International Journal of
HEMATOLOGY

Plasma Leptin and Leptin Receptor Expression in Childhood Acute Lymphoblastic Leukemia

Heike Wex,[a] Edita Ponelis,[a] Thomas Wex,[b] Regina Dressendörfer,[c] Uwe Mittler,[a] Peter Vorwerk[a]

[a]*Department of Paediatric Haematology and Oncology and* [b]*Clinic for Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke-University, Magdeburg;* [c]*Diagnostic Systems Laboratories, Sinsheim, Germany*

Received January 15, 2002; received in revised form June 19, 2002; accepted July 26, 2002

Abstract

Recently, leptin has been shown to play a regulatory role for differentiation within the myeloid and erythroid cell lineage, whereas results of its regulatory effects on lymphocytes and related tumor cells have been contradictory. To investigate whether leptin plays a role in acute lymphoblastic leukemia (ALL), we investigated the levels of leptin in plasma with enzyme-linked immunosorbent assays and the expression of the leptin receptor on malignant lymphoblasts with reverse transcriptase polymerase chain reaction (RT-PCR). At diagnosis, the leptin levels of bone marrow–derived plasma in children with ALL were found to be significantly lower than the levels of healthy control subjects (0.92 ± 0.79 ng/mL versus 3.01 ± 2.27 ng/mL, respectively). Notably, at complete hematologic remission (at day 33 of chemotherapy), leptin levels had normalized to 2.6 ± 2.4 ng/mL. To elucidate the underlying mechanism of this phenomenon, we analyzed the expression of the leptin receptor on the mononuclear cell populations of the patients. RT-PCR analysis revealed gene expression rates of 33% at diagnosis versus 71% at remission, compared with 100% for healthy control subjects. Results of immunohistochemical staining supported these findings by showing that the tumor clones themselves do not express the leptin receptor. Finally, some hypotheses that might explain the decrease of leptin levels in the presence of the tumor clone are discussed. *Int J Hematol.* 2002;76:446-452.

Key words: Leukemia; Leptin; Leptin receptor; Blood cells; RT-PCR; ELISA

1. Introduction

The microenvironment of the bone marrow provides the structural basis for the differentiation of bone marrow–derived hematopoietic cells. Different cell types, such as epithelial and stromal cells, adipocytes (as a structural component of the bone marrow), and precursor and mature immune cells, are involved in hematopoiesis. Recently, it was reported that leptin, a secretory protein of adipocytes, is involved in regulation of body weight and is capable of stimulating the proliferation and activation of monocytes and macrophages [1-3]. Furthermore, leptin has been shown to induce differentiation, proliferation, and activation of mature hematopoietic and $CD34^+$ stem cells [4]. Cioffi et al identified the leptin receptor on hematopoietic stem cells [5], and Nakao et al demonstrated the expression of this receptor in erythropoietic-, myeloid- and lymphoblast-derived cell lines [6], but studies of the expression of leptin receptors on lymphocytes are rare. Fantuzzi et al described a role of leptin on mature T-cells in the context of inflammation processes [7], and Martin-Romero et al reported that leptin may act as modulator of T-lymphocyte activation toward the Th1 phenotype [8]. In addition, some immune cell–derived tumor cells express the leptin receptor, suggesting a functional importance of leptin in hematopoietic differentiation and proliferation [9].

Correspondence and reprint requests: Dr. H. Wex, Otto-von-Guericke-University, Department of Paediatric Haematology and Oncology, E.-Larisch-Weg 17-19, Magdeburg D-39112, Germany (e-mail: heike.wex@medizin.uni-magdeburg.de).

To investigate the potential regulatory role of leptin for lymphoblastic cells, we analyzed leptin concentrations in bone marrow plasma and the expression of the leptin receptor on the mononuclear cell (MNC) fraction of children suffering from acute lymphoblastic leukemia (ALL).

2. Materials and Methods

2.1. Determination of Leptin

Leptin concentrations in bone marrow–derived plasma samples were analyzed by enzyme-linked immunosorbent assay (ELISA) with the Active Human Leptin Kit from Diagnostic Systems Laboratories (Sinsheim, Germany). Briefly, antileptin antibody–coated microtiter plates were loaded with 25 μL of standards, samples, or controls, and after the addition of 100 μL assay buffer, the plate was incubated at room temperature on a horizontal rocking platform (500-700 rpm) for 2 hours. The plate was washed 5 times (350 μL washing solution per well), and after the addition of 100 μL antibody-enzyme conjugate (anti–human leptin antibody conjugated to horseradish peroxidase), the plate was incubated at room temperature for 1 hour. The washing procedure was repeated, and then 100 μL substrate (3,3′,5,5′-tetramethylbenzidine) was added per well, and the plate was incubated at room temperature for 10 minutes. The reaction was terminated by adding 100 μL stopping solution (0.2M sulfuric acid), and the samples were measured with the Multiscan RT microtiter plate reader (Labsystems, Helsinki, Finland) at 450 nm within 10 minutes. Samples were measured in duplicate (average SD, 6.1%), and leptin concentrations were calculated from a standard curve of human recombinant leptin concentrations ranging from 0 to 50 ng/mL.

2.2. Separation of Mononuclear Cells

MNC were separated as previously described [10] with the standard protocol of gradient centrifugation with Ficoll-Paque (Pharmacia, Uppsala, Sweden). At the time of diagnosis, the level of leukemic cell clones in general came to 50% to 90% of the MNC fraction, whereas at remission the percentage of tumor clones was below 5%.

2.3. RNA Extraction and Reverse Transcriptase Polymerase Chain Reaction

Total RNA from MNC was extracted with QIAshredder, RNeasy, and RNase-free DNase Set (Qiagen, Hilden, Germany) following the manufacturer's protocol. The RNA was stored at –80°C until use. Total RNA (2 μg) was reverse-transcribed into complementary DNA (cDNA) with Omniscript reverse transcriptase (Qiagen) as recommended by the manufacturer. Subsequently, a cDNA aliquot (0.5 μL) was used in a Hybaid Gene Thermocycler (Hybaid, Egelsbach, Germany) to enzymatically amplify the extracellular domain of leptin receptor in a 50-μL reaction volume containing 1× reaction buffer, 1 U *Taq* (PCR Core Kit, Qiagen), and 0.2 pmol of both gene-specific primers. An initial denaturation step at 95°C for 5 minutes was followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 62°C for 1 minute, and elongation at 72°C for 40 seconds. The final elongation step was extended to 15 minutes. One fifth of the reaction mix was loaded onto a 1% agarose gel, separated by electrophoresis at 5 V/cm in Tris-acetate-EDTA buffer, and stained with ethidium bromide. The following primers were used: leptin receptor (GenBank accession No. U43168), 2204-2224 (forward) and 2846-2828 (reverse). The primers were deduced from the extracellular domain of the leptin receptor. For β-actin (GenBank accession No. M10277) the primers were 1131-1148 (forward) and 047-2038 (reverse).

The resulting polymerase chain reaction (PCR) products were 642 base pairs (bp) and 351 bp in length for the leptin receptor and β-actin, respectively.

2.4. Immunhistochemical Staining Procedure for the Detection of Leptin Receptor

Mononuclear cells (2.5×10^5 per slide) were centrifuged with a cytospin centrifuge onto slides, fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at room temperature for 10 minutes, and stored in 0.4% paraformaldehyde at 4°C for up to 4 weeks. The staining procedure was performed with an affinity-purified goat polyclonal anti–OB-receptor antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and the Vectastain ABC-AP kit (Vector, Burlingame, CA, USA) and followed the manufacturer's instructions. Briefly, the slides were washed in PBS and blocked for 20 minutes. Subsequently, the samples were incubated with the primary antibody (dilution, 1:300) at 4°C overnight, washed for 5 minutes, and incubated with the biotinylated secondary antibody for 30 minutes. Then, the slides were washed and treated with Vectastain ABC-AP reagent for 30 minutes, washed, and incubated with the substrate solution for another 30 minutes. The slides were rinsed with water, dehydrated with increasing concentrations of ethanol, treated 2 times with xylol, and, finally, mounted. Specific signals appeared red in color.

2.5. Patient Samples

The study included children with ALL. The cytometric immunophenotyping of hematopoietic malignancies was carried out in local and reference laboratories and in accordance with the consensus protocol [11]. For practical reasons the analysis of plasma leptin levels (n1 = 38) and the study of leptin receptor gene expression (n2 = 39) were performed on 2 different study groups that only partially overlapped (n = 9).

2.5.1. Study Group n1

Bone marrow plasma samples from 38 patients were obtained from different ALL-BFM (Berlin-Frankfurt-Münster) study centers (median age, 6.0 years; range, 1.2-21.9 years); diagnoses were B-cell precursor ALL (n = 10), B-cell ALL, (n = 2), T-cell ALL (n = 1), common ALL (c-ALL) (n = 23), pre–T-cell ALL (n = 1), and unknown phenotype (n = 1). None of the ALL patients received any

drugs before the first blood or bone marrow sample was taken. In accordance with the ALL-BFM 90/95 protocol of the Society of Paediatric Oncology and Haematology in Germany, bone marrow–derived plasma samples were taken on the day of diagnosis and at day 33 after the beginning of chemotherapy. The initial chemotherapy treatment included prednisone, vincristine, daunorubicin, and asparaginase. All 38 patients were in complete hematologic remission at day 33. Weight and height data for 25 of the 38 patients were available at diagnosis and at remission for the calculation of body mass indices (BMI = [weight, kg]/[height, m]^2).

2.5.2. Study Group n2

MNC of bone marrow samples were obtained from 39 patients at the day of diagnosis (median age, 6.2 years; range, 1.2-20.9 years); diagnoses were B-cell precursor ALL (n = 4), B-cell ALL (n = 3), T-cell ALL (n = 3), c-ALL (n = 23), and unknown phenotype (n = 6). At day 33, 21 samples were obtained (median age, 6.1 years; range 1.2-20.9 years); diagnoses were B-cell precursor ALL (n = 3), B-cell ALL (n = 1), T-cell ALL (n = 3), and c-ALL (n = 14).

The control group included peripheral MNC and plasma samples from 13 healthy children (median age, 7 years; range, 3-13 years).

In adherence with the guidelines of the Ethics Committee, informed consent was obtained from the participating patients or their parents.

3. Results

3.1. Plasma Leptin Levels in the Bone Marrow and Peripheral Blood

The concentration of leptin was analyzed at diagnosis of ALL and at complete hematologic remission (day 33 of chemotherapy treatment). Leptin concentrations on average were 0.92 ± 0.79 ng/mL (range, 0.14-3.39 ng/mL) at diagnosis and 2.6 ± 2.4 ng/mL (range, 0.65-11.62 ng/mL) at day 33. The 2.8-fold increase between these values was found to be significant ($P < .01$, t test). Furthermore, analysis of individuals revealed that 32 of 38 patients showed an increase in their plasma leptin levels at remission, compared with the level at diagnosis (group I, Figure 1). Five of the remaining 6 patients (83%) with a decrease in the leptin level at remission (group II) already had revealed at diagnosis a leptin concentration above the average of 0.92 ng/mL, whereas only 7 of 32 patients from group I had leptin levels above 0.92 ng/mL. Both groups had significant differences in leptin level at diagnosis ($P < .05$, t test). We also found a significant higher risk of meningeosis ($P < .02$, t test) in group II. There were no significant differences between the groups regarding relapse risk, age, sex, phenotype, or BMI.

Leptin concentrations of healthy donors averaged 3.01 ± 2.27 ng/mL (range, 0.9-9.1 ng/mL) and were consequently in the same range as the plasma of children with ALL at complete remission.

3.2. Calculation of BMI

BMI was calculated according to the formula: BMI = (weight, kg)/(height, m)^2. The average BMI at diagnosis was 16.8 and was 16.5 at remission. Seven of 25 patients had a slightly higher BMI at remission than at diagnosis, whereas 18 of 25 showed a decrease in BMI during initial chemotherapy that was caused by weight reduction. Most patients displayed a lower BMI at remission. Overall, no significant difference was found between the BMI at both time points ($P < .46$, t test) (Figure 2).

3.3. Expression of the Leptin Receptor on MNC

To investigate further the potential functional role of leptin for the tumor clone, we analyzed a second study group

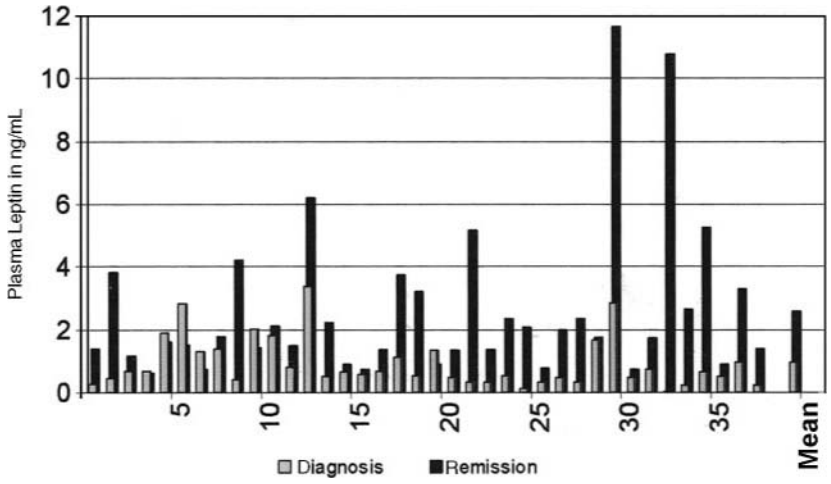

Figure 1. Leptin concentrations (ng/mL) of bone marrow–derived plasma samples from patients with acute lymphoblastic leukemia at the day of diagnosis (gray) or at day 33 (black). Average leptin concentrations (rightmost columns) were 0.92 ± 0.79 and 2.6 ± 2.4 ng/mL on the day of diagnosis and day 33, respectively (n = 38; $P < .01$, Student t test).

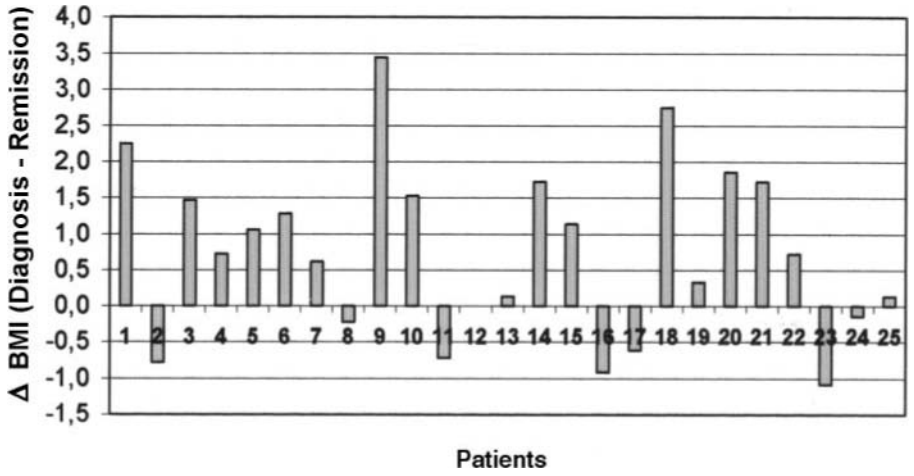

Figure 2. Differences in body mass index (BMI) (BMI at diagnosis – BMI at remission) from 25 patients. Seven of 25 patients displayed a negative BMI difference, ie, a higher BMI at remission than at diagnosis. Because of weight reduction during initial chemotherapy treatment, most patients had a lower BMI at remission than at diagnosis. The differences were not significant (Student *t* test, $P < .46$).

for the gene expression of leptin receptor on bone marrow–derived MNC. Leptin receptor gene expression was analyzed in MNC samples from a total of 51 ALL patients. Because the limited amounts of material were used primarily for diagnostic purposes, this study group was heterogeneous, and the corresponding materials were only available in part. Specifically, the 51 samples were as follows: at diagnosis, n = 39; at remission, n = 21; and for samples at both time points, n = 9.

Analysis by reverse transcriptase polymerase chain reaction (RT-PCR) of 39 samples obtained at diagnosis revealed gene expression of the leptin receptor for 33% (13 of 39 patients) of these samples (results shown in part in Figure 3A). The 21 samples obtained from ALL patients at remission showed a higher leptin receptor expression rate of 71% (15 of 21; Figure 3B). This increase is supported by individual analysis of the 9 patients from whom we received samples at both time points (Figure 3D). Here, samples from 4 of 9 patients showed a consistently positive or negative receptor expression at diagnosis and in remission. Another 4 of 9 of the MNC samples were found to be negative for receptor expression at diagnosis but expressed the leptin receptor gene at remission, and 1 patient had the opposite result.

RT-PCR analysis of 13 peripheral MNC samples obtained from healthy probands revealed gene expression of the leptin receptor for all 13 samples (Figure 3C).

3.4. Immunohistochemical Analysis of the Leptin Receptor on Peripheral MNC Samples of ALL Patients

On the basis of the RT-PCR analysis, we concluded that the tumor clone probably does not express the leptin receptor. To confirm this assumption, we further analyzed leptin receptor expression with immunohistochemistry. As a representative sample shows (Figure 4), leukemic blasts did not express the leptin receptor, whereas normal lymphocytes exhibited strong staining in the cytoplasm and on the cell surface. Immunohistochemical analysis was performed on samples from 3 patients with ALL and revealed identical results for all experiments. Staining of control samples (without primary antibody) did not reveal any signals (data not shown).

3.5. Statistical Analysis

All statistical analyses were done by the *t* test and analyzed with SPSS software version 10 (SPSS, Chicago, IL, USA). *P* values below .05 were considered significant.

4. Discussion

Leptin, a secretory protein, is produced mainly by the adipocytes of fat tissue. Furthermore, stromal cells and other cell types are able to secrete this protein. Leptin mediates its regulatory functions through interactions via its receptor. Although leptin is not produced by immune cells themselves, it stimulates monocytes and macrophages [1,2]. There are several reports of hematopoietic progenitor cells, as well as some subsets of immune cells, expressing the leptin receptor, and these findings imply a physiological role for leptin during expansion and differentiation of these cells [4,5,12]. This conclusion suggests that leptin is involved in the leukemogenic process.

To investigate the potential regulatory role of leptin in childhood leukemia, we analyzed the plasma levels of leptin and at the time of diagnosis found them to be significantly decreased in the bone marrow–derived samples of 38 children. Interestingly, these reduced levels returned to normal levels after 33 days of chemotherapy, and this change was accompanied by the complete hematologic remission of all patients. The fact that analysis of individual patients revealed this normalization in 32 of 38 patients led us to infer that this phenomenon may be a general one. Furthermore, there was a significantly higher risk of meningeosis in the patient group without an increase of plasma leptin level at day 33. However, because 5 of these 6 patients had leptin levels higher

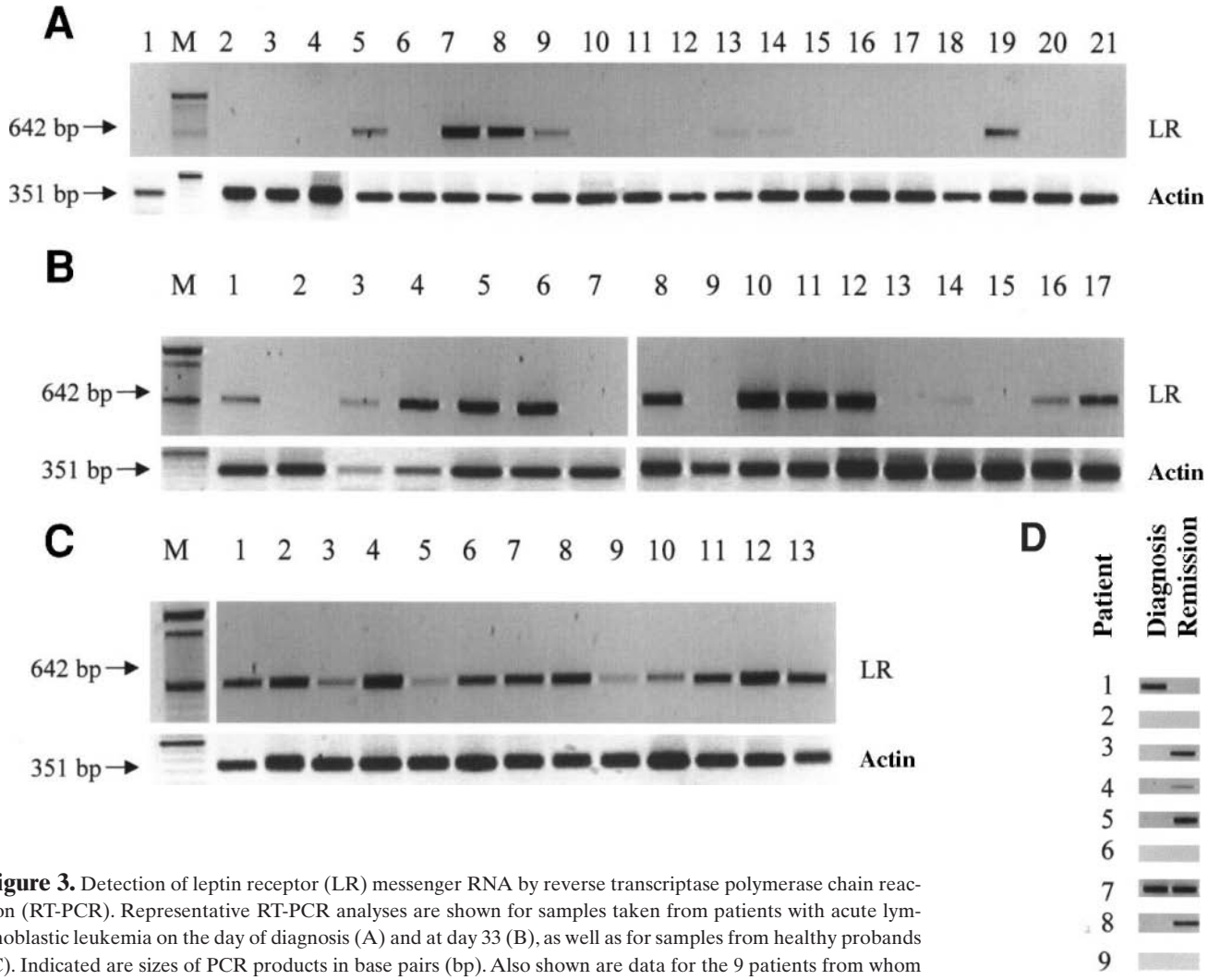

Figure 3. Detection of leptin receptor (LR) messenger RNA by reverse transcriptase polymerase chain reaction (RT-PCR). Representative RT-PCR analyses are shown for samples taken from patients with acute lymphoblastic leukemia on the day of diagnosis (A) and at day 33 (B), as well as for samples from healthy probands (C). Indicated are sizes of PCR products in base pairs (bp). Also shown are data for the 9 patients from whom bone marrow-derived samples were available for both time points (D).

than the average concentration of 0.92 ng/mL, we believe that this finding may be the reason for the loss of leptin normalization after 33 days of chemotherapy. The observation that most patients did have an increase in leptin level, as well as the fact that the leptin levels in the peripheral plasma of healthy children were similar to those found in the samples from children in remission, suggests a direct link between the presence of the tumor clone and the decreased levels of leptin.

It is well known that leptin levels depend on BMI. Because this parameter did not change significantly in our patients during initial chemotherapy treatment, it is probably not the cause for the changes in plasma leptin level.

The expansion of the tumor clone could lead to the enhanced binding of the free plasma leptin via receptor interaction and to a consequent decline of peripheral leptin level. Considering the fact that leukemic cell clones can reach cell numbers of up to 10^{12} at the time of diagnosis, we think it likely that the interaction between leptin and its corresponding receptor could cause the substantial decrease in peripheral leptin levels. Notably, diminished leptin levels have been described in this context for other tumors, such as lung carcinoma [13] or colorectal cancer [14], that have been shown to express the leptin receptor. To investigate this hypothesis, we studied the gene expression of the leptin receptor in the MNC fraction by RT-PCR. It is notable that the leptin receptor exists as several isoforms that differ only in their intracellular parts [5]. Because we were interested in studying the presence of a potential leptin-binding structure, we used PCR primers that correspond to a fragment of the extracellular domain that is identical in all isoforms. Because the tumor clone usually represents most of the cells within the MNC fraction at the time of diagnosis (50%-90%), one would expect positive RT-PCR results in all samples at this time if the tumor clones express the leptin receptor. The low expression rate of about 33% detected at diagnosis, however, strongly implies that ALL clones do not generally express this receptor. The findings that the leptin receptor expression rate of children in remission was significantly higher (71%) and that the expression rate was even 100% in healthy probands indicated that normal immune cells do express the leptin receptor. Therefore, the result of a 33%

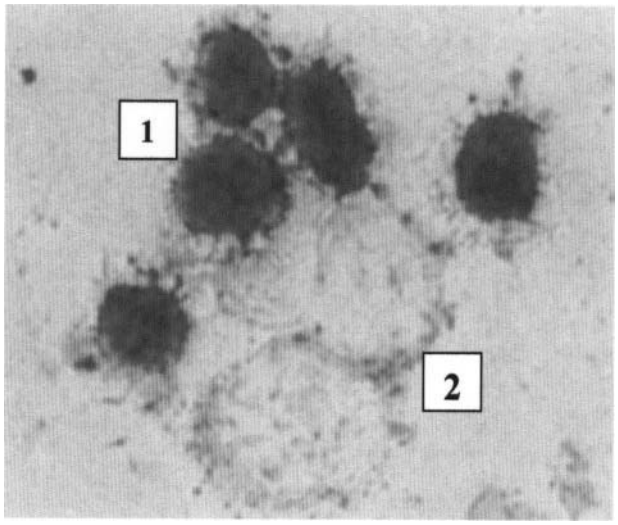

Figure 4. Immunohistochemical staining for the leptin receptor for a mononuclear cell fraction obtained from the peripheral blood of a patient with acute lymphoblastic leukemia. The level of leukemic blasts in this sample was about 50% (1, normal lymphocyte; 2, leukemic blast).

expression rate at the time of diagnosis most likely originated from these cells within the MNC sample. This assumption is further supported by the immunohistochemical analysis that showed negative results for receptor staining for ALL blasts, whereas other immune cells such as lymphocytes expressed considerable amounts of the leptin receptor. Because we did not purify the ALL clones, we cannot completely rule out the possibility that ALL clones can express the leptin receptor in some cases; however, in general they do not. This observation is supported by other studies [6,15]. Notably, Konopleva et al [15] demonstrated the receptor on samples of acute myeloid precursors but found the leptin receptor on samples of chronic or acute lymphocytic leukemia only at a very low level. Nakao et al [6] described the expression of the leptin receptor in 4 of 15 ALL samples. However, both studies did not consider the possibility that the detected level of leptin receptor gene expression could partially or completely originate from the presence of normal immune cells in the sample.

Indirect regulatory effects mediated by cytokines secreted from the tumor clone can lead to the repression of leptin expression by adipocytes and stromal cells. It is known that proinflammatory cytokines such as IL-1β, IL-6, tumor necrosis factor α, and immune interferon (IFN-γ) inhibit the expression of leptin by bone marrow adipocytes [9] and by human subcutaneous and omental adipocytes [16,17]. These cytokines are overexpressed in cancer cells, and this response leads to cachexia, a wasting syndrome attributed to loss of appetite and elevated energy expenditure [18-20].

The fact that at diagnosis the immense presence of the tumor clone leads to a decrease of other leptin-secreting cell components of the bone marrow (adipocytes, stromal cells) and subsequently to a lower leptin concentration of the bone marrow at diagnosis may explain the increase of leptin levels during initial chemotherapy. The normalization of leptin concentrations at remission could reflect the change in cell population. However, adipocytes and stromal cells in the bone marrow are only a small fraction of leptin-producing cells in the whole body. The low leptin concentrations detected in bone marrow at the time of diagnosis probably reflect the general impoverishment of leptin in the peripheral circulation.

Furthermore, that the patients received 33 days of prednisone therapy as one part of their treatment has to be considered. There are several reports that steroids influence leptin expression. Some investigators have indicated that administration of corticosteroids increases serum leptin levels [21,22] and leptin gene expression in organ cultures from human adipose tissue [23]. Others have reported no direct influence of intravenously administered [24] or inhaled glycocorticoids [25] on serum leptin levels. The discussion is still controversial. Because the leptin levels in our patients were normalized and not elevated after initial chemotherapy, it is unlikely that prednisone caused the increase in serum leptin levels. Taken together, the data of the study revealed a decrease in the leptin levels in the bone marrow of children with ALL at the time of diagnosis, as well as a low leptin receptor expression rate in the corresponding MNC fractions. At remission, the values of both parameters had become normalized. Furthermore, it was shown that the ALL clones in general do not express the leptin receptor.

Although the underlying mechanism for this phenomenon still needs to be discovered, some potential explanations can be ruled out. On the basis of the evidence of this study, changes in BMI and leptin receptor–leptin interactions on leukemic blasts do not explain the decreased serum leptin levels in children with ALL. Furthermore, an evaluation of the current literature implies that neither numeric changes within the bone marrow cell population nor the administration of prednisone represents a solid basis for explaining the phenomenon. The hypothesis that cytokines secreted by the tumor clones themselves lead to down-regulation of leptin expression is a possible one. It may be useful to study the cytokine pattern of ALL clones and to analyze the effect of these cytokines on leptin expression.

Acknowledgments

This work was supported by the Magdeburger Förderkreis krebskranker Kinder e.V. and the W. A. Drenkmann Foundation. We thank Beatrix Kramer, Ruth-Hilde Hädicke (Department of Paediatric Haematology and Oncology) and Ursula Stolz (Clinic of Gastroenterology) for their technical assistance. We also thank all participating hospitals of the BFM study group for sending the patient samples.

References

1. Santos-Alvarez J, Goberna R, Sanchez-Margalet V. Human leptin stimulates proliferation and activation of human circulating monocytes. *Cell Immunol*. 1999;194:6-11.
2. Gainsford T, Willson TA, Metcalf D, et al. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. *Proc Natl Acad Sci U S A*. 1996;93:14564-14568.
3. Umemoto Y, Tsuji K, Yang FC, et al. Leptin stimulates the prolif-

eration of murine myelocytic and primitive hematopoietic progenitor cells. *Blood*. 1997;90:3438-3443.
4. Bennett BD, Solar GP, Yuan JQ, Mathias J, Thomas GR, Matthews W. A role for leptin and its cognate receptor in hematopoiesis. *Curr Biol*. 1996;6:1170-1180.
5. Cioffi JA, Shafer AW, Zupancic TJ, et al. Novel B219/OB receptor isoforms: possible role of leptin in hematopoiesis and reproduction. *Nat Med*. 1996;2:585-589.
6. Nakao T, Hino M, Yamane T, Nishizawa Y, Morii H, Tatsumi N. Expression of the leptin receptor in human leukaemic blast cells. *Br J Haematol*. 1998;102:740-745.
7. Fantuzzi G, Faggioni R. Leptin in the regulation of immunity, inflammation, and hematopoiesis. *J Leukoc Biol*. 2000;68:437-446.
8. Martin-Romero C, Santos-Alvarez J, Goberna R, Sanchez-Margalet V. Human leptin enhances activation and proliferation of human circulating T lymphocytes. *Cell Immunol*. 2000;199:15-24.
9. Laharrague P, Truel N, Fontanilles AM, Corberand JX, Penicaud L, Casteilla L. Regulation by cytokines of leptin expression in human bone marrow adipocytes. *Horm Metab Res*. 2000;32:381-385.
10. Wex H, Vorwerk P, Mohnike K, et al. Elevated serum levels of IGFBP-2 found in children suffering from acute leukaemia is accompanied by the occurrence of IGFBP-2 mRNA in the tumour clone. *Br J Cancer*. 1998;78:515-520.
11. Rothe G, Schmitz G. Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies: Working Group on Flow Cytometry and Image Analysis. *Leukemia*. 1996;10: 877-895.
12. Gainsford T, Alexander WS. A role for leptin in hemopoieses? *Mol Biotechnol*. 1999;11:149-158.
13. Lopez-Soriano J, Carbo N, Tessitore L, Lopez-Soriano FJ, Argiles JM. Leptin and tumor growth in rats. *Int J Cancer*. 1999;81:726-729.
14. Tessitore L, Vizio B, Jenkins O, et al. Leptin expression in colorectal and breast cancer patients. *Int J Mol Med*. 2000;5:421-426.
15. Konopleva M, Mikhail A, Estrov Z, et al. Expression and function of leptin receptor isoforms in myeloid leukemia and myelodysplastic syndromes: proliferative and anti-apoptotic activities. *Blood*. 1999;93:1668-1676.
16. Gottschling-Zeller H, Birgel M, Scriba D, Blum WF, Hauner H. Depot-specific release of leptin from subcutaneous and omental adipocytes in suspension culture: effect of tumor necrosis factor-alpha and transforming growth factor-beta1. *Eur J Endocrinol*. 1999;141:436-442.
17. Fawcett RL, Waechter AS, Williams LB, et al. Tumor necrosis factor-alpha inhibits leptin production in subcutaneous and omental adipocytes from morbidly obese humans. *J Clin Endocrinol Metab*. 2000;85:530-535.
18. Kurzrock R. The role of cytokines in cancer-related fatigue. *Cancer*. 2001;92(suppl):1684-1688.
19. Tisdale MJ. Wasting in cancer. *J Nutr*. 1999;129(suppl 1S): 243S-246S.
20. Matthys P, Billiau A. Cytokines and cachexia. *Nutrition*. 1997;13: 763-770.
21. Lerario DD, Ferreira SR, Miranda WL, Chacra AR. Influence of dexamethasone and weight loss on the regulation of serum leptin levels in obese individuals. *Braz J Med Biol Res*. 2001;34: 479-487.
22. Nishiyama M, Makino S, Suemaru S, et al. Glucocorticoid effects on the diurnal rhythm of circulating leptin levels. *Horm Res*. 2000; 54:69-73.
23. Masuzaki H, Ogawa Y, Hosoda K, et al. Glucocorticoid regulation of leptin synthesis and secretion in humans: elevated plasma leptin levels in Cushing's syndrome. *J Clin Endocrinol Metab*. 1997;82: 2542-2547.
24. Laferrere B, Fried SK, Hough K, Campbell SA, Thornton J, Pi-Sunyer FX. Synergistic effects of feeding and dexamethasone on serum leptin levels. *J Clin Endocrinol Metab*. 1998;83:3742-3745.
25. Heuck C, Wolthers OD. Serum leptin in children with asthma treated with inhaled budesonide. *Respir Med*. 1999;93:268-271.