CONCISE REPORT

No signs of immunoactivation in the cerebrospinal fluid during treatment with infliximab

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Neuroinflammatory (demyelinating) disease is a rare but feared complication of treatment with anti-tumour necrosis factor (TNF) α in patients with polyarthritis. In this study, blood and cerebrospinal fluid markers of inflammation were analysed in 10 people with polyarthritis before and during treatment with infliximab. An increased systemic expression of interferon (IFN) γ was detected. Systemic administration of IFN γ is known to exacerbate multiple sclerosis. However, the present study failed to detect signs of inflammation in the cerebrospinal fluid samples—that is, pleocytosis, oligoclonal immunoglobulin G bands, increased expression of IFN γ , TNF α or interleukin 10, or increased levels of nitric oxide oxidation products. Our initial hypothesis, that the few cases of clinical neuroinflammatory disorders observed during treatment of polyarthritis with anti-TNF α represent the extreme end of a commonly occurring minor intrathecal immune activation, which in most cases does not give any overt neurological dysfunction, was not supported. Induction of systemic IFN γ production may still be relevant in neuroinflammation associated with treatment with anti-TNF α .

Randomised double-blind, placebo-controlled trials show that treatment with anti-tumour necrosis factor (TNF) α improves clinical symptoms and signs in patients with rheumatoid arthritis, spondyloarthropathies and inflammatory bowel disease.¹ However, rare cases of neuroinflammatory disorders, such as multiple sclerosis and optic neuritis, have been reported after treatment with anti-TNF α .² Moreover, treatment with anti-TNF α of patients with multiple sclerosis has been reported to cause clinical worsening or increased inflammatory activity as measured by magnetic resonance imaging of the brain and cell counts and immunoglobulin (Ig)G index of the cerebrospinal fluid (CSF).^{3,4} The possible link between treatment with anti-TNF α and neuroinflammation prompted us to test the hypothesis that the few cases of clinical neuroinflammatory disorders observed after treatment with anti-TNF α merely represent the extreme end of a commonly occurring minor intrathecal immune activation, which in most cases does not give rise to overt neurological symptoms.

To test the hypothesis, CSF and peripheral blood were sampled before and during treatment with infliximab in 10 patients with polyarthritis. Cells in the CSF and peripheral blood mononuclear cells were isolated and the relative mRNA expressions of interferon (IFN) γ , TNF α and interleukin (IL)10 were assayed using real-time quantitative polymerase chain reaction (PCR). Cell counts in the CSF reflect inflammatory cell infiltration into the CNS. Cellular expression of IFN γ , TNF α and IL10 reflect the activation state of inflammatory cells. IFN γ is typically produced by Th1 and natural killer cells, TNF α mainly by monocytes or macrophages and IL10 by several cell types. A raised nitric oxide

(NO $_x$) level in the CSF is a marker of neuroinflammatory activity in multiple sclerosis and systemic lupus erythematosus.⁵ The IgG index and detection of oligoclonal bands are classic measures of intrathecal immunoglobulin production.

PATIENTS AND METHODS

Patients

Between 2003 and 2004, patients who started treatment with infliximab were asked to undergo lumbar puncture before treatment and at week 8 of treatment. Table 1 gives the characteristics of the patients (all women) who agreed to participate.

A standard schedule was followed with intravenous infliximab given at weeks 0, 2 and 6. Standard doses of 3 mg/kg were used. In one patient, the treatment was temporarily withdrawn after the second infliximab infusion because of a knee arthroplasty. In this patient, infliximab was given at weeks 0, 2 and 14. The CSF and blood were sampled after the third infliximab treatment, 14 weeks after the first sampling. The local ethics committee approved the study design.

CSF, blood samples and real-time quantitative PCR determination of cytokine mRNA expression

The procedures for preparation of peripheral blood mononuclear cells, cells in the CSF and real-time quantitative PCR, including sequences of primers and probes, were similar to those previously published.⁶

Analysis of NO $_x$

NO $_x$, nitrite and nitrate, were analysed using capillary electrophoresis as described previously.⁷

Routine determination of CSF and C reactive protein

The cells in the CSF were counted using a Jessen chamber, the IgG index was obtained, albumin fractions were calculated and isoelectric focusing was carried out to detect oligoclonal bands. Serum levels of C reactive protein (CRP) were determined with a routine high-sensitivity assay. All routine assays were performed at the Department of Clinical Chemistry, Karolinska University Laboratory, Stockholm, Sweden.

Statistical analysis

Differences in the relative expression of cytokines and levels of CRP and NO $_x$ before and during treatment, were tested for significance with non-parametric tests (JMP V.5.1, SAS Institute, North Carolina, USA).

Abbreviations: CNS, central nervous system; CRP, C reactive protein; CSF, cerebrospinal fluid; IFN, interferon; NO $_x$, nitric oxide oxidation product; PCR, polymerase chain reaction; TNF, tumour necrosis factor

Table 1 Characteristics of patients, levels of CRP and response to infliximab

Age (years)	Diagnosis (ACR criteria)	Disease duration (years)	Prednisolone dosage at W0, W8 (mg)	DMARD	CRP level at W0, W8 (mg/l)	DAS 28 Baseline	ACR response
42	JCA	34	7.5, 5.0	MTX	4.0, 0.54	2.35	ND
36	JCA	27	7.5, 7.5	MTX	7.6, 7.4	4.10	ACR 20
76	RA	14	12.5, 12.5	MTX	93.3, 43.3	7.50	NR
38	RA	2	0	MTX	3.0, 2.3	4.77	NR
53	RA	2	0	MTX, AZA	15.6, 1.6	5.94	ACR 20
33	RA	10	0	MTX	19.8, 0.52	ND	ND
64	RA	2	0	MTX	5.4, 5.0*	3.80	NR
54	PsoA	4	0	MTX	78.8, NA	6.34	ACR 20
62	PsoA	11	0	MTX	28.6, 0.92	5.51	ACR 20
27	SpA	5	5.0, 5.0	MTX	56.5, 1.7	ND	ND

ACR, American College of Rheumatology; ACR response, ACR definition of improvement to measure patients' response to treatment; AZA, azathioprine; CRP, C reactive protein; DAS 28, 28-joint Disease Activity Score; DMARD, disease-modifying antirheumatic drug; JCA, juvenile chronic arthritis; MTX, methotrexate; NA, not available; ND, not determined; NR, non-responder; PsoA, psoriatic arthritis; RA, rheumatoid arthritis; SpA, spondyloarthritis; W0, week 0; W8, week 8. *Standard CRP measure, not high-sensitivity assay.

RESULTS

Clinical outcome and high-sensitivity CRP

Table 1 shows the clinical outcome data. Of the seven patients with polyarthritis who were evaluated, four reached ACR 20 clinical response after 3 months of treatment with infliximab. The median CRP levels were significantly decreased with treatment (17.7 v 1.7 mg/l; $p < 0.01$), suggesting clinical effectiveness of the treatment in the patient group studied.

No sign of pleocytosis, increased IgG indices, oligoclonal IgG bands or cytokine mRNA increases in the CSF

The relative expression of IFN γ , TNF α or IL10 mRNA in cells in the CSF did not change markedly in patients treated with infliximab compared with their baseline levels (fig 1A–C). Cell counts of the CSF and IgG indices were all within normal limits and no oligoclonal bands were detected, either before or during treatment with infliximab (data not shown). The ratio of CSF albumin to serum albumin was not affected, indicating preserved integrity of the blood–brain barrier in all the patients sampled (data not shown).

Increased mRNA expression of IFN γ , but not of TNF α or IL10, in peripheral blood mononuclear cells

The relative expression of IFN γ mRNA peripheral blood mononuclear cells was significantly higher after 8 weeks of treatment with infliximab as compared with baseline levels ($p < 0.05$). Of the 10 patients studied, 9 showed varying levels of increased systemic IFN γ mRNA expression (fig 2B). The relative expression of TNF α and IL10 did not change markedly (fig 2A,C).

NO $_x$ levels in the CSF and serum are not affected by treatment with infliximab

The mean (standard deviation (SD)) levels of NO $_x$ in the CSF were slightly raised: 10.1 (3.0) μ mol/l at week 0 and 10.0 (2.5) μ mol/l at week 8 (serum levels and individual CSF data not shown) as compared with previous findings in healthy people and people with tension-type headache.⁷ We found no marked change during treatment and no correlations with the clinical response or the cytokine expression.

DISCUSSION

To our knowledge, this is the first systematic study to investigate markers of inflammatory activity in the CSF on consecutive patients with polyarthritis before and during TNF α -blocking treatment. None of the assays used indicated that intrathecal immune activation was a consequence of treatment with infliximab in any of the patients studied. The slightly raised levels found in the patients investigated both

before and during treatment may reflect spinal degenerative changes,⁸ and are unlikely to reflect CNS inflammatory activity. Thus, our initial hypothesis that the few cases of clinical neuroinflammatory disorders observed after treatment of patients with polyarthritis with anti-TNF α merely

Figure 1 Cytokine mRNA expression in cells in the cerebrospinal fluid from patients with polyarthritis, before (W0) and during (W8) treatment with infliximab. Expression of (A) tumour necrosis factor (TNF) α , (B) interferon (IFN) γ and (C) interleukin (IL)10 mRNA relative to glyceraldehyde-3-phosphate dehydrogenase was determined using real-time reverse transcriptase polymerase chain reaction. No significant change related to treatment was observed.

Figure 2 Cytokine mRNA expression in the peripheral blood mononuclear cells of patients with polyarthritis, before (W0) and during (W8) treatment with infliximab. Expression of (A) tumour necrosis factor (TNF) α , (B) interferon (IFN) γ and (C) interleukin (IL)10 mRNA relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was determined using real-time reverse transcriptase polymerase chain reaction. The p value was calculated using a non-parametric test (JMP V.5.1).

represent the extreme end of a commonly occurring minor intrathecal immune activation was not supported.

However, we showed that peripheral blood mononuclear cells expressed markedly higher levels of IFN γ mRNA during treatment with infliximab. This finding is in accordance with earlier reports in which the number of cells spontaneously producing IFN γ was increased during treatment with anti-TNF α .⁹ An increased systemic production of IFN γ is a potential triggering factor for neuroinflammatory (demyelinating) disease, as systemic administration of IFN γ is known to exacerbate multiple sclerosis.¹⁰

Enhanced lymphocyte reactivity to both microbial antigens¹¹ and autoantigens⁹ during treatment with anti-TNF α has also been observed. These findings indicate that a systemic pro-inflammatory component is part of the diverse effects of treatment with anti-TNF α . At least two possible mechanisms can explain these findings. By blocking the action of TNF α , the activation of peripheral monocytes is decreased, and this deactivation of peripheral monocytes may result in enhanced activation and reactivity of peripheral T cells.¹² A decreased T cell reactivity was observed in patients with rheumatoid arthritis compared with healthy people,¹³ arguing for the relevance of this mechanism. An alternate

explanation for the increased systemic T cell activity is decreased T cell trafficking from the systemic compartment over the synovial membrane due to the down regulation of adhesion molecules, such as intercellular adhesion molecule-1, and reduction in the chemotactic property of TNF α .¹⁴ Regardless of the mechanism, an increased systemic T cell activity may include the activation of autoreactive T cells, including cells directed against cerebral autoantigens, as such cells are part of the normal T cell repertoire. Even in situations with an intact blood–brain barrier, such T cells may transmigrate to the CNS to be stimulated again by local antigen-presenting cells.¹⁵ Thus, the inability of infliximab to pass the blood–brain barrier does not exclude effects on neuroinflammation.

Our results suggest that immune activation in the CNS during TNF α -blocking treatment is not a common finding. Possible mechanisms in the few people with precipitated or worsened neuroinflammation during TNF α blockade may include a systemic IFN γ induction. Further studies are needed to clarify whether induction of autoreactive, nervous system-specific T cells is an additional factor.

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