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Natalizumab treatment in multiple sclerosis: Marked decline of chemokines and cytokines in cerebrospinal fluid.

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Abstract

Background

Natalizumab exerts impressive therapeutic effects in patients with multiple sclerosis (MS). The proposed main mode of action is reducing transmigration of leukocytes into the CNS, but other immunological effects may also be operative. Cytokines and chemokines are involved in the regulation of inflammatory responses and may reflect the disease process in MS.

Objective

To study the effects of natalizumab treatment on cytokine and chemokine profiles systemically and intrathecally in multiple sclerosis.

Methods

We used luminex to analyse a panel of cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNF-α, IFN-γ, GM-CSF) and chemokines (CXCL9, CXCL10, CXCL11, CCL17, CCL22) in blood and cerebrospinal fluid (CSF) from 31 patients with relapsing MS before and after one year of natalizumab treatment.

Results

There was a marked decline in CSF levels of cytokines and chemokines, thus including pro-inflammatory cytokines (IL-1β, IL-6 and IL-8) as well as chemokines associated with both Th1 (CXCL9, CXCL10, CXCL11) and Th2 (CCL22). Circulating plasma levels of some cytokines (GM-CSF, TNF-α, IL-6 and IL-10) also decreased after one year of treatment.

Conclusions

This is the first study to show that natalizumab treatment is associated with a global decline in cytokine and chemokine levels at a protein level. This finding was most pronounced in CSF, in line with the reduced transmigration of cells into CNS, whereas reduction in plasma levels indicates other possible mechanisms of natalizumab treatment.

Introduction

Migration of immune cells over the blood-brain-barrier is an important step in the inflammatory process of multiple sclerosis (MS), a chronic autoimmune disease of the central nervous system (CNS). Natalizumab, a humanised mouse monoclonal antibody against the integrin very late activation antigen (VLA)-4 on leukocytes, blocks attachment of activated T-cells to brain endothelial cells and thereby prevents their entrance into the CNS [1,2]. Consequently, a decrease in the number of cells in the cerebrospinal fluid (CSF) has been observed in patients with MS treated with natalizumab [3]. In the animal model of MS, experimental allergic encephalomyelitis (EAE), natalizumab hampers the development of inflammation and demyelination [4]. In randomized placebo-controlled trials [5,6] treatment of relapsing-remitting MS (RRMS) with natalizumab reduces the relapse rate with about 70% and is thus more efficient than other available treatments. Apart from reduced entrance of inflammatory cells into the CNS, other unknown immunological effects may also be operative and long term consequences of treatment with natalizumab are yet unknown.

Cytokines and chemokines are of fundamental importance for the regulation of inflammatory responses and tissue repair and have been ascribed important roles in MS pathogenesis [7,8] Chemokines comprise a large protein family being responsible for the trafficking of leukocytes to sites of inflammation and the regulation of leukocyte maturation [9]. Chemokines have a crucial role in establishing the balance between subpopulations of T helper (Th) cells and they are widely used as markers for Th1/Th2 immunity. Accordingly, associations have been found between Th1-like diseases, e.g. sarcoidosis, tuberculosis and Crohn´s disease [10,11] and CXCL11 (also known as interferon-γ inducible T cell α chemoattractant, I-TAC), whereas Th2-like conditions as atopic dermatitis and asthma have been associated with CC ligand 17 (CCL17, also named thymus- and activation-regulated chemokine (TARC)) and CCL22 (macrophage-derived chemokine (MDC)) [12-14]. Since chemokines occur at measurable concentrations in the circulation and in other body fluids, they can be used as bio-markers of immune status. Cytokines, on the other hand, in general are harder to detect in body fluids, yet they provide information as to different directions of an immune response including; inflammation (e.g. tumour necrosis factor (TNF), interleukin-1β (IL-1β), IL-6, IL-8 and granulocyte-monocyte colony stimulating factor (GM-CSF)); anti-inflammation (IL-10); Th1 (interferon-γ (IFN-γ)), Th2 (IL-4, IL-5), and T cell activation (IL-2).

Decreased mRNA expression in CSF cells of pro-inflammatory cytokines was recently reported to occur after one year of natalizumab treatment while, interestingly, an increased expression was found in peripheral blood mononuclear cells (PBMC) [15]. However, the effects of natalizumab on cytokine protein levels have not been assessed previously, neither have chemokines been evaluated. To gain increased insight in the immunomodulating effects of natalizumab, we therefore examined levels of a panel of cytokines and chemokines in plasma and CSF from patients with MS treated with natalizumab for one year.

Material and methods

Patients

Natalizumab treatment (Tysabri®, Biogen Idec Inc; 300 mg IV once a month) was initiated in 31 patients with active multiple sclerosis (MS), 18 men and 13 women (mean age 36 years, range 23-50), that had either unsatisfactory effect or intolerable side-effects of treatment with approved disease modifying agents (Table 1). All patients fulfilled the McDonald criteria of MS [16] and were consecutively recruited from the Department of Neurology at the University Hospital, Linköping. Patients were diagnosed as relapsing-remitting MS (RRMS, n=23) or relapsing-remitting MS with secondary progression (RRMS/SP, n=8). Sampling of peripheral blood and CSF was obtained before (mean 1.25 months, range 0.25-5.0) start of natalizumab treatment and after one year of treatment (mean 12 months, range 8.5-14.5). Within the last three months before sampling, 26 patients were on immunomodulatory treatment and 5 patients were not. The washout period before baseline sample was ≥ 1 month (10 patients), whereas 21 patients had no washout period at all. Patients were examined by a neurologist, which included definition of Expanded Disability Status Scale (EDSS) before and after one year of treatment. Ten patients with non-inflammatory neurological diseases (OND) and 15 healthy blood donors served as control groups for CSF and plasma samples respectively. OND patients were diagnosed with paresthesia/hypesthesia (n= 7), pain syndrome (n=1), vertigo (n=1) and hereditary neuropathy with pressure palsies (n=1). Routine CSF analyses were normal in the OND patients (Table 1). The study was approved by the regional ethics committee in Linköping and written consent was obtained from all participants.

Table 1 Patient characteristics at baseline.

Clinical and laboratory data	RRMS	RRMS/SP	OND
No. of subjects	23	8	10
Mean age (years) [a]	35 (range 23-50)	38 (range 30-49)	42 (range 21-54)
Sex (men/women)	15/8	3/5	3/7
Mean disease duration (years) [b]	8.1 (range 2-19)	11.5 (range 4-19)	NA
EDSS (no. of subjects)			NA
0-3,5	17	0	
4,0-5,5	4	1	
6,0-8,0	2	7	
Mean EDSS	3.0 (range 0-7)	6.5 (range 4-8)	NA
Treatment within last 3 months			NA
Interferon-β	13	4	
Glatiramer acetate	5	1	
Mitoxantrone	1	1	
IVIG	1	0	
Corticosteroids	2 [e]	0	
No treatment	3	2	
Mean no. of relapses last two years [c]	2.3 (range 0-8)	1.5 (range 0-4)	NA
No. of patients with relapse last month [d]	1	0	NA
Mean total CSF cell counts	5.5×10^6 cells/l (range 0.2-16.0)	5.2×10^6 cells/l (range 0.0-31.0)	1.1×10^6 cells/l (range 0.0-2.6)
Mean IgG index	1.22 (range 0.56-3.01)	1.31 (range 0.61-4.06)	0.46 (range 0.32-0.55)
Mean Albumine ratio	4.64 (range 2.20-11.40)	3.98 (range 2.90-6.10)	4.90 (range 1.90-8.30)
Oligoclonal IgG bands	22/23	7/8	0/10

[a] Mean age at baseline sampling of blood and CSF. [b] Mean number of years from first symptoms of MS to baseline sampling of blood and CSF. [c] Mean no. of relapses the last two years before baseline sampling of blood and CSF. [d] No. of patients with relapse last month before baseline sampling of blood and CSF. [e] These two patients were treated with corticosteroids, in addition to interferon and glatiramer acetate treatment respectively, due to relapses. EDSS; Expanded Disability Status Scale. RRMS; relapsing-remitting multiple sclerosis. RRMS/SP; relapsing-remitting multiple sclerosis with secondary progression. OND; other neurological diseases. NA; not applicable.

CSF handling and multiplex bead assay

One aliquot of the CSF sample was immediately transferred to the Department of Clinical Chemistry, where phase contrast microscopy counting of cells was done within one hour, and where also measurements of albumin and IgG were performed, all according to the clinical routines. The other aliquots of the CSF sample were centrifuged (200g for 10 min) within one hour and the supernatant immediately frozen in -70. Samples were stored until use. For CSF and cytokines in plasma, measurements were done on samples that were not thawn before, whereas for chemokines in plasma, measurements were done on samples that had been re-frozen once. It has been shown in our lab (M. Sandberg, unpublished) that a cycle of three thawings does not alter chemokine levels. For assessment of cytokine concentrations in plasma and CSF, an ultra-sensitive 10-plex kit was used in accordance with the instructions provided (Invitrogen® Cytokine Ultrasensitive Human 10-plex Panel). Analyzed cytokines included (lower detection limit given within brackets) IL-1β (0.38 pg/mL), IL-2 (0.45 pg/mL), IL-4 (1.45 pg/mL), IL-5 (1.26 pg/mL), IL-6 (0.56 pg/mL), IL-8 (0.80 pg/mL), IL-10 (0.47 pg/mL), IFN-γ (0.73 pg/mL), TNF-α (0.51 pg/mL) and GM-CSF (0.75 pg/mL). Values above zero but under the detection limit were given half the value of the lowest standard point. An in-house assay was used to analyse concentrations of the CXC chemokines CXCL9 (MIG), CXCL10 (IP-10) and CXCL11 (ITAC) and the CC chemokines CCL17 (TARC) and CCL22 (MDC) in plasma and CSF. Briefly, 50 µL of samples (or PBS / 1%BSA for blanks) were added to the wells of a Multiscreen[TM] filter plate (Millipore/Fisher). For quantification, a three-fold seven step standard curve was set up and added in duplicates (CXCL9 13.7-10000 pg/mL, CXCL10 5.5-4000 pg/mL, CXCL11/CCL17/CCL22 1.9-1400 pg/mL). A mixture of sonicated beads was added to each well, and samples and beads were incubated overnight under agitation at 4°C. After washing, a mixture of biotinylated antibodies was incubated with the beads for 1 h under agitation. Washing was repeated, after which Streptavidin-phycoerythrin was added. Following another 30 min agitated incubation and dilution in PBS / 1%BSA, analysis ensued. Blank samples were used in both assays, as well as inter-assay control. The samples were analysed on a Luminex[100] instrument (Biosource), and the data were acquired using the StarStation 2.0 software (Applied cytometry systems, Sheffield, UK).

Statistics and subgroups

Differences in levels of cytokines and chemokines before and after one year of natalizumab therapy were analysed using Wilcoxon signed rank test (GraphPad Software 5.2, San Diego,

CA, USA). Dividing patients into disease related subgroups was based on three different parameters; EDSS before treatment (0–3.5 and 4.0-8.0), diagnosis before treatment (RRMS and RRMS/SP), the occurrence of relapses during natalizumab therapy (yes or no). Subgroup analyses of the effect of natalizumab treatment on cytokine and chemokine levels were done by calculating ratios of concentrations post- and pre-treatment followed by Mann-Whitney´s U-test. This test was also used when analysing differences between MS patients and healthy blood donors (plasma), differences between MS and the OND group (CSF) as well as differences between patients with or without washout period. All p-values were based on two-tail statistical tests. A p-value of <0.05 was considered statistical significant.

Results

Baseline cytokine and chemokine levels compared with controls

The CSF levels of IL-1β, IL-8, CXCL10, CXCL11 and CCL22, were significantly higher in MS patients at baseline compared with OND patients (Table 2). The levels in CSF of GM-CSF, IFN-γ, TNF-α, IL-2, IL-4, IL-5, IL-10 and CCL17 were too low to be detectable by the method used. In plasma, levels of IFN-γ, IL-2, IL-5 and IL-8 were significantly higher among MS patients at baseline compared with healthy blood donors (Table 2). No differences in plasma chemokine levels were seen between MS patients and blood donors.

Clinical and CSF variables after one year of natalizumab treatment

Median total cell counts in CSF decreased from 2.5 x 10^6 cells/L (range 0.0-31.0) to 0.9 x 10^6 cells/L (range 0.1-3.6, p<0.001, Figure 1) Median IgG index decreased from 0.97 (range 0.56-4.1) to 0.83 (range 0.49-3.8, p<0.001, Figure 1). Median albumin ratios were not different pre-treatment (4.2, range 2.2-11.4) to post-treatment (4.4, range 1.8-8.2). 22 patients had no relapse, 7 patients had 1 relapse and 2 patients had 2 relapses. Only one patient had a relapse within the last month before follow up sampling. Among the 9 patients with relapses, 6 (67%) had EDSS ≥ 3.5 at inclusion. The mean annual relapse rate was 0.35 (11 relapses/31 person-years). EDSS after natalizumab treatment was stable or improved (median 3.0, range 0.0-8.0), but was not significantly different from EDSS pre-treatment (median 3.5, range 0.0-8.0). One patient was treated twice with corticosteroids because of relapses during natalizumab therapy. All patients but one underwent lumbar puncture at follow up after one year.

Table 2 Cytokine and chemokine concentrations (pg/ml) in OND and healthy blood donors compared to MS patients before treatment.
Medians are given and interquartile range within brackets.

Plasma	No. of MS patients with measurable levels	MS (n=31)	No. of healthy blood donors with measurable levels	Healthy blood donors (n=15)
Cytokines				
IL-1β	31/31	0.2 (0.2-0.5)	15/15	0.2 (0.2-0.4)
IL-2	31/31	**172.5 (117.6-194.4) ***	15/15	123.4 (72.9-150.1)
IL-4	31/31	77.4 (51.4-136.5)	15/15	95.4 (43.0-112.0)
IL-5	31/31	**18.1 (11.8-23.1) ***	15/15	12.8 (6.3-19.0)
IL-6	31/31	15.9 (7.4-27.3)	15/15	22.8 (7.7-27.5)
IL-8	31/31	**39.7 (23.3-46.3) ***	15/15	30.3 (17.2-35.0)
IL-10	31/31	19.2 (9.1-27.4)	15/15	15.2 (8.3-27.1)
TNF-α	31/31	67.6 (48.8-81.8)	15/15	56.9 (31.4.70.0)
IFN-γ	31/31	**88.3 (58.1-98.4) ***	15/15	51.3 (37.7-78.0)
GM-CSF	31/31	57.4 (30.6-90.4)	15/15	62.1 (26.1-108.5)
Chemokines				
CXCL9	31/31	79.5 (58.5-135.0)	15/15	84.6 (41.3-144.1)
CXCL10	31/31	49.6 (35.0-80.3)	15/15	44.8 (33.0-67.5)
CXCL11	31/31	48.3 (34.6-92.4)	15/15	48.7 (35.0-96.1)
CCL17	31/31	22.3 (15.9-41.5)	15/15	36.6 (21.2-74.6)
CCL22	31/31	196.8 (149.5-317.2)	15/15	192.2 (153.8-281.8)

CSF	No.of MS patients with measurable levels	MS (n=31)	No. of OND patients with measurable levels	OND (n=10)
Cytokines				
IL-1β	29/30 [a]	**4.4 (3.7-5.3) ****	9/9 [b]	2.7 (2.6-3.8)
IL-2	0/30	-	0/9	-
IL-4	0/30	-	0/9	-
IL-5	29/30	0.6 (0.6-0.6)	9/9	0.6 (0.6-0.6)
IL-6	28/30	1.1 (0.7-1.9)	7/9	1.4 (0.3-2.8)
IL-8	29/30	**83.8 (70.2-111.3) ****	9/9	51.1 (43.3-71.3)
IL-10	24/30	0.2 (0.2-0.2)	0/9	-
TNF-α	26/30	0.3 (0.3-0.3)	5/9	0.3 (0.3-0.3)
IFN-γ	0/30	-	0/9	-
GM-CSF	8/30	0.4 (0.4-0.4)	0/9	-
Chemokines				
CXCL9	31/31	50.1 (37.3-73.6)	10/10	3.3 (28.8-55.6)
CXCL10	31/31	**188.3 (146.4-294.1) *****	10/10	87.1 (74.2-118.9)
CXCL11	31/31	**5.4 (4.7-7.2) ***	10/10	4.4 (3.2-4.9)
CCL17	31/31	1.0 (1.0-1.0)	10/10	1.0 (1.0-1.0)
CCL22	31/31	**21.4 (12.8-35.3) *****	10/10	8.8 (6.1-9.7)

Values in bold represent significant differences compared to healthy blood donors (plasma) or patients with OND (CSF). [a] n=30, while analyse was not done in one patient. [b] n= 9 while analyse was not done in one patient. * p<0.05 compared to healthy blood donors. ** p<0.01 compared to patients with OND. *** p<0.001 compared to patients with OND. NA; not applicable.
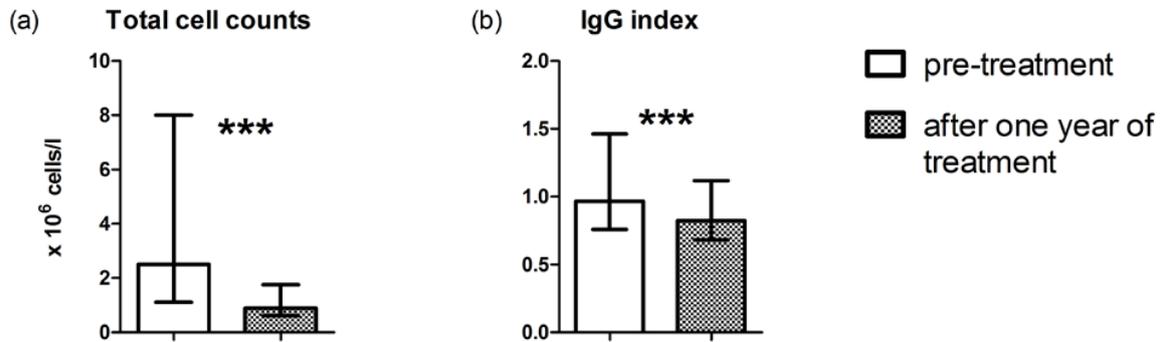
Figure 1. Total cell counts in cerebrospinal fluid (a) and IgG index (b) of MS patients before and after one year of natalizumab treatment. Bars represent median values and whiskers mark the interquartile range. *** p<0.001.

Chemokines in CSF and plasma after one year of treatment

The levels of all measurable chemokines in CSF, *i.e* all but CCL17, decreased significantly on follow up (Figure 2). For CCL17 no conclusion could be made while the levels were too low in CSF. The significant changes were not depending on whether or not the patient had a wash out period prior to start with natalizumab. In plasma, no differences were found between pre- and post-treatment levels (Figure 2). No significant differences, neither in CSF nor in plasma, were observed in the immunological effects of natalizumab therapy between different subgroups (low or high EDSS at inclusion, RRMS or RRMS/SP or presence or absence of relapse during treatment).
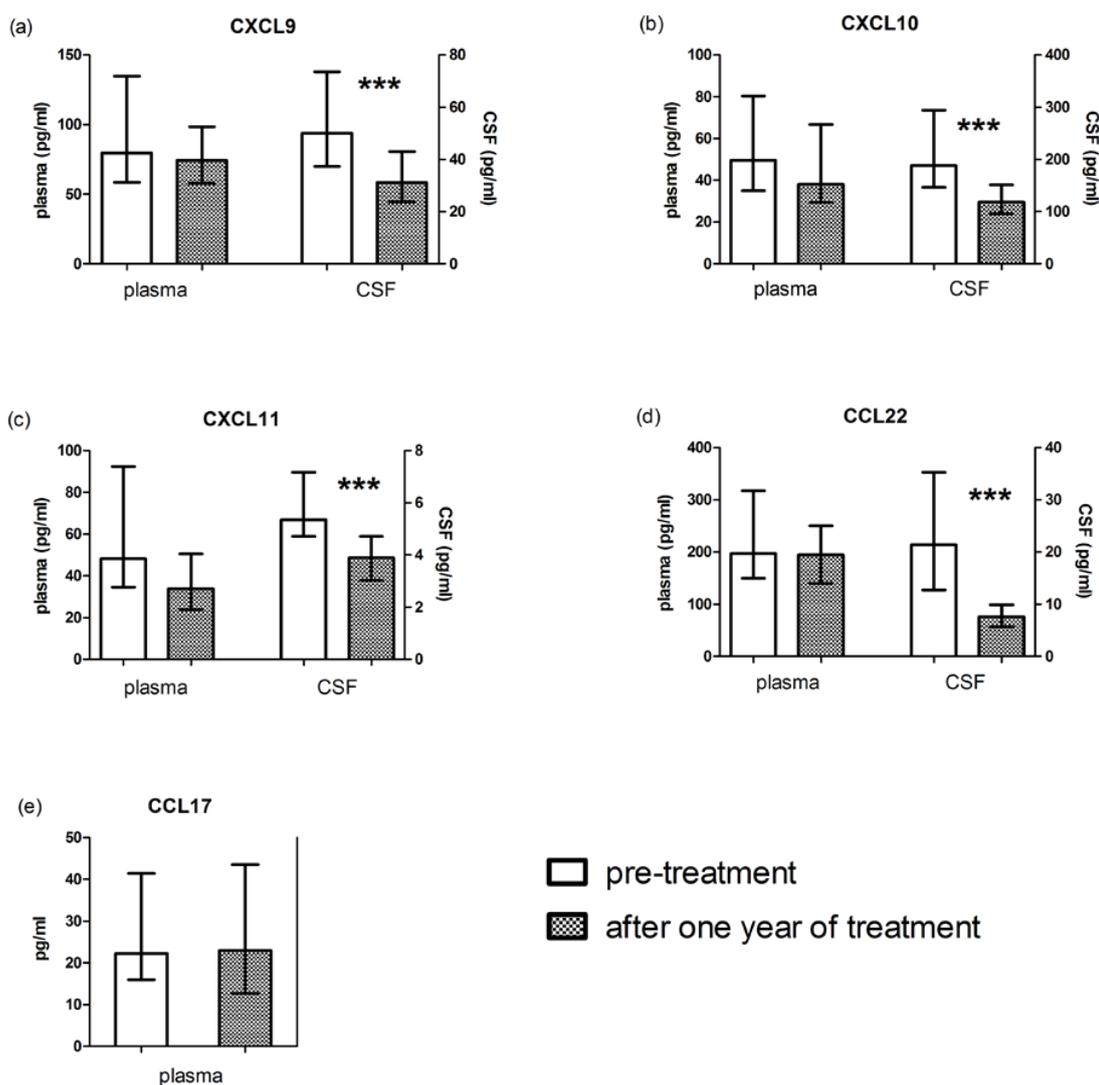
Figure 2. Concentrations of chemokines in plasma and cerebrospinal fluid (CSF) in MS patients before and after one year of natalizumab treatment as measured by Luminex. Levels in CSF of CXCL9 (a), CXCL10 (b), CXCL11 (c) and CCL22 (d) decreased significantly after one year of natalizumab treatment. In plasma no significant changes in levels of these chemokines were found after treatment. Data on CCL17 concentrations in CSF are lacking while levels were too low to be detected by the method used. Bars represent median values and whiskers mark the interquartile range. *** $p < 0.001$.

Cytokines in CSF and plasma after one year of treatment

The CSF levels of IL-1β, IL-6 and IL-8 decreased significantly after one year of natalizumab treatment (Figure 3). Concentrations of GM-CSF, IFN-γ, TNF-α, IL-2, IL-4, IL-5 and IL-10 were too low, both before and after treatment, to be detected. In plasma, there was a significant decrease in levels of GM-CSF, IL-6, IL-10 and TNF-α after one year of treatment, while there were no significant changes in levels of IL-1β, IL-2, IL-4, IL-5, IL-8 or IFN-γ

(Figure 3). The significant changes were not depending on whether or not the patient had a wash out period prior to start with natalizumab. In subgroup analyses, there was an increase in plasma levels of IL-2 and IL-8 after one year of treatment in patients diagnosed with RRMS/SP compared with those diagnosed with RRMS ($p< 0.05$). No other changes across subgroups related to disease activity were found, neither in plasma, nor in CSF.

Discussion

This is the first study to show the effects of natalizumab treatment on intrathecal and systemic chemokines and cytokines at a protein level. We demonstrate that natalizumab therapy for one year is associated with a marked global decline in CSF levels of cytokines and chemokines, thus including pro-inflammatory cytokines (IL-1β, IL-6 and IL-8) as well as chemokines associated with both Th1 (CXCL9, CXCL10, CXCL11) and Th2 (CCL22). Interestingly, also circulating plasma levels of some cytokines (GM-CSF, TNF-α, IL-6 and IL-10) decreased after one year of natalizumab treatment, partly in disagreement with previous mRNA-based data [15] and suggesting other immunological effects than modulation of CNS traffic. Although interquartile range may overlap comparing pre- to post-treatment samples, the described changes are still consistent and significant. When looking at the relative decrease in terms of pg/mL the changes are quite substantial; the relative reduction ranged from 14 % to 65 % in the cytokine/chemokine levels that were reported as significant. The biological and clinical significance of such changes are however unknown.

In accordance with earlier studies we found that natalizumab strongly reduced total cell counts intrathecally [3,17], and this was also accompanied by a decline in IgG index. Since natalizumab diminishes functional VLA-4 expression and migratory capacity of immune cells [2], it is conceivable that total cell counts in CSF are reduced and consequently there are less cells in the CNS to produce pro-inflammatory mediators and interact with the chemokine system. Although most cells in CSF are T cells [18], the lowered IgG index indicates that the reduced cell content in CSF also includes B cells and plasma cells, in line with VLA-4 expression also on B cells. However, it cannot be excluded that a lowered number of T cells could indirectly affect IgG production by diminished T cell help. At the same time acknowledging the effect of natalizumab, there is a possibility that regression towards mean could be a confounder causing some of the results reported here. Nevertheless, all patients
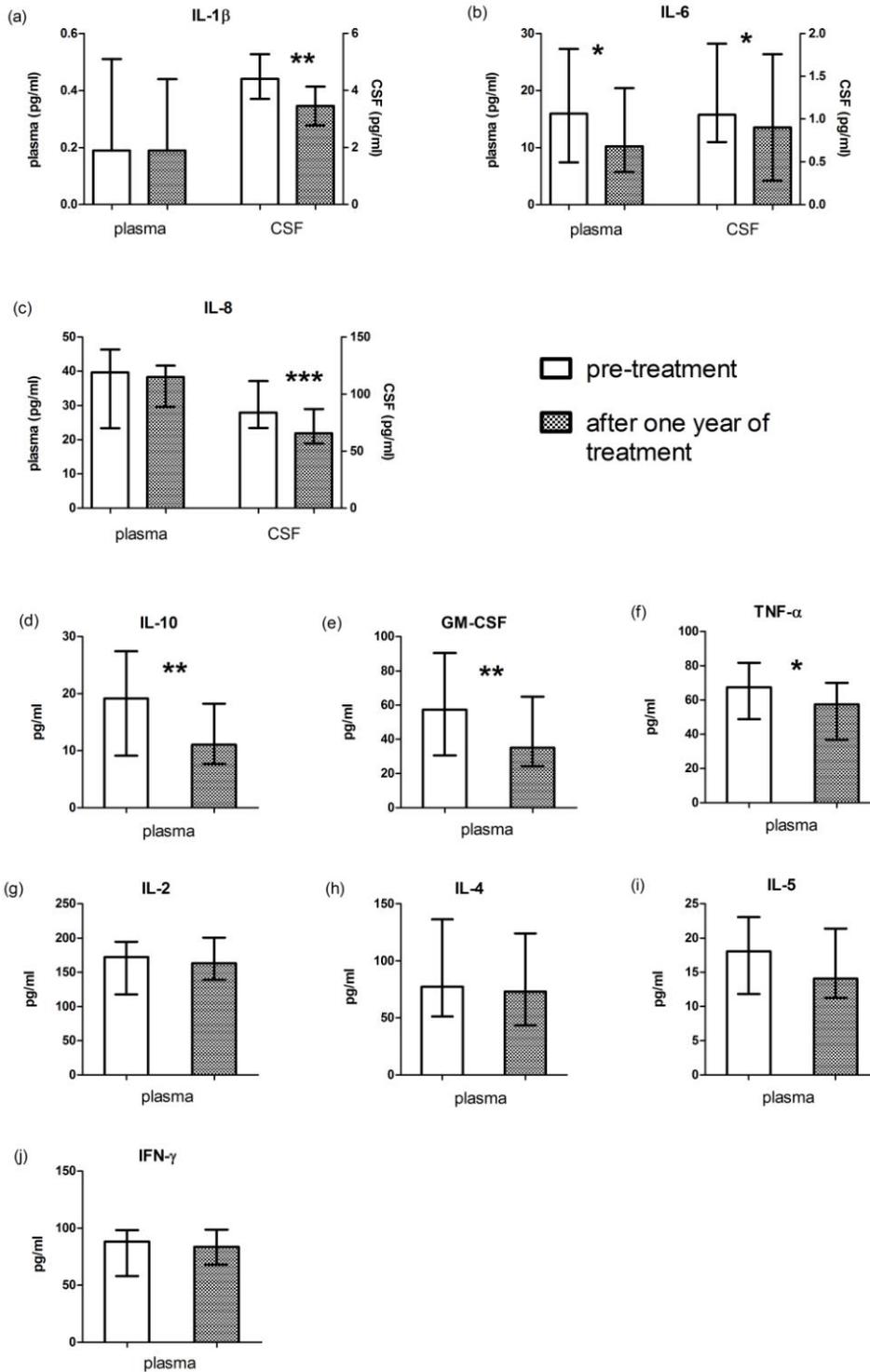
Figure 3

Figure 3. Concentrations of cytokines in plasma and cerebrospnal fluid (CSF) in MS patients before and after one year of natalizumab treatment as measured by Luminex. Levels of IL-1b (a), IL-6 (b) and IL-8 (c) in CSF were decreased after treatment. Levels of IL-6 in plasma (b) also decreased after treatment. Levels of IL-10 (d), GM-CSF (e) and TNF-α (f) in plasma were decreased after treatment, while no significant changes were found in levels of IL-2 (g), IL-4 (h), IL-5 (i) and IFN-g (j) in plasma after treatment. Data on levels in CSF of IL-2, IL-4, IL-5, GM-CSF, IFN-γ, IL-10 and TNF-α are lacking while concentrations of these cytokines were too low to be detected by the method used. Bars represent median values and whiskers mark the interquartile range. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

experienced a constantly active disease, most of them for many years, which indicates these patients to be more prone to have an active disease also in the future.

We also found a marked decline in CSF levels of pro-inflammatory cytokines, which is in accordance with previously published data on lowered intrathecal mRNA expression of IFN-γ and IL-23 after one year of treatment [15], all being in line with decreased CNS inflammation. In addition to cytokines, we also included a set of chemokines, all showing markedly lower levels after treatment, with the exception of CCL17, which could not be evaluated because of mostly undetectable levels in CSF. The chosen chemokines were selected to represent Th1 and Th2, and, clearly, both Th1 and Th2-associated chemokines decreased significantly. However, since the discontinuation of the preceding immunomodulative treatment, like IFN-β and glatiramer acetate, could be a confounding factor, the specific effect of natalizumab treatment on Th1 and Th2 balance is difficult to evaluate.

Interestingly, the marked decline in chemokine and cytokine levels intrathecally were not accompanied by an increase in pro-inflammatory mediators systemically as would have been expected, considering the proposed main mode of action of natalizumab with sequestration of activated lymphocytes in the peripheral circulation. On the contrary, our data showed that levels of pro-inflammatory mediators GM-CSF, IL-6 and TNF-α in fact decreased systemically after one year of natalizumab treatment. This is in contrast to earlier published data where mRNA expression of the pro-inflammatory cytokines IFN-γ and TNF-α were shown to increase in PBMCs of patients treated with natalizumab for one year. The main difference is that we report data at the protein level and not gene expression data. Also, previous data [15] reflect expression in blood cells, whereas our data reflect circulating levels, where the secreting cells may not be located in blood, but rather in secondary lymphoid organs. Considering that VLA-4 is expressed on activated T cells throughout the body, including lymphoid organs, it is indeed likely that functional blocking of this molecule would have other immunological effects, besides diminishing cell recruitment to CNS.

In the present study we used a broad panel of cytokines and chemokines. As expected, chemokines were in general easily detected both in plasma and CSF. As to cytokines, only IL-1β, IL-6 and IL-8 occurred at sufficiently high concentrations to be detected by our method. Of note, IL-8 is indeed a chemokine, although we here presented IL-8 in the context of cytokines, since it was included in the cytokine panel kit. Notably, IL-1β occurs at higher

levels in CSF than in plasma, perhaps indicating its prominent role in regulation of hypothalamic activity. Whether decreased IL-1β levels in CSF are associated with decreased fatigue remains to be settled.

Since most patients in our study (68%) had no wash-out period before the baseline sample was drawn, one can argue that the preceding immunomodulating treatment would influence the levels of cytokines and chemokines in the pre-treatment samples. However, we found that the significant changes pre-to post-treatment could not be explained by whether patients had a washout period or not. In addition, the majority (92%) of these patients with no wash-out period was treated with IFN-β or glatiramer acetate, and Jansson et al reported reduction in cells secreting IFN-γ, IL-4, IL-5 with those treatments [19]. Moreover, Khademi et al showed that the expression of IFN-γ and TNF-α systemically decreased with IFN-β treatment [20]. As to plasma levels, Jensen et al reported an increase in TNF-α after three months of IFN-β treatment[21], whereas Duddy et al showed no sustained alteration of IL-1β, TNF-α, IL-6 and IL-10 after the same time [22]. A study from 2001 showed that also circulating levels of CXCL10 were unaffected by treatment with interferon-β1a [23]. Although slight diverging results, taken together, there is no firm evidence that IFN-β treatment would increase cytokine and chemokine levels, thus our observation of decreasing levels can not be explained by withdrawal of IFN-β treatment.

There is much evidence that chemokines may play an important role in autoimmune inflammation in CNS. Chemokines and their receptors seem to be actively involved in the pathogenesis of multiple sclerosis in at least two ways; (1) their capacity to attract leukocytes to sites of inflammation via direct chemoattraction and (2) interact with leukocyte integrins to increase adherence and migration through the blood-brain barrier [24,25]. More specific, in CSF from MS patients levels of CXCL9 and CXCL10 were found to be elevated during acute attacks and the expression of CXCR3, a CXCL9/CXCL10 receptor, was the predominant chemokine receptor in the perivascular inflammatory infiltrate in active MS brain lesions at autopsy [26]. In addition, in the same study by Sorensen et al concentrations of CXCL10 correlated with the leukocyte count in CSF of patients with relapse. CXCR3 is of particular interest when studying migration of proinflammatory cells into the CNS. It is preferentially expressed on activated Th1 cells but also on astrocytes, microglia/monocytes, dendritic cells and natural killer cells [27,28]. This chemokine receptor is up-regulated in response to IFN-γ and TNF stimulation in vitro in human brain endothelial cells as well as in astrocytes and

microglia [29,30]. At the same time CXCR3 expression is shown to be reduced in non-inflammatory multiple sclerosis lesions and CXC3-positive cells were not detected in normal appearing white matter of MS brains [26,29,31]. Since targeting the chemokine signalling receptors might open up new therapeutic avenues for autoimmune diseases like MS, much effort have been made to find candidates for chemokine and chemokine receptor antagonism. So far results have been disappointing, for a review see Hamaan et al [7]. Still, as possible marker for disease activity in MS the CXCR3/CXCL10 axis might be useful.

In conclusion, our data show that natalizumab treatment is associated with a significant decline in levels of chemokines and pro-inflammatory cytokines in CSF, in line with the reduced cellular migration and lowered number of CSF cells. The lowering of cytokine levels also in plasma indicates other immunological effects of natalizumab besides affecting cell migration to CNS.

Acknowledgements

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