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Elevated antinuclear antibodies and altered anti-Epstein-Barr virus immune responses

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ABSTRACT

It has been shown that Epstein-Barr virus (EBV) is able to alter the immune response towards self-antigens and may enhance risk of autoimmune diseases such as systemic lupus erythematosus (SLE) in genetically predisposed individuals. In this study, we evaluated the specific antibody immune response against EBV in patients with anti-nuclear autoantibodies (ANA) in comparison with ANA-negative healthy controls. For this purpose, 92 patients with an high anti-ANA reactivity with or without concomitant extractable nuclear antigen (ENA) or double stranded DNA (dsDNA) positivity were selected and compared with 146 healthy donors. We found that anti-EBV-VCA and EA IgG concentrations were significantly higher in ANA-positive patients in comparison to the controls (VCA $P < 0.0001$ and EA $P < 0{,}03$) as well as in those ANA-positive patients that showed a concomitant ENA positivity ($P = 0.0002$). Interestingly, elevated anti-EBNA-1 IgG was found in a group of patients who had anti SSA/Ro antibodies. Anti-VCA IgM Abs were more frequently found in those patients with a very high titer of ANA ($P = 0.06$); moreover detection of anti-VCA IgM/IgG in absence of anti-EBNA-1 IgG was more frequent in the patient than in the control group. Both these conditions correlate with a recent EBV infection or reactivation. The data suggest that EBV, particularly during acute infection or in its reactivation phase, could be involved in the ANA and ENA autoantibody formation.

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1. Introduction

EBV is a ubiquitous gamma herpesvirus which infects more than 90% of the human population worldwide (Klein et al., 2007). The virus establishes a latent infection in B cells by expressing only a limited number of viral antigens (Babcock et al., 2000). The number of infected cells remains generally constant over a lifetime in healthy individuals with occasional flare in viral replication mainly related to B cell homeostasis and developmental cues received by the infected cells. During acute infection, which causes infectious mononucleosis (IM) in 50% of the young adults, a massive viral replication occurs. It is estimated that IM patients have 20 fold increased risk of developing multiple sclerosis and other autoimmune diseases (Ascherio and Munger, 2007; James et al., 2001). While the link between EBV and lymphomagenesis is becoming increasingly clearer, the contribution of the virus in autoimmunity still remains fairly obscure. It is suspected that deregulated virus-specific immune response could underlie autoimmune diseases (Poole et al., 2009).

The development of autoimmune diseases such as Systemic Lupus Erythematosus (SLE) seems to be associated with both genetic and environmental factors (Klein-Gitelman and Miller, 2004). In the former category, HLA-DR and HLA-DQ alleles strongly predispose to SLE development whereas in the latter category, EBV, cytomegalovirus (CMV) and human endogenous retroviruses (HERVs) have been proposed to be involved (Klein-Gitelman and Miller, 2004). The role of EBV in triggering autoimmune diseases has become evident first from serological and subsequently from in situ studies in maladies like SLE, RA and multiple sclerosis (Denman, 2000; James and Robertson, 2012; Niller et al., 2008). As far as

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SLE is concerned, the serological studies initially indicated that almost 100% of SLE patients are EBV seropositive against about 90% seropositivity in healthy population, characterized by higher VCA-IgG (James et al., 1997a). Furthermore, increased viral load in blood and saliva are frequent (Moon et al., 2004; Strauch et al., 1974). These data are further backed by DNA analysis in SLE patients (Yu et al., 2005).

The increased EBV load described in SLE patients could be dependent on an increased replication or a higher number of latenly infected B cells. Recently, Gross et al. (2005) showed that SLE patients have higher number of cells carrying EBV in blood and that it is particularly high during the flares, typically observed in SLE. It was thus suggested that the immune system perturbation associated with SLE can be the cause of EBV spread and of the increased number of infected cells (Gross et al., 2005).

The immunological dysfunction in SLE is correlated both to B and T cell abnormalities which result in production of autoantibodies to nuclear antigens generating immune complexes which consequently damage various organs. The B cell dysfunction in SLE creates auto-reactive B cell population which produces anti-Sm and anti SSA/Ro antibodies (Arbuckle et al., 2003). Interestingly, antibodies directed against Sm and Ro proteins cross-react with the EBV antigens EBNA-1 and EBNA-2, suggesting molecular mimicry as a potential mechanism (Sabbadini et al., 1993; Incaprera et al., 1998; McClain et al., 2005). The role of the cross-reaction between antibodies anti-EBNA1 and autoantibodies to Sm and dsDNA in the onset of SLE is further strengthened by the observation that rabbits and mice immunized with EBNA1 DNA show symptoms of SLE (Poole et al., 2008; James et al., 1997b). Dysfunction of T cell responses in SLE has also been described. Specifically, a disruption in EBNA1 related antibody-dependent cellular cytotoxicity and a reduced T cell cytotoxicity against EBV infected cells are noted in SLE (Rothfield et al., 1973; McClain et al., 2006). Additionally, EBV specific T cell cytotoxicity is also compromised in SLE patients (Berner et al., 2005).

In this study, we have extended previous observations on altered EBV specific responses in SLE patients, to subjects with high ANA titers and various ENA positivity. Moreover, we found that a recent EBV infection or reactivation might have an important role in eliciting autoantibody deregulation.

2. Materials and methods

2.1. Patient population

Ninety-two patients attending the outpatients' department at our hospital were recruited for this study. Criteria for selection were anti-nuclear positive staining ≥ 1:160 and/or ENA/dsDNA positive detection (Table 1). The cut-off titer was chosen according to the existing guidelines which indicate that a titer over 1:160 could suggest the presence of an autoimmune disease (Kavanaugh et al., 2000). One hundred-forty-six healthy volunteers were recruited among the blood donors from the Hematology Department. All healthy donors were negative for anti-nuclear antibody.

2.2. Indirect Immunofluorescence (IFI) staining

Anti-nuclear antibodies detection was performed by IFI using Hep-2 laryngeal carcinoma cells as substrate (Delta Biological). The dsDNA autoantibodies were detected on Crithidia Luciliae cells (Chematil) according to manufacturer instructions. The anti-ENA autoantibodies, U1RNP (A,B,C), Sm-D, Scl70, CENP B, Jo-1, SSA/Ro, SSB/La were detected in ELISA (Alifax) and the results were confirmed by immunoblotting (Euroimmun).

Table 1
ANA, ENA and dsDNA antibodies expression in 92 patients.[a]

Number of patients ($n=92$)	ANA-main fluorescence pattern	ENA positive ($n=57$)	dsDNA positive ($n=10$)
19	Anti-centromere	17 CENP B 1 CENPB/SSA/SSB	1
24	Homogeneous	4 Scl70 3 SSA/Ro	8
3	Nucleolus organized region (NOR 90)	–	–
6	Nucleolar	–	–
40	Speckled	13 SSA/Ro 11 SSA/Ro + SSB/La 5 RNP 1 Jo-1 1 SSA/Ro + RNP 1 SSA/Ro + Sm + RNP	1

[a] Patients included in the study showed ANA titer ≥1:160.

2.3. EBV-specific antigen detection

EBV-VCA IgM, -VCA IgG and -EBNA-1 IgG detection were performed by ELISA (Diasorin). EA IgG detection method is based on the ELISA principle and was performed using CHORUS kit and instrument (DIESSE).

2.4. Statistical analysis

Statistical analysis was performed using the GraphPad software. The bee-swarm plots were prepared with the help of R software. The P value and the statistical significance were evaluated by unpaired t-test. The analysis between percentage of positive and negative groups was performed by Fischer's exact test.

3. Results

3.1. Increased anti-VCA-IgG in ANA positive patients

Ninety-two ANA positive patients with different reactivity patterns (Table 1) and 146 healthy blood donors were compared for EBV specific antibody response. We found that VCA-IgG was significantly higher in the ANA-positive patients group ($P=0.0001$, Table 2, Fig. 1). A significant difference in VCA-IgG was also found when the analysis was restricted to patients with a concomitant ANA and ENA ($n=57$) positivity ($P=0.0002$) (Fig. 2). The ENA positive patients showed a various pattern of reactivity (Table 1). No individual from the control group showed any anti-nuclear reactivity.

In contrast, the anti-VCA IgM and anti-EBNA-1 IgG antibody levels were not significantly different in the ANA positive and healthy control groups (Table 2). Additionally, the percentage of positive patients for these antibodies did not show significant differences among the two groups (Table 2).

Among the ANA-positive subjects, we found a higher percentage of VCA-IgG and/or VCA IgM positive patients that were concomitantly negative for anti-EBNA-1 antibodies. The VCA IgM/IgG positivity associated with EBNA-1 negativity is indicative of a recent EBV infection (Table 2) (Klutts et al., 2009).

3.2. Increased EBNA1 IgG in SSA/Ro positive patients

A high homology between SSA/Ro and EBNA-1 proteins has been previously shown (McClain et al., 2005). Based on this, we selected those patients positive for SSA/Ro antigen without any other ENA positivity and compared their antibody response against

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