Adsorptive depletion of blood monocytes reduces the levels of circulating interleukin-17A in Langerhans cell histiocytosis

Magda Lourda,1,2 Selma Olsson-Åkefeldt,1 Désirée Gavhed,1 Ulla Axedorph Nygell,3 Gösta Berlin,4 Evaldas Laurencikas,5 Tatiana von Bahr Greenwood,1 Mattias Svensson,2 and Jan-Inge Henter1

1Childhood Cancer Research Unit, Department of Women’s and Children’s Health, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden; 2Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden; 3Center for Apheresis and Stem Cell Processing, Clinical Immunology/Transfusion Medicine, Karolinska University Hospital and Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden; 4Department of Clinical Immunology and Transfusion Medicine and Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden; 5Division of Radiology, Department of Clinical Sciences, Karolinska Institutet, Danderyd Hospital, Stockholm, Sweden.

Correspondence: Magda Lourda, PhD
Center for Infectious Medicine, F59, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Huddinge, 141 86 Stockholm, Sweden.
e-mail: magdalini.lourda@ki.se, telephone: +46 858589688, fax: +46 87467637.

and

Selma Olsson-Åkefeldt, MD, PhD (for clinical questions)
Childhood Cancer Research Unit, Q6:05, Astrid Lindgren Children’s Hospital, 171 76
LOURDA et al

MONOCYTE APHERESIS IN LCH

Stockholm, Sweden.

e-mail: selma.olsson-akefeldt@karolinska.se, telephone: +46 707332005.

Text word count: 1200 words

Figures: 2; References: 16; Supplemental data: Materials and methods
To the Editor:

Langerhans cell histiocytosis (LCH) has been described as an inflammatory myeloid neoplasia that affects various organs. CNS involvement may result in endocrinopathies and progressive neurodegeneration (ND-LCH), characterized by low-grade inflammation and degenerative changes, resulting in cognitive deficits, behavioral disturbances and neuromotor dysfunction. Prolonged disease activity is a risk factor for ND-LCH. Potential biomarkers in cerebrospinal fluid (CSF) indicative of ND-LCH have been suggested (e.g. neurofilament protein light chain; NF-L), while increased interleukin(IL)-17A levels in blood and extracranial lesions of LCH patients have been associated with local and systemic inflammation. Independently, IL-17A has been shown to disrupt the blood-brain-barrier and increase production of reactive oxygen species in brain endothelial cells, pointing towards a crucial step for the development of experimental autoimmune encephalitis and possibly also for ND-LCH.

There is currently no established therapy for ND-LCH, although many have been proposed. We aimed to find an effective therapy for a child with severe, rapidly progressive ND-LCH unresponsive to standard treatments. The patient was followed clinically (for >15 years), by MRI and by measuring NF-L in the CSF and later also IL-17A in CSF and plasma.

Briefly, the patient was diagnosed with LCH at five months of age, which rapidly progressed into multisystem LCH with risk-organ involvement. Following initial treatment (LCH-II protocol) she stabilized but after several reactivations she developed a chronic active disease with elevated erythrocyte sedimentation rate (ESR). At four
years of age, clinical signs of ND were noted (tripping, clumsiness) and brain MRI a year later revealed widespread ND-LCH affecting the basal ganglia, corpus callosum, cerebellum and medulla. Despite many treatments, the patient deteriorated markedly within the next four years. Prompted by the need for effective treatment, at the age of eight years we initiated granulocyte-and-monocyte-apheresis (GMA), initially in parallel with conventional LCH treatment (Figure 1; at week 0), aiming at removing activated granulocytes and monocytes, thus reducing the inflammatory load.10

GMA was initially performed weekly for ten weeks, followed by clinical improvement, decreased NF-L and regression of cerebellar lesions. After a ten-week break, GMA was restarted and performed every second week (eleven sessions). During a subsequent four-month break, higher NF-L levels were noted and GMA was restarted but performed less frequently and with less noticeable benefits. Therefore, her treatment was replaced with a combination of vinblastine and simvastatin (without GMA) to benefit from the anti-inflammatory effect of simvastatin. However, the patient deteriorated the following six months with more fatigue, impaired balance and gait, and increased NF-L (Figure 1A). Nystagmus was confirmed a few months later. Given the positive effects of the first GMA treatments, GMA was restarted (w115). Cytarabine was given in parallel for six months based on positive reports from others.11 Due to absence of clinical improvement, lack of alternative treatment and considering the positive effects of natalizumab on patients with multiple sclerosis (MS),12 we then administered natalizumab after thorough discussions with neurologists and the parents. GMA continued once every four weeks. Overall the patient tolerated the treatment well. After six months of natalizumab treatment NF-L levels were lower, but at the one-year-follow-up NF-L had increased again (Figure 1B, w195 and w216,
respectively), walking had deteriorated and ESR indicated active inflammation (Figure 1A). Due to lack of improvement and increased risk of progressive multifocal leukoencephalopathy with prolonged treatment, natalizumab was ceased.

Attracted by the lack of side-effects from GMA in this patient who had not responded to other treatments, GMA was now (w220) intensified to once weekly in parallel to low corticosteroid pulses (initially every 2w, later every 3-4w) for more than two years. NF-L decreased during this period, and the neurological deterioration eventually slowed down; the patient stabilized clinically and MRI remained stable. In addition, but most apparent initially, positive effects were noted on the patient’s fatigue, possibly due to the marked decrease in circulating cytokine levels (Figures 1B-G).

The impact of GMA over time on IL-17A in CSF, in plasma and inside blood monocytes is illustrated in Figures 1B-D, respectively. The methods used are described in the Supplemental data. When GMA was performed weekly, both the plasma IL-17A levels (Figure 1E) and the percentage of IL-17A+ monocytes in blood (Figure 1F) tended to be lower, compared to less frequent treatment, but the influence of spontaneous improvement or parallel treatments on this outcome cannot be ruled out. A remarkable reduction was observed in IL-17A and IL-23 plasma levels during the last years (Figure 1G, w326) while ESR remained slightly elevated. The increased IL-17A production on w242 was accompanied by increased ESR and IL-23 levels, maybe due to subclinical infection. Stable low IL-17A plasma levels for the last two years and apparent neurological stabilization suggested less value from future GMA. Because of this and emerging difficulties with vascular access, GMA was stopped on w333 after 169 sessions. At 6- and 18-month follow-up after GMA termination, NF-L
and IL-17A levels in CSF remained low (Figure 1B) as did plasma levels of IL-17A and IL-23 (Figure 1G, w416).

Analysis of blood samples before and immediately after GMA showed 50% reduction in the percentage of circulating IL-17A⁺ monocytes in the patient’s blood immediately after treatment (Figure 2A). Notably, up to 90% of peripheral blood mononuclear cells captured in the GMA-column were IL-17A⁺ monocytes (Figure 2B) and a positive correlation was observed between plasma IL-17A levels and percentage of IL-17A⁺ monocytes in the GMA-column (Figure 2C), as well as between IL-17A levels in plasma and CSF (Figure 2D).

Although LCH pathogenesis remains unclear, increased IL-17A production has been associated with higher disease activity. Our observation that high IL-17A levels in blood and CSF accompany the disease progression supports a possible role for IL-17A in ND-LCH pathogenesis. Notably, when IL-17A levels decreased, NF-L levels also decreased and the patient’s fatigue improved. Measurement of IL-17A plasma levels in three additional LCH patients described to have isolated CNS involvement also revealed significantly increased IL-17A plasma levels (394, 369 and 234 pg/ml, with corresponding ESR of 3, 5, and 26 mm/hr, respectively) compared to 28 controls (median IL-17A: 120 pg/ml, p<0.01, Mann-Whitney). In line, high IL-17A and IL-23 levels have been reported in serum and CSF of patients with amyotrophic lateral sclerosis, a progressive neurodegenerative disease.

Only a few children with either acute relapse of MS, neuromyelitis optica or acute disseminated encephalomyelitis have been reported to receive short-term apheresis
Recently, short-term GMA has also been beneficial in sarcoidosis. To the best of our knowledge, our study is the first in which GMA has been used regularly for long-term (>6 years) and, importantly, without major side-effects. The treatment removed cytokine-producing cells from blood and decreased the levels of IL-17A in plasma and CSF. Possibly, the number of cytokine-producing cells in tissues was also reduced. Since various treatments were administered and the natural development of the neurodegenerative process is not well known, where some patients stabilize with age, we cannot be certain if, and if so to what extent, GMA contributed to the stabilization of our patient. Nevertheless, our study suggests that (long-term) GMA may be considered for experimental treatment in patients with systemic LCH, aiming to reduce hypercytokinemia and disease activity. Finally, our novel finding that IL-17A may be used as an additional CSF biomarker for ND-LCH provides an additional method for detecting and monitoring ND-LCH.
Acknowledgments

The authors would like to thank Dr. Ingrid van’t Hooft Hagberg at Karolinska Institutet for providing detailed information on the neuropsychological evaluations of the patient, Dr. Anna Fogdell-Hahn at Karolinska Institutet for providing control CSF samples, the Clinical neurochemistry lab in Gothenburg for performing the NF-L analyses and Puran Chen at Karolinska Institutet for helping illustrate the time-line changes of the patient’s condition.

This work was supported by grants from the Swedish Children’s Cancer Foundation, the Swedish Research Council, Karolinska Institutet and the Stockholm County Council (ALF project) to JIH and MS, the Cancer and Allergy Foundation of Sweden to JIH and Karolinska Institutet to ML. ML also received post-doc scholarships from Karolinska Institutet, Mary Béve Foundation and Märta and Gunnar V Philipsons Foundation.

Authorship

Contribution: ML designed and performed the experiments, analyzed the data, interpreted the results and prepared the figures; SOÅ cared for the patient, provided blood samples and clinical data and contributed to interpretation of the results and figure layout; DG arranged for NF-L measurements in CSF, recorded the neurological status of the patient over time and contributed to interpretation of the results and figure layout; UAX supervised the GMA treatments in Stockholm; GB supervised the GMA treatments in Linköping and retrieved the ESR values for that period; EL reviewed the MRI examinations of the patient; TvBG cared for the patient and provided blood and CSF samples; MS supervised the study and provided feedback on the experimental set-
up, the analysis and interpretation of the results and figure layout; JIH cared for the patient, initiated all the treatments described in the manuscript, provided blood and CSF samples, contributed to interpretation of the results and generally supervised the study; ML and SOÅ wrote the manuscript and all authors contributed to editing and finalizing the manuscript.

Conflict-of-interest disclosure: The authors have no competing financial interests.

Correspondence: Magda Lourda, Center for Infectious Medicine, F59, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Huddinge, 141 86 Stockholm, Sweden; e-mail: magdalini.lourda@ki.se; and for clinical questions Selma Olsson-Åkefeldt, Childhood Cancer Research Unit, Q6:05, Astrid Lindgren Children’s Hospital, 171 76 Stockholm, Sweden; e-mail: selma.olsson-akefeldt@karolinska.se.
References


10. Cortese I, Chaudhry V, So YT, Cantor F, Cornblath DR, Rae-Grant A.


Figure 1. Granulocyte-and-monocyte-apheresis (GMA) treatment resulted in decreased inflammation in blood and CSF. (A) Description of disease status in relation to age and treatments over time. GMA (int) indicates the interval of GMA in weeks for the indicated period, as specified by the numbers inside the boxes in the GMA row. GMA (week) indicates the number of weeks from GMA onset (0), specified by the numbers in italics below the boxes. For MRI, ND and NF-L the dashes indicate unchanged status compared to previous examination. The arrowheads pointing down indicate regression/decreased levels compared to the previous examination, while arrowheads pointing up indicate increased levels compared to the previous examination. The dashed lines next to each treatment indicate the treatment period. (B) NF-L and corresponding IL-17A protein levels in CSF. Results from only one NF-L ELISA kit (from week 109 onwards) are shown for simplicity due to different reference values and sensitivity of the two NF-L ELISAs used (see Supplemental data). (C-D) IL-17A protein levels in plasma (C) and the percentages of IL-17A+ monocytes in PBMCs (D) measured from week 115 onwards. The interval of GMA in weeks, is indicated in the boxes on top of the graphs in B-D. The dotted line indicates the mean value of controls (healthy) and the dashed line indicates + 3 SD from control mean. (E-F) IL-17A protein levels in plasma (E) and percentage of IL-17A+ monocytes in PBMCs (F) in relation to the frequency of GMA. (G) Relative changes in plasma levels of IL-17A and IL-23 on weeks 326 (7 weeks before GMA termination) and 416 (18 months after GMA termination) as compared to week 115. DI, diabetes insipidus; ESR, erythrocyte sedimentation rate; MRI: magnetic resonance imaging; PPBS: Posterior pituitary bright spot; NF-L: neurofilament protein light chain; CST, corticosteroids; 6-MP, 6-mercaptopurine. The ESR values are presented as the mean value for the
indicated period.

**Figure 2. GMA successfully removed IL-17A-producing monocytes from blood.**

(A) Contour plots of peripheral blood mononuclear cells (PBMCs) that were isolated from the patient’s blood on week (w) 159 before and immediately after GMA were stained intracellularly with an anti-IL-17A antibody in combination with surface marker staining for monocytes (CD14). (B) Histograms of the cells that were trapped in the GMA-column on week 159 and stained with antibodies for either IL-17A (black line) or Alexa 647 isotype (grey area). The percentage refers to IL-17A⁺ cells among PBMCs. (C) Correlation between plasma IL-17A levels and percentage of IL-17A⁺ cells trapped in the GMA-column. (D) Correlation between IL-17A levels in CSF and plasma. CSF was collected 1-2 times/year and mostly on occasions separate from when plasma was collected, therefore graph D includes only 5 time points: One time point following a long break in GMA (i), three time points with plasma and CSF collection at maximum one week apart (ii-iv), and one time point ≥6 months after GMA termination (v).
Figure 2

A

CD14

IL-17A

B

IL-17A

89.1%

C

IL-17A plasma levels (pg/ml)

% IL-17A+ cells trapped in GMA column

D

IL-17A levels in CSF (pg/ml)

IL-17A levels in plasma (pg/ml)

r^2 = 0.7

w159

w129

w224

w326

r^2 = 0.8

(i) (ii) (iii) (iv) (v)

Week of sample collection

CSF

Plasma

159 240

263 320 368

115 242 262 340 416

(i) (ii) (iii) (iv) (v)
Adsorptive depletion of blood monocytes reduces the levels of circulating interleukin-17A in Langerhans cell histiocytosis

Magda Lourda, Selma Olsson-Åkefeldt, Désirée Gavhed, Ulla Axdorph Nygell, Gösta Berlin, Evaldas Laurencikas, Tatiana von Bahr Greenwood, Mattias Svensson and Jan-Inge Henter