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Short communication

Effects of interferon-beta therapy on elements in the antiviral immune response towards the human herpesviruses EBV, HSV, and VZV, and to the human endogenous retroviruses HERV-H and HERV-W in multiple sclerosis

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ABSTRACT

Effects of treatment of multiple sclerosis patients with IFN-β on elements of the antiviral immune response to herpesviruses were analysed in a longitudinal study. We found significantly increased seroreactivity to EBV EBNA-1, and to VZV, in patients who did not respond to IFN-β therapy. We found no significant changes in seroreactivity to EBV EA, or to HSV. For the same patient cohort, we have previously demonstrated significant decreases in seroreactivities to envelope antigens for the two human endogenous retroviruses HERV-H and HERV-W, closely linked to efficacy of therapy.
We further searched for correlations between seroreactivities to EBV, HSV, and VZV, and levels of mannan-binding lectin (MBL), and MBL-associated serine protease 3. We found no such correlations.
Our results are in accord with recent reports of increased seroreactivity to EBV EBNA-1, and to VZV in active MS, and they support that the herpesviruses EBV and VZV together with HERV-H/HERV-W and the antiviral immune response may play a role in MS development.

1. Introduction

Multiple sclerosis (MS) is a complex, demyelinating disease of the central nervous system (CNS). MS pathogenesis involves inflammatory and apoptotic processes (Kornek and Lassmann, 2003; Barnett and Prineas, 2004; Hafler, 2004), while the aetiology seems to involve interactions between genetic and environmental factors (Hogancamp et al., 1997). Human herpesviruses and human endogenous retroviruses (HERVs) are often suggested as etiologic factors in MS (Antony et al., 2004; Christensen, 2005; 2010).

Interferons comprise a group of cytokines with immunomodulatory, antiproliferative, and antiviral effects (Baron et al., 1991; Vilcek, 2006; Fernald et al., 2007) and interferon-beta (IFN-β) therapy is a widely used treatment for relapsing–remitting MS (Koch-Henriksen et al., 2006).

In earlier studies, we demonstrated a significant correlation between elevated antibody-levels to Envelope (Env) epitopes from the Gammaretrovirus HERV-H and disease activity, and also indications of protective effects of high levels of two components in innate immunity: the pathogen-associated molecular pattern (PAMP) recognition molecule mannan-binding lectin (MBL), and the MBL-associated serine protease, MASP-3 (Christensen et al., 2007). We have also demonstrated significant decreases in anti-Env antibody reactivities for both HERV-H and HERV-W, as a consequence of IFN-β therapy, closely linked to efficacy of therapy/low disease activity. In the same study we confirmed strong indications of a protective effect of high levels of MBL, and MASP-3, and found no overall changes in Th1/Th2 ratios for selected MS-relevant cytokines (Petersen et al., 2009).

Among the herpesviruses, suggested as viral candidates in MS pathogenesis, are herpes simplex virus (HSV), varicella zoster virus (VZV), human herpes virus type 6 (HHV-6) and, particularly, Epstein-Barr virus (EBV) (reviewed in Simmons, 2001; Christensen, 2005, 2007; Ascherio and Munger, 2010; Giovannoni, 2011).

In the following, we extend our longitudinal study of antiviral responses in MS patients, initiating *de novo* treatment with IFN-β, to include analyses of serologic reactivity to EBV, VZV, and HSV.

2. Materials and methods

2.1. Blood samples

Blood samples were collected from 26 individuals after informed consent.

Blood samples were drawn into VT-100UX Venoject tubes (Meda, Denmark) without additions. Serum was aliquoted into 1 ml portions and stored at −70 °C. All assays were performed blinded for treatment, and clinical status of the patients.

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2.2. Study population

The data for the MS patients are presented in Table 1. Relapsing–remitting (RR) MS was defined in accordance with the revised McDonald criteria (Polman et al., 2005), EDSS (extended disability status scale) was assessed according to Kurtzke (Kurtzke, 1983). The mean annual relapse rate the year before inclusion for all patients was 1.4. There was no significant difference in the pre-inclusion annual relapse rates for the 6 patients who subsequently were classified as IFN-β non-responders compared with the remaining patients, and there were no differences between the three treatment groups: Treatments were: Avonex: (IFN-β1a, Biogen Idec), (6 MIU) intramuscularly once weekly; Betaseron (IFN-β1b, Bayer Schering Pharma), (8 MIU): subcutaneously every other day, or Rebif (IFN-β1a, Serono), (6 or 12 MIU): subcutaneously three times weekly.

The baseline sample was drawn immediately before the patients initiated the IFN-β treatment while the subsequent sample was drawn at the 1-year follow-up.

2.3. Herpesvirus serology: enzyme linked immunosorbent assay (ELISA)

Herpesvirus serology was assessed using commercial kits as described by the manufacturer: anti-HSV IgG by Enzygnost kit (Dade Behring (product no. OWMX155), anti-VZV IgG by Vidas kit (product no. 30217), and anti-EBV serology by EA IgM ELISA (Biotest product no. 807015), EA IgG ELISA (Biotest product no. 807016), and EBNA-1 ELISA (Biotest product no. 807017), IgM and IgG antibodies against the non-herpesvirus rubella (Vidas RUB IgM- and IgG kits, product numbers 30214 and 30221) and parvovirus B19 (Biotrin Parvovirus B19 IgM kit product no. V619IM and IgG kit product no. V519IG) were also measured by ELISA. Results are presented as ELISA readings and evaluated positive or negative according to cut-off values calculated as recommended by the manufacturer. All these methods are approved according to the European IVD directive, and their performance was checked by the external quality assessment service Labquality.

2.4. HERV serology: time-resolved immunofluorometric assay (TRIFMA) for anti-peptide antibodies

The TRIFMA assays and peptides used are described in detail in Petersen et al. (2009). Briefly, antibodies were detected in peptide-coated microtiter wells using europium labelled secondary antibodies The capture peptides were synthesised by Genosys Biotechnologies (Cambridge, UK): H3: NGTEELPVPLMTPTQQ (HERV-H Env), and W3: TEQDLYSYVISKPRNK (HERV-W/syncytin 1 Env).

TRIFMA Ratios (TR) are defined as individual measurements in relation to the median values of sera from healthy controls (Christensen et al., 2007). For individual peptides, TRs ≥1.2 are considered positive, and TRs<1.2 are considered negative.

2.5. MASP-2: TRIFMA assay

MASP-2 levels were measured as previously described using a sandwich type immunoassay and measured by time-resolved fluorometry (Moller-Kristensen et al., 2003).

2.6. MASP-3 TRIFMA assay

A sandwich type TRIFMA was used to measure MASP-3 levels (Thiel et al., 2006).

2.7. MBL TRIFMA assay

MBL levels were measured as previously described (Christiansen et al., 1999).

2.8. Statistics

Non-parametric Mann–Whitney tests, Wilcoxons matched pairs tests and Spearman's tests for correlation were performed using GraphPad Instat ver.3.

3. Results

An overview of the results of the analyses is presented in Table 2. Serological herpesvirus data for each individual patient can be found in Supplementary Table 1.

Six of the patients (R2, R7, A6, A7, B5, and B8) experienced a relapse within three weeks before the 1-year follow-up. These 6 patients were classified as IFNβ-non-responders (active MS) during the year-long period of IFN-β therapy and had significantly increased annual attack rates (2.4 (range two to four) compared to <1 for the other MS patients in the cohort, $p=0.01$, Mann Whitney rank sum test)) (Petersen et al., 2009) (please also refer to the legend for Supplementary Table 1).

3.1. Herpesvirus serology: reactivity to EBV, VZV, and HSV

All patients were previously EBV infected as determined by serological parameters. IgG reactivity to EBNA-1 ($p=0.0275$, Wilcoxon matched pairs) was significantly increased from baseline to follow-up. When stratified for disease activity, we found this due to a significant increase for the six patients with active MS during therapy (IFNβ-non-responders, $p=0.0313$, Wilcoxon matched pairs); no other significant change in EBV serology was found.

Median EBNA-1 IgG levels were similar for the two patient groups at entry (3.215 (active MS) vs 3.330 (inactive MS), but increased markedly for patients with active disease at follow-up (3.935 (IFNβ-non-responders) vs 3.520 (IFNβ-responders MS)). One of the patients with active MS showed a serology pattern indicating reactivated EBV both at baseline and at follow-up, as determined by positive EBV EA IgM and IgG, and EBNA-1 IgG. This patient had high disease activity with relapse about two months before entry and four relapses from baseline to follow-up. All other patients were seronegative for EBV EA IgM. All six patients classified as IFNβ-non-responders were seropositive for EBV EA IgG, as were 11 of 17 patients who were IFNβ-responders.

All patients were previously VZV infected (VZV is not included in the Danish childhood vaccination programme). IgG reactivity to VZV

Table 1
The clinical and demographic data at baseline for the MS patients treated with IFN-β.

	Avonex	Betaseron	Rebif	All
N	9	8	9	26
Age (years)	32 (23–54)*	38 (29–50)	40 (25–51)	37 (23–54)
Sex (F/M)	6/3	5/3	7/2	18/8
Age at onset (years)	27 (17–53)	31 (17–44)	28 (21–49)	28 (17–53)
Time to last relapse (days)	80 (26–188)	150 (1–410)	141 (25–271)	123 (1–410)
Duration of MS (months)	63 (6–249)	26 (3–312)	11 (6–228)	26 (3–312)
EDSS	2.5 (0–3)	3 (1–4.5)	3.0 (0–4)	3 (0–4.5)

The participants were from a homogenous population of Caucasians with Northern European descent.
The different IFN-β therapies used here have very similar clinical effects (Weinstock-Guttman et al., 2008), justifying the inclusion of RR-MS patients treated with either Avonex, Betaseron, or Rebif in this study.
It has been shown that IFN-β therapy may induce anti-interferon antibodies, which, in high concentrations, are associated with reduction of treatment response and increase in disease progression. Such antibodies were monitored using an antiviral neutralisation bioassay (Sorensen et al., 2003). MS patients B3, B4, and B6 were shown to have developed >80% anti-IFN-β neutralising antibodies and were therefore not included in the detailed analyses.
* : median (range).