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Interleukin 17F Level and Interferon Beta Response in Patients With Multiple Sclerosis

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Abstract

Importance—High serum levels of interleukin 17F (IL-17F) at baseline have been associated with suboptimal response to interferon beta in patients with relapsing-remitting multiple sclerosis.

Objective—To further investigate the role of IL-17F in predicting treatment response to interferon beta-1b in patients with relapsing-remitting multiple sclerosis using the Singulex Erenna IL-17F immunoassay.

Design, Setting, and Patients—Serum samples were analyzed from 239 randomly selected patients treated with interferon beta-1b, 250 μg, for at least 2 years in the Betaferon Efficacy Yielding Outcomes of a New Dose Study.

Exposure—Treatment with interferon beta-1b, 250 μg, for at least 2 years.

Main Outcome Measures—Levels of IL-17F at baseline and month 6 as well as the difference between the IL-17F levels at month 6 and baseline were compared between the following: (1) patients with less disease activity vs more disease activity; (2) patients with no disease activity vs some disease activity; and (3) responders vs nonresponders.

Results—Levels of IL-17F measured at baseline and month 6 did not correlate with lack of response to treatment after 2 years using clinical and magnetic resonance imaging criteria. Relapses and new lesions on magnetic resonance imaging were not associated with pretreatment serum IL-17F levels. When patients with neutralizing antibodies were excluded, the results did not change. All patients with levels of IL-17F greater than 200 pg/mL were associated with poor response with some clinical or radiological activity.

Conclusions and Relevance—An increase of IL-17F before and early after treatment with interferon beta-1b was not associated with poor response. These data do not support the value of IL-17F as a treatment response indicator for therapy of patients with multiple sclerosis with interferon beta, although high levels of IL-17F greater than 200 pg/mL may predict nonresponsiveness.

Interleukin 17F (IL-17F) is one of the signature cytokines of helper T 17 cells that play a key role in the defense against pathogens and autoimmunity.[1–3] Helper T 17 cells have been invoked as key determinants of aberrant immune responses in multiple sclerosis.[3–4],

Interleukin 17F has been shown to function in part differently from another member of the IL-17 cytokine family, IL-17A.[2] Interleukin 17F is a central mediator of cellular immunity governing the expression of critical cytokines that exert proinflammatory effects.[3] High serum levels of IL-17F at baseline have been associated with suboptimal response to interferon beta-1b in patients with relapsing-remitting multiple sclerosis.[5–6] To further investigate the role of IL-17F in predicting treatment response to interferon beta-1b, we used the Singulex Erenna IL-17F immunoassay to analyze serum samples collected at baseline

and after 6 months of treatment from 239 patients who participated in the Betaferon Efficacy Yielding Outcomes of a New Dose (BEYOND) Study.

Methods

Study Design

Serum samples were analyzed from 239 randomly selected patients treated with interferon beta-1b, 250 μg, for at least 2 years in the BEYOND Study.[7] In these patients, IL-17F was measured at baseline and after 6 months of treatment using the Singulex Erenna immunoassay. The Erenna IL-17F immunoassay kit makes use of a microparticle-based single-molecule counting technology.[8] Human serum samples were diluted in assay buffer and concentrations of IL-17F were determined using a reference and standard curve. The limit of detection of the assay was 0.5 pg/mL. The expected median value of IL-17F in human serum from healthy control subjects was 20 pg/mL. The low limit of quantification was 1.6 pg/mL (20% coefficient of variation and ±20% recovery). The short-term effects of interferon beta were confirmed by measuring 2 known interferon-inducible proteins (interferon gamma–induced protein 10 and interferon-inducible T-cell α chemoattractant) using the Human Discovery MAP250 version 1.0 Luminex-based, multianalytes profiling assay (Myriad RBM).

Statistical Analysis

Using the entire patient group (n = 239), IL-17F levels at baseline and month 6 as well as the difference between IL-17F at month 6 and baseline (IL-17F) were compared between the following: (1) patients with less disease activity (no relapse and   4 lesions on magnetic resonance imaging [MRI]) vs more disease activity (1 relapse or >4 MRI lesions) within 2 years of treatment; (2) patients with no disease activity (no relapse and no MRI lesion) vs some disease activity (1 relapse or   1 MRI lesion); and (3) responders (no relapse and no confirmed progression on the Expanded Disability Status Scale) vs nonresponders (1 relapse and confirmed progression on the Expanded Disability Status Scale). Additional group comparisons excluded patients with neutralizing antibodies to interferon beta. Outcomes of patients having baseline IL-17F concentrations greater than 50 pg/mL or greater than 200 pg/mL were described in line with previous studies that found patients with pretreatment IL-17F values greater than 50 pg/mL to be clinical nonresponders.[5] Finally, correlation of IL-17F serum levels with the number of new MRI lesions and the annualized relapse rate was determined for the entire population.

Nonparametric methods were used for all analyses: Wilcoxon rank sum test for group comparisons and Spearman ρ for correlation. Values were compared graphically by means of scatterplots. P values were not corrected for multiple comparisons.

Results

Baseline characteristics and disease course during the first 2 years of interferon beta-1b treatment were similar between the patient group used for IL-17F measurements and the entire BEYOND Study cohort (Table 1). There was no statistically significant difference between IL-17F levels at baseline and month 6 (median, 16.5 vs 16.8 pg/mL, respectively; P

= .22). However, levels of interferon gamma–induced protein 10 and interferon-inducible T-cell α chemoattractant increased significantly from baseline to month 6. There was a 2.4-fold increase of median interferon gamma–induced protein 10 levels (P < .001) and a 2.4-fold increase of median interferon-inducible T-cell α chemoattractant levels (P < .001) (data not shown).

When clinical and MRI criteria were used to measure response to treatment, 115 patients (48.1%) had less disease activity (no relapse and 4 MRI lesions) and 124 (51.9%) had more disease activity (1 relapse or >4 MRI lesions). Fifty-six patients (23.4%) had no disease activity (no relapse and no MRI lesion) and 183 (76.6%) had some disease activity (1 relapse or 1 MRI lesion). Using clinical response criteria, 124 patients (51.9%) were responders (no relapse and no confirmed progression on the Expanded Disability Status Scale), while 27 (11.3%) were nonresponders (1 relapse and confirmed progression on the Expanded Disability Status Scale).

Levels of IL-17F at baseline and month 6 as well as IL-17F did not differ significantly between patient groups (Table 2, eTable 1, and eTable 2). Similar results were obtained for the 149 patients who did not develop neutralizing antibodies during the entire study (data not shown).

The frequency of high (>50 pg/mL) or very high (>200 pg/mL) IL-17F values at baseline was similar for the different definitions of active vs inactive disease used in our study (Table 2). Of note, we found that no patients without any clinical or radiological disease activity with interferon beta-1b treatment demonstrated baseline IL-17F values higher than 200 pg/mL, whereas 8 (4.4%) of the patients with at least some disease activity showed such values.

There was no significant correlation between baseline IL-17F level and the annualized relapse rate (ρ = 0.08; P = .21) and the number of new MRI lesions observed within the first 2 years of interferon beta-1b treatment (ρ = −0.13; P = .06) (eFigure). Finally, there was no significant correlation between the month 6 IL-17F level and the annualized relapse rate (ρ = 0.01; P = .91), between IL-17F and the annualized relapse rate (ρ = −0.02; P = .70), between the month 6 IL-17F level and the number of new MRI lesions observed within the first 2 years of interferon beta-1b treatment (ρ = −0.10; P = .16), or between IL-17F and the number of new MRI lesions observed within the first 2 years of interferon beta-1b treatment (ρ = 0.01; P = .92).

Discussion

We found that serum concentrations of IL-17F alone did not predict response to interferon beta-1b therapy in patients with relapsing-remitting multiple sclerosis. Baseline IL-17F levels in the group of patients with higher disease activity were in the same range as the group having lower disease activity. Relapses and new MRI lesions occurring in the first 2 years of interferon beta-1b exposure did not correlate with pretreatment IL-17F levels. Negative results were also obtained for the comparison of responders and nonresponders defined on the basis of stringent clinical criteria. Similar results were obtained for IL-17F

measured at month 6 and for IL-17F. When patients with neutralizing antibodies were excluded, the results did not change. Finally, IL-17F levels in patient serum did not differ during administration of interferon beta-1b, in contrast to large and significant changes in levels of acute interferon beta-1b bioactivity markers interferon gamma–induced protein 10 and interferon-inducible T-cell α chemoattractant.

Of note, the proportions of patients with pretreatment IL-17F values higher than 50 pg/mL, which had been the threshold for nonresponse in the study by Axtell et al,[5] were similar in our patient strata. When focusing on patients with even higher pretreatment IL-17F values, we found that all patients with values higher than 200 pg/mL had at least some clinical or radiological disease activity while being treated with interferon beta-1b. Analyses of extreme outcomes are often illuminating because such analyses often eliminate patients who may be misclassified.[9–10] However, considering all our results makes it difficult to draw a robust conclusion from this finding.

Conceptual and methodological differences may have contributed to the discordance of findings between the study by Axtell et al[5] and this study. Axtell and colleagues collected samples from a single center, and patients were treated with interferon beta-1a or interferon beta-1b. Our study was multicenter, and patients were treated with interferon beta-1b exclusively. The IL-17F serum concentrations were determined in our study using an assay optimized to measure IL-17F level in human serum, which is different from the multiplex assay used by Axtell et al.[5] The Singulex IL-17F assay is recognized to be one of the most accurate, reproducible, and sensitive assays available for measuring IL-17F concentrations in human serum.[11] Although Axtell et al[5] found a positive correlation between pretreatment IL-17 serum concentrations and nonresponse to interferon beta, these results were based on a much smaller patient cohort (n = 26) and exclusively used clinical criteria for nonresponse. In a recent study, Bushnell et al[12] were also unable to correlate IL-17F serum levels with treatment response to interferon beta. However, in their study, IL-17F values did not exceed 50 pg/mL, which makes it difficult to compare their results with those reported by Axtell et al[5] and those from our study.

Because the biology of interferon beta is complex, it is not unexpected that serum IL-17F concentration by itself is inadequate to predict treatment response. It has also been demonstrated in mice that IL-17F and IL-17A as single factors do not contribute vitally to the development of autoimmune neuroinflammation.[13] Measurement of a combination of other cytokines in concert with IL-17F may be required. Although predictors of response to interferon beta remain elusive, much progress has been made in this area.[14–20] Recognizing the synergistic interactions between IL-17F and other potent mediators on immune regulation, identification of predictors of response to interferon beta treatment may still be possible. Given the multifaceted pathophysiology associated with disease progression and response to treatment by patients with relapsing-remitting multiple sclerosis, using extreme patient cohorts in combination with immune-based biomarker signatures may actually be the most efficient way of initially identifying response markers. One approach might be to focus first on subjects with the highest levels of IL-17F (>200 pg/mL) in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline Characteristics and Disease Course of Patients Receiving Interferon Beta-1b in the Original Cohort of the BEYOND Study vs Those Selected for Interleukin 17F Measurement

	Mean (Median; Q1–Q3)	
Characteristic	**Original Cohort [a]** **(n = 897)**	**Selected Cohort [b]** **(n = 239)**
Baseline		
Age, y	35.8 (35; 28–43)	36.5 (37; 29–44)
Women, %	627 (69.9)	172(72.0)
Disease duration, y	5.2 (3; 1–7)	5.3 (3; 1–8)
Relapses in last y, No.	1.6(1; 1–2)	1.5(1; 1–2)
T2 lesion volume, cm^3	9.3 (5.7; 2.2–12.0)	10.2 (6.7; 2.4–13.4)
1 Gd-enhancing lesion, %	46	46
Disease course		
Newly active lesions within first 2 y with interferon beta-1b treatment, cumulative No.	5.4 (2; 0–5)	5.5 (2; 0–5)
Annualized relapse rate	0.4 (0; 0–0.5)	0.4 (0; 0–0.5)
Confirmed EDSS progression within first 2 y with interferon beta-1 b treatment, %	21	23

Abbreviations: BEYOND, Betaferon Efficacy Yielding Outcomes of a New Dose; EDSS, Expanded Disability Status Scale; Gd, gadolinium; Q1, first quartile; Q3, third quartile.

[a] Patients randomized to treatment with interferon beta-1b, 250 g, in the BEYOND Study.

[b] Patients randomly selected from the original cohort for measurement of interleukin 17F concentration.

Table 2

Comparison of Baseline Interleukin 17F Concentrations

IL-17F Concentration	Less vs More Disease Activity		No vs Some Disease Activity		Clinically Defined Responders vs Nonresponders	
	Patients With No Relapse and 4 MRI Lesions (n = 115)	Patients With 1 Relapse or >4 MRI Lesions (n = 124)	Patients With No Relapse and No MRI Lesion (n = 56)	Patients With 1 Relapse or 1 MRI Lesion (n = 183)	Patients With No Relapse and No Progression (n = 124)	Patients With 1 Relapse and Progression (n = 27)
Mean (median), pg/mL	35.22(16.40)	41.62(17.15)	30.3(17.8)	41.1 (16.4)	41.48(16.25)	28.13(18.00)
Q1–Q3, pg/mL	10.00–24.45	10.38–28.20	10.9–28.2	10–27.0	10.38–27.88	10.20–24.25
>50 pg/mL, %	12.2	9.7	10.7	10.9	12.1	7.4
>200 pg/mL, %	2.6	4.0	0.0	4.4	3.2	3.7
P value[a]	.48		.62		.94	

Abbreviations: IL-17F, interleukin 17F; MRI, magnetic resonance imaging; Q1, first quartile; Q3, third quartile.

[a] Wilcoxon rank sum test.