

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