Acta Microbiologica et Immunologica Hungarica, 58 (1), pp. 41–50 (2011)
DOI: 10.1556/AMicr.58.2011.1.5

DECREASED HORMONE CONTENT OF IMMUNE CELLS IN CHILDREN DURING ACUTE LYMPHOCYTIC LEUKEMIA (ALL) – EFFECT OF TREATMENT

ÉVA PÁLLINGER[1], ZSUZSANNA HORVÁTH[2], MÓNIKA CSÓKA[2], G. T. KOVÁCS[2] and G. CSABA[3]*

[1]Research Group for Inflammation Biology and Immunogenomics, Hungarian Academy of Sciences and Semmelweis University
[2]2nd Department of Pediatrics and [3]Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest, Hungary

(Received: 21 December 2010; accepted: 18 February 2011)

Histamine, serotonin and triiodothyronine (T_3) content of different circulating lymphocyte subsets of leukemic (acute lymphocytic leukemia, ALL) and non-leukemic (control) children were investigated by multicolor flow cytometry. The hormone contents of the cells were followed from the time of diagnosis till the end of treatment. Each hormone could be detected in every time in the investigated cell types, although the amounts of them changed during the treatment.

T lymphocytes: Significantly lower amount of serotonin was found in each T cell subsets (Th, Tc and activated T lymphocytes) of leukemic children compared to the healthy control group at the time of diagnosis and it was permanently low during the maintenance therapy. The decreased amount of serotonin could be demonstrated in Tc and Th cells even at one year after the end of treatment. However, there was no alteration in the histamine and T_3 content of T cell subsets in the time of diagnosis, but significant decrease was detected during the maintenance therapy and after treatment.

NK cells: The serotonin and T_3 contents of NK cells (both NK and NKT subsets) were significantly lower at the time of diagnosis and during the maintenance therapy. Similar decrease was detected in the case of serotonin in B cells. Although there was no difference in the T_3 content of B cells at the time of diagnosis, significantly lower amounts could be detected during the therapy compared to the healthy control group. The serotonin concentration remained low for years after the end of treatment, both in B and NK cells. These observations might have diagnostic and prognostic importance.

Keywords: acute lymphocytic leukemia, hormones, immune cells, serotonin, histamine, triiodothyronine

* Corresponding author: György Csaba MD, PhD, DSc, Professor Emeritus; Department of Genetics, Cell and Immunobiology, Semmelweis University, P.O. Box 370, H-1445 Budapest, Hungary; Phone and fax: (+36-1) 210-2950; E-mail: csagyor@dgci.sote.hu

Introduction

There is a close connection between the immune and neuroendocrine systems. Trop-hormones of the pituitary gland influence the function of immune cells, and products of other endocrine glands also have targets on the specific receptors of immune cells [1–10]. In addition, the immune cells (lymphocytes, monocytes, granulocytes and mast cells) contain hormone-like neurotransmitters and real hormones, which are also able to influence immune functions [10–13]. Thus, histamine and serotonin, which are present in immune cells, participate in normal and pathological immune reactions [1, 14–16], endorphin calms pain during inflammation and endorphin insulin, ACTH and triiodothyronine (T_3) have an immunomodulatory function [17–22]. The T_3 of the immune cells is regulated by TSH, similar to those of the thyroid gland [23]. The receptors of immune cells for hormones are identical to those of the other target cells [8].

Most of the experiments aiming to clarify the presence and function of hormones in immune cells were carried out in animals. However, these hormones are present also in human immune cells, in which histamine, serotonin, epinephrine, T_3, endorphin and ACTH were demonstrated and mapped for different cell types and subsets in healthy persons [24, 25]. The question is, whether the hormone content of human immune cells is altered or not during pathological conditions. The aim of the present experiments to evaluate the changes of hormone contents in children having acute lymphocytic leukemia (ALL) before, during and after treatment and to compare that of the control persons.

Materials and Methods

Subjects

Thirty-four children (1.5–17 years old) treated in the 2nd Dept. of Pediatrics of Semmelweis University were enrolled in the study: 6 controls (not having hematological disease), 10 in the time of ALL diagnosis, 9 during maintenance therapy (at eight months as a mean after ending the intensive therapy) and 9 after finishing the treatment (2.2 years as a mean after finishing the maintenance therapy). Children were treated according to the ALL IC-BFM2002 international protocol. Written consent for the experiments was obtained from the parents after a statement of participation was read. The study was approved by the Ethical Community of the Semmelweis University according to the Helsinki Declaration.

Blood collection

Peripheral venous blood samples were prevented from clotting using Vacutainer® Brand PLUS K2 EDTA Tubes (Becton Dickinson, San Jose, CA, USA). Collected venous blood was divided into two parts: one for the clinical investigations and the other to the research measurements.

Sample preparation for flow cytometric analysis

All the antibodies used for the characterization of different cell populations were manufactured by BD Biosciences (San Jose, Ca, USA). Erythrocytes were lysed after incubation with monoclonal antibodies, following a protocol for simultaneous cell surface and intracellular labeling (FACSLysing, BD Biosciences, San Jose, Ca, USA) [26] and leukocytes were washed with PBS. Lysed and washed cells were fixed by 4% paraformaldehyde (Sigma-Aldrich, St. Louis, Mo) and permeabilized by 0.1% saponin solution (Sigma) for staining of intracellular T_3 and serotonin. For histamine detection EDAC was used for fixation instead of paraformaldehyde. Before the measurements, cells were washed with 0.1% saponin solution and fixed by 2% paraformaldehyde solution (Sigma-Aldrich, St. Louis, Mo).

Detection of lymphocyte populations in the peripheral blood

Anticoagulated whole blood (100 µl/tube) was incubated with fluorochrome-conjugated monoclonal antibodies for 25 minutes at room temperature. Optimal amounts of antibodies were determined earlier. The antibodies were diluted according to the product description and the dilutions were controlled at the first application [19]. Fluorochrome-conjugated anti-human CD3, anti-human CD19 and anti-human CD56 antibodies were used for the determination of the frequencies of T cells, B cells and NK cells, respectively.

Cell surface expression of CD4, CD8, and HLA-DR were determined to assess the T cell subpopulations and activated T cells.

The lymphocyte gate was determined according to the size and granularity by the FS/SS (forward scatter/side scatter) dot plot. The purity of gate was detected by CD45/CD14 labeling. The lymphocyte gate was set considering the

amount of CD45+ lymphocytes (more than 95%) and the ratio of CD45+/CD14+ monocytes (less than 3%).

Parallel measurements were done. Isotype control antibodies were used for the detection of unspecific staining.

Flow cytometric analysis of the hormone content

The hormone content of permeabilized cells was detected with affinity isolated highly antigen-specific antibodies to histamine [H-7403, Lot, 51K4855], serotonin [S-5545, Lot 075K4779] and triiodothyronine (T_3, produced in rabbit, by Sigma, USA) used as primary antibody, and FITC-labelled anti-rabbit FITC-IgG (Sigma; [F-9887, Lot. 063K4816]), as secondary antibody. For controlling the specificity, autofluorescence of the cells and unspecific staining with the secondary antibodies were detected. The measurement was done with a FACSCalibur flow cytometer (Becton Dickinson, San Jose, USA), using 25,000 cells for each measurement. For the analysis of results CellQuest Pro program was used.

During the evaluation, lymphocyte populations were defined by their immunophenotype. Histamine, serotonin and T_3 content (concentration) of different cell types were compared at different time points (at the time of diagnosis, during maintenance therapy and after treatment, related to the control). The numerical comparison of detected values was done by the comparison of percentual changes of geometric mean channel values (Geo-mean). Student's *t*-test of Origin program was used for determining significance between the groups.

Results and Discussion

Considering the observation that human immune cells contain hormones, the aims of the present experiments were to examine 1) the effect of a hematological malignant disease, ALL, on this hormone level, and 2) to investigate the influence of the treatment on the hormone level. Three hormones were chosen as indices: two biogenic amines, histamine and serotonin, both having well-known important regulatory functions in the immune system [1, 14, 15], and T_3, the function of which is less known in the immune system. However, T_3 is present in each immune cell and its level is influenced similarly to the identical hormone of the thyroid gland [11, 23]. The amount of hormones was demonstrated by flow cytometry. The values obtained in samples of children with active disease, during

and after treatment were compared with each other, and to the control (hematologically healthy) children. However, not the values measured in the same patients, but that of the groups of different patients being in similar conditions were followed.

The first and perhaps the most important observation is that the amount of serotonin significantly decreased (compared to the controls) in the ALL children in each set and subset of lymphocytes (Tables II and V). This decrease sustained during maintenance therapy. Years after finishing the treatment, this decrease was also observed, except in activated T lymphocytes. On the other hand, histamine and T_3 content (Tables I, III, IV and VI) did not change at the time of diagnosis of

Table I

Histamine content of T lymphocytes in control (healthy) and acute lymphoid leukemic (ALL) children

Group	Whole T ly. (CD3+)	Tc (CD3+/CD8+)	Th (CD3+/CD4+)	Tact (CD3+HLA–DR+)
Control	262.1+/–104.4	257.3+/–106.7	264.6+/–105.0	344.1+/–96.3
ALL (before tr.)	432.3+/–260.0	403.3+/–257.2	467.8+/–260.1	463.0+/–229.1
Signif. to contr.	n.s.	n.s.	p<0.06	n.s.
During maintenance tr.	234.8+/–128.2	213.4	267.7+/–155.2	182.8+/–37.7
Signif. to contr.	n.s.	n.s.	n.s.	p<0.01
Signif. to ALL	p<0.03	p<0.03	n.s.	p<0.05
After treatment	210.9+/–118.9	217.5+/–116.4	219.5+/–112.8	305.0+/–117.3
Signif. to contr.	n.s.	n.s.	n.s.	n.s.
Signif. to ALL	p<0.05	n.s.	p<0.05	n.s.

n.s. = not significant; T = T cells; Tc = cytotoxic T cells; Th = helper T cells; Tact = activated T cells

Table II

Serotonin content of T lymphocytes in control (healthy) and acute lymphoid leukemic (ALL) children

Group	Whole T ly. (CD3+)	Tc (CD3+/CD8+)	Th (CD3+/CD4+)	Tact (CD3+HLA–DR+)
Control	1362.5+/–553.4	1362.6+/–545.7	1354.3+/–592.5	1521.2+/–816.7
ALL (before tr.)	592.1+/–220.1	575.5+/–182.4	595.1+/–200.5	486.4+/–200.7
Signif. to contr.	p<0.01	p<0.01	p<0.02	p<0.01
During maintenance tr.	387.5+/–182.1	485.5+/–306.1	434.0+/–240.6	320.1+/–155.4
Signif. to contr.	p<0.01	p<0.01	p<0.01	p<0.01
Signif. to ALL	p=0.08	n.s.	n.s.	n.s.
After treatment	574.8+/–224.7	565.0+/–203.9	573.4+/–236.7	837.2+/–68.3
Signif. to contr.	p<0.01	p<0.01	p<0.01	n.s.
Signif. to ALL	n.s.	n.s.	n.s.	p<0.01

n.s. = not significant; T = T cells; Tc = cytotoxic T cells; Th = helper T cells; Tact = activated T cells

Table III

T_3 content of T lymphocytes in control (healthy) and acute lymphoid leukemic (ALL) children

Group	Whole T ly. (CD3+)	Tc (CD3+/CD8+)	Th (CD3+/CD4+)	Tact (CD3+HLA–DR+)
Control	1104.2+/–193.7	1065.7+/–182.9	1186+/–209.6	1262.8+/–410.5
ALL (before tr.)	1076.0+/–302.5	960.4+/–259.9	1036.6+/–292.2	1128.6+/–445.5
Signif. to contr.	n.s.	n.s.	n.s.	n.s.
During maintenance tr.	552.3+/–129.1	566.6+/–142.5	568.1+/–132.0	276.4+/–84.3
Signif. to contr.	p<0.01	p<0.01	p<0.01	p<0.01
Signif. to ALL	p<0.01	p<0.01	p<0.01	p<0.01
After treatment	626.7+/–244.8	609.2+/–235.2	639.236–239.0	1064.3+/–188.1
Signif. to contr.	p<0.01	p<0.01	P<0.01	n.s.
Signif. to ALL	p<0.01	p<0.01	P<0.01	n.s.

n.s. = not significant; T = T cells; Tc = cytotoxic T cells; Th = helper T cells; Tact = activated T cells

Table IV

Histamine content of B lymphocytes and NK cells in control (healthy) and acute lymphoid leukemic (ALL) children

Group	Whole B ly. (CD19+)	NK ly. (CD3–/CD56+)	NKT ly. (CD3+/CD56+)
Control	328.7+/–204.6	266.2+/–139.5	291.6+/–121.3
ALL (before tr.)	505.4+/–313.1	352.8+/–206.1	385.8+/–226.7
Signif. to contr.	n.s.	n.s.	n.s.
During maintenance tr.	226.0+/–112.9	187.0+/–82.0	251.2+/–158.6
Signif. to contr.	n.s.	p<0.06	n.s.
Signif. to ALL	p<0.02	p<0.07	n.s.
After treatment	216.4+/–122.0	205.1+/–129.5	219.2+/–106.7
Signif. to contr.	n.s.	n.s.	n.s.
Signif. to ALL	p<0.02	p=0.08	p<0.06

n.s. = not significant; B = B cells

ALL. However, during maintenance therapy, both hormones decreased in each subset, except for histamine in Tc and Th cells. And while histamine content did not decrease after treatment, the amount of T_3 was significantly reduced, but only in the Tc and Th cells.

The presence of histamine in ALL cells was demonstrated earlier and it was supposed that histamine is an autocrine regulator of ALL cell proliferation [27]. Other authors observed histamine-induced cyclic AMP synthesis in ALL cells [28]. Considering these data, the unchanged histamine level in the ALL cells is surprising. However, there are no data on the role of T_3 in ALL, and in normal immune cells, since even the knowledge on the presence of this hormone in the immune cells is relatively new [11]. Nevertheless, it is interesting that plasma T_3

Table V

Serotonin content of B lymphocytes and NK cells in control (healthy) and acute lymphoid leukemic (ALL) children

Group	Whole B ly. (CD19+)	NK ly. (CD3–/CD56+)	NKT ly. (CD3+/CD56+)
Control	987.8+/–385.6	1096.0+/–204.5	1378.8+/–526.0
ALL (before tr.)	575.1+/–326.9	425.0+/–178.1	644.2+/–356.5
Signif. to contr.	p<0.05	p<0.01	p<0.01
During maintenance tr.	351.7+/–193.1	250.8+/–107.2	258.6+/–27.9
Signif. to contr.	p<0.01	p<0.01	p<0.01
Signif. to ALL	n.s.	p<0.03	p<0.05
After treatment	602.6+/–139.8	534.5+/–270.4	689.9+/–304.0
Signif. to contr.	p<0.05	p<0.01	p<0.01
Signif. to ALL	n.s.	n.s.	n.s.

n.s. = not significant; B = B cells

Table VI

T_3 content of B lymphocytes and NK cells in control (healthy) and acute lymphoid leukemic (ALL) children

Group	Whole B ly. (CD19+)	NK ly. (CD3–/CD56+)	NKT ly. (CD3+/CD56+)
Control	826.1+/–195.4	1189.3+/–639.5	1194.1+/–281.7
ALL (before tr.)	847.8+/–265.4	591.0+/–278.2	375.6+/–246,3
Signif. to contr.	n.s.	p<0.05	p<0.01
During maintenance tr.	355.6+/–194.2	278.2+/–170.2	412.3+/–246.3
Signif. to contr.	p<0.01	p<0.01	p<0.01
Signif. to ALL	p<0.01	p<0.02	n.s.
After treatment	683.5+/–317.3	866.5+/–604.6	1152.2+/–856.6
Signif. to contr.	n.s.	n.s.	n.s.
Signif. to ALL	n.s.	n.s.	p<0.06

n.s. = not significant; B = B cells

level decreases during chemotherapy [29] and this was found also in our studies on lymphocytes.

A decrease of the serotonin hormone content (compared to the control) was observed in each state of the investigation. However, for the other two hormones the decrease appeared only during maintenance treatment and this remained unchanged years after the end of the therapy. This indicates – and makes likely also in the case of serotonin – that the therapy causes the persistence of the hormone decrease.

The presence of serotonin in lymphocytes was demonstrated by us earlier [25]. In the present investigation the decrease of serotonin content was the most characteristic change observed of all the hormones studied. The significant de-

crease detected at the time of diagnosis points to the possibility that serotonin could serve as a diagnostic index. However, the role of serotonin in the leukemic process is not known. Still, its persistent and therapy independent decrease points to its possible role in the essential immune modulation of the leukemic process. It would be interesting to see how serotonin levels change on the long term, for example 10 years after treatment, or in relapsed patients. This way it may serve as a prognostic factor as well.

The results call attention to the selective change of cellular hormone levels during a pathological process, and points to the selective hormone response. Considering these observations, the possibility of prognostic value of hormone content is also suggested. At present, two biogenic amines and one real hormone were studied. However, this latter is also an amino-acid type simple hormone (T_3). It can be suggested that the study of more – especially peptide – hormones in ALL, which are also present in normal immune cells, can broaden our knowledge on the prognostic value of hormone levels in hematological diseases.

Acknowledgements

This work was supported by the Scientific Research Council, Ministry of Health, Hungary (083/2009). The authors thank Ms. Angela Kozák for her expert technical assistance.

References

1. Mossner, R., Lesch, K. P.: Role of serotonin in the immune system and in neuroimmune interactions. Brain Behav. Immun. **12,** 249–271 (1998).
2. Athreya, B. H., Pletcher, J., Zulian, F., Weriner, D. B., Williams, W. V.: Subset-specific effects of sex hormones and pituitary gonadotropins on human lymphocyte proliferation in vitro. Clin. Immunol. Immunopathol. **66,** 201–211 (1993).
3. Cavagnaro, J., Waterhouse, G. A., Lewis, R. M.: Neuroendocrine-immune interactions: immunoregulatory signals mediated by neurohumoral agents. Year Immunol. **3,** 228–246 (1988)
4. Carr, D. J.: Neuroendocrine peptide receptors on cells of the immune system. Chem. Immunol. **52,** 84–105 (1992).
5. Csaba, G., Kovács, P., Pállinger, É.: In vitro effect of biogenic amines on the hormone content of immune cells of the peritoneal fluid and thymus. Is there a hormonal network inside the immune system? Cell Biol. Int. **31,** 224–228 (2007).
6. Blalock, J. E.: Proopiomelanocortin and the immune-neuroendocrine connection. Ann. N. Y. Acad. Sci. **885,** 161–172 (1999).

7. Blalock, J. E., Bost, K. L., Smith, E. M.: Neuroendocrine peptide hormones and their receptors in the immune system. Production, processing and action. J. Neuroimmunol. **10,** 31–40 (1985).
8. Weigent, D. A., Blalock, J. E.: Interactions between the neuroendocrine and immune systems: common hormones and receptors. Immunol. Rev. **100,** 79–108 (1987).
9. Carr, D. J., Blalock, J. E.: From the neuroendocrinology of lymphocytes toward a molecular basis of the network theory. Horm. Res. **31,** 76–80 (1989).
10. Carr, D. J., Weigent, D. A., Blalock, J. E.: Hormones common to the neuroendocrine and immune systems. Drug Des. Deliv. **4,** 187–195 (1989).
11. Csaba, G., Kovács, P., Pállinger, É.: Immunologically demonstrable hormone-like molecules (triiodothyronine, insulin, digoxin) in rat white blood cells and mast cells. Cell Biol. Int. **28,** 487–490 (2004).
12. Csaba, G., Pállinger, É.: In vitro effect of hormones on the hormone content of rat peritoneal and thymic cells. Is there an endocrine network inside the immune system? Inflamm. Res. **56,** 447–451 (2007).
13. Musso, N. R., Brenci, S., Setti, M., Indiveri, F., Lotti, G.: Catecholamine content and in vitro catecholamine synthesis in peripheral human lymphocytes. J. Clin. Endocrinol. Metab. **81,** 3353–3357 (1996).
14. Falus, A.: Histamine: biology and medical aspects. Karger, Basel, 2004.
15. Jutel, M., Blaser, K., Akdis, C. A.: The role of histamine in regulation of immune responses. Chem. Immunol. Allergy **91,** 174–187 (2006).
16. Quiu, Y. H., Cheng, C., Dai, L., Peng, Y. P.: Effect of endogeneous catecholamine in lymphocytes on lymphocyte function. J. Neuroimmunol. **167,** 45–52 (2005).
17. Blalock, J. E.: β-endorphin in immune cells. Immunol. Today **19,** 191–192 (1998).
18. Panerai, A. E., Sacerdote, P.: β-endorphin in immune system: A role at last. Immunol. Today **18,** 317–319 (1997).
19. Csaba, G., Pállinger, É.: β-endorphin in granulocytes. Cell Biol. Int. **26,** 741–743 (2002).
20. Ottaviani, E., Franchini, A., Genedani, S.: ACTH and its role in immune-neuroendocrine functions. A comparative study. Curr. Pharm. Des. **5,** 673–681 (1999).
21. Csaba, G., Kovács, P., Pállinger, É.: Influence of in vitro and in vivo insulin treatment on the hormone (histamine, serotonin, endorphin and triiodothyronine) content of thymus and spleen cells. Life Sci. **78,** 1034–1037 (2006).
22. Chatterjee, S., Chandel, A. S.: Immunomodulatory role of thyroid hormones: In vivo effect of thyroid hormones on the blastogenic response of lymphoid tissues. Acta Endocrinol. **103,** 95–100 (1983).
23. Csaba, G., Pállinger, É.: Thyrotropic hormone (TSH) regulation of triiodothyronine T_3 concentration in immune cells. Inflamm. Res. **58,** 151–154 (2009).
24. Pállinger, É., Csaba, G.: A hormone map of human immune cells showing the presence of adrenocorticotropic hormone, triiodothyronine and endorphin in immunophenotyped white blood cells. Immunology **123,** 584–589 (2007).
25. Pállinger, É., Csaba, G.: Presence and distribution of biogenic amines (histamine, serotonin and epinephrine) in immunophenotyped human immune cells. Inflamm. Res. **57,** 530–534 (2008).
26. Robinson, J. P. (ed.): Handbook of flow cytometry methods. Purdue University Cytometry Laboratories, West Lafayette, IN, USA, 1993.
27. Malaviya, R., Uckun, F. M.: Histamine as an autocrine regulator of leukemia cell proliferation. Leuk. Lymphoma **36,** 367–373 (2000).

28. Maurer, W. M., Litos, M., Lutz, D.: Distinct histamine induced cyclic AMP synthesis in acute leukemia. Scand. J. Haematol. **37,** 438–442 (1986).
29. Santen, H. M. van, Thonissen, N. M., Kraker, J. de, Vulsma, T.: Changes in thyroid hormone state in children receiving chemotherapy. Clin. Endocrinol. **62,** 250–257.