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Xenotropic Murine Leukemia Virus-Related Virus (XMRV) in Patients with Systemic Lupus Erythematosus

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Abstract
Objectives Xenotropic murine leukemia virus-related virus (XMRV)-specific proviral DNA has been recently detected in peripheral blood mononuclear cells of patients with chronic fatigue syndrome. Since chronic fatigue is commonly reported in patients with systemic lupus erythematosus (SLE) we aimed at testing the presence of this virus in these patients.
Methods Ninety-five SLE patients, 45 of whom had a Fatigue Severity Scale score higher than 3, were included. Molecular analyses were performed by PCR from DNA obtained from the whole blood of both SLE patients and 50 healthy controls.
Results None of the 145 samples analyzed yielded the specific XMRV PCR product.
Conclusions We conclude that XMRV is not detected in blood neither from SLE patients nor from healthy controls. It leads to infer that other environmental and biological triggers (different from XMRV) may account for the increased levels of fatigue over the course of SLE.

Keywords Systemic lupus erythematosus · XMRV · chronic fatigue

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Fatigue is commonly reported by systemic lupus erythematosus (SLE) patients [1]. It is present in up to 90% of the patients [2], and around 50% of them consider it as the most disabling disease symptom [3]. It may occur even in the absence of other clinical manifestations indicative of active disease. As a matter of fact, neither disease activity nor damage is associated with increased levels of fatigue in SLE patients [4, 5]. On the other hand, the use of medications does not seem to account for the fatigue perceived by the patient [5]. This suggests that factors other than disease activity and treatment may be involved.

A recent study has identified xenotropic murine leukemia virus-related virus (XMRV)-specific proviral DNA in peripheral blood mononuclear cells (PBMCs) of patients with chronic fatigue syndrome (CFS) [6]. Antibodies against the virus were also found in these patients and the authors showed that it was transmittable from clinical samples to cell cultures. CFS is characterized by debilitating fatigue, chronic inflammation, and other abnormalities of the immune system. Immune alterations are a hallmark and perfectly recognizable features in patients with SLE. All these facts led us to hypothesize that SLE patients (especially those affected with chronic fatigue) might be infected with XMRV.

Patients and Methods

Ninety-five Spanish Caucasian individuals who fulfilled at least four of the American College of Rheumatology criteria for SLE [7] were included. Fatigue was measured using the original instrument developed by Krupp et al., the Fatigue Severity Scale (FSS) [3] that has previously been used in patients with lupus. Fatigue was defined as present if the FSS score was equal or higher than 3.0. Forty-five of

our SLE patients (43 women and two men; mean age, 42.8 years; range, 21–62 years) had an FSS score higher than 3 (mean±SD, 4.5±1.3), whereas 50 SLE patients (46 women and four men; mean age, 40.8 years; range, 18–82 years) had an FSS score lower than 3. As for the SLE activity, it was assessed by the SLE disease activity index (SLEDAI), and those with an SLEDAI equal or higher than 5 were considered to have active disease [8]. None of the patients with chronic fatigue presented a SLEDAI higher than 5. Among the non-chronic fatigue SLE patients, 22 had an SLEDAI higher than 5. With regard to the treatment, ten patients from the chronic fatigue SLE group were receiving nothing, 15 were receiving just corticosteroids, two were receiving immunosuppressors only, two just antimalarial drugs, and 16 patients were on corticosteroids plus immunosuppressors and/or antimalarial drugs. In the non-chronic fatigue SLE group, eight patients were receiving nothing, six were receiving just corticosteroids, six were just taking immunosuppressors, and 30 patients were on corticosteroids plus immunosuppressors and/or antimalarial drugs or biologicals (monoclonal antibodies). An ethnically matched random healthy control population (blood donors) was also included in the study (n=50, 47 women and three women; mean age, 42.2 years; range, 20–64 years).

Genomic DNA was obtained from 10 mL of whole blood using the QIAamp DNA Blood Maxi Kit (QIAGEN GmbH, Hilden, Germany). DNA concentrations ranged from 100 to 300 ng/μL. All samples were screened by single-round polymerase chain reaction (PCR) to amplify a *gag* XMRV fragment. GeneCraft (Heidelberg, Germany) supplied all the reagents but the primers (which were purchased from Operon Biotechnologies GmbH from Cologne, Germany) and the reaction buffer (buffer number 2, from the "Expand Long Template PCR System" kit (Roche Diagnostics, Barcelona, Spain). Besides 1 μL of DNA, each 25-μL reaction contained: 0.5 mM dNTPs, 1X Taq polymerase buffer, 2 units of Taq DNA polymerase,

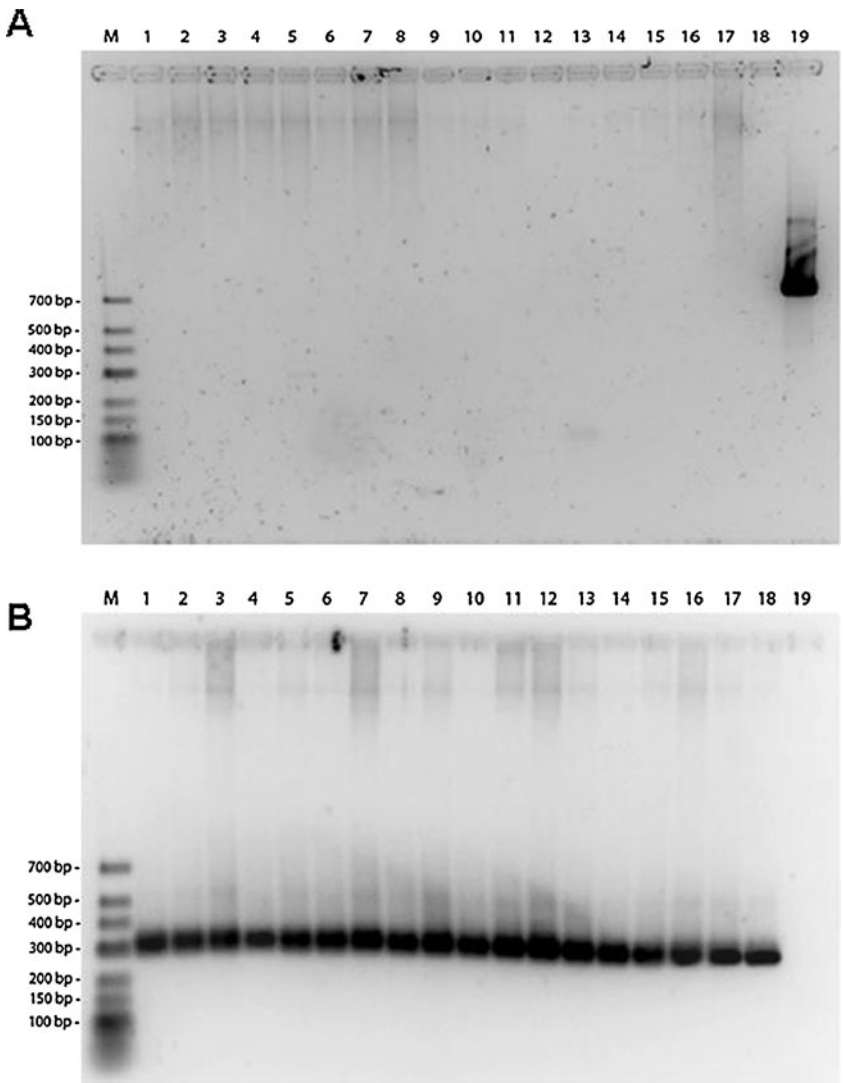

Fig. 1 PCR results for XMRV *gag* **a** and β-actin **b** sequences from whole blood of healthy blood donors (*lanes 1–8* in **a** and **b**) and SLE patients with chronic fatigue (*lanes 9–17* in **a**, and *lanes 9–18* in **b**). A negative control (water) was included in each run (*lane 18* in **a** and *lane 19* in **b**). Positive control consisted of a $1:10^{10}$ dilution of a plasmid with a full-length XMRV (isolate VP62) insert provided at a concentration of 1 μg/μL (*lane 19* in **a**). *M* DNA marker. Two percent agarose gel stained with SYBR Green I

and 7.5 pmol of primers P1 and P2 (P1: 5′-ATCAGT TAACCTACCCGAGTCGGAC-3′; P2: 5′-GCCGCCTCTTCTTCATTGTTCTC-3′). These are the same primers used by Lombardi et al. [6] which amplify a 736-bp-long XMRV *gag* PCR product. Reactions were carried out in a Whatman Biometra TGradient thermocycler (Goettingen, Germany) under the following conditions: denaturation at 94°C for 4 min; 45 amplification cycles consisting of 30 s at 94°C, 30 s at 57°C, and 1 min at 72°C; and seven additional minutes at 72°C. The positive control was a $1{:}10^{10}$ dilution of a plasmid with a full-length XMRV (isolate VP62) insert provided at a concentration of 1 μg/μL and obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (XMRV VP62 cDNA from Drs. Robert H. Silverman and Beihua Dong) [9]. Measures were taken to prevent contamination, and a negative control (water) was included in each run. To ensure that the DNA was amplifiable, all the samples were also subjected to another PCR with specific primers for the housekeeping β-actin gene (P3: 5′-TCACCCA CACTGTGCCCATCTACGA-3′; P4: 5′-CAGCG GAACCGCTCATTGCCAATGG-3′) under the same conditions described above and run for 30 cycles. Ten microliters of each reaction were loaded in 2% agarose gel. Following electrophoresis, fragments were visualized by staining with SYBR Green I (Sigma, Madrid, Spain).

Results and Discussion

In this work, we have addressed the potential link between XMRV and SLE. Other authors have also evaluated the presence of XMRV sequences in another autoimmune disease, rheumatoid arthritis [10]. In this case, no association was detected. Similarly, none of the 145 samples we analyzed yielded the specific XMRV PCR product, whereas the β-actin fragment was amplified from all of them (see Fig. 1). Therefore, we conclude that XMRV is not detected in PBMCs neither from SLE patients nor from healthy controls under the conditions used in our study. Lombardi et al. [6] identified XMRV DNA in 67% of patients with CFS and 3.7% healthy controls. Recently, Lo et al. [11] have also detected murine leukemia virus (MLV)-related sequences in 86.5% of their CFS samples and 6.8% of control blood donors, whereas other studies from USA [10, 12] have reported negative results. On the other hand, all the studies performed in Europe so far have failed to detect it both in healthy populations and CFS patients [13–15]. Although the prevalence of XMRV in Spanish CFS patients is currently unknown, fibromyalgia patients (who share many features with CFS) have recently found to be negative for MLV-related sequences in Spain [16]. Overall, it leads us to infer that European distribution of XMRV seems to be low. Furthermore, a study performed in Chinese patients with CFS has also reported negative results [17]. In view of all these conflicting results, the association between XMRV and CFS remains controversial. The heterogeneity in *gag* gene sequences observed by Lo et al. [11] suggests that geographic differences in different MLV-related viruses may be considerable and could affect both the sensitivity and the specificity of the PCRs.

In an attempt to follow the Fukuda criteria for CFS [18] in SLE, we selected patients with chronic fatigue but with an inactive SLE. Among our SLE patients without chronic fatigue, some had active SLE and others were stable for the disease. XMRV was not associated neither to chronic fatigue nor to SLEDAI. Although it has recently been published that XMRV-specific sequences may be detected in respiratory secretions of patients with respiratory tract infection under immunosuppression treatment [19], the absence of proviral XMRV DNA in all of our patients' PBMCs subjected to different treatments led us to conclude that it did not depend on whether the patient was receiving or not a particular drug.

Many factors could potentially be associated with increased levels of fatigue over the course of SLE. It is perfectly feasible that other environmental and biological triggers (different from XMRV) may account for the disease. In multivariable analyses, the presence of constitutional symptoms (such as weight loss and fever), among others, has been found to be associated to increased levels of fatigue [4]. Furthermore, it may be mediated by the coexistence of psychological and behavioral variables, such as inadequate social support and the patient's perception that she/he has an incurable disease. Thus, fatigue in SLE is likely multifactorial in origin. To think that just a virus could be responsible for it could even seem naïve. Nevertheless, although we have not detected XMRV in their PBMCs, we cannot rule out the possibility that other known or unknown microorganisms (exogenous or induced as a consequence of the characteristic immune deregulation of SLE) may contribute to exacerbate the chronic fatigue so frequently observed in these patients.

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References

1. Tench CM, McCurdie I, White PD, D'Cruz DP. The prevalence and associations of fatigue in systemic lupus erythematosus. Rheumatology (Oxford). 2000;39(11):1249–54.
2. Zonana-Nacach A, Roseman JM, McGwin Jr G, Friedman AW, Baethge BA, Reveille JD, et al. Systemic lupus erythematosus in three ethnic groups. VI: Factors associated with fatigue within

5 years of criteria diagnosis. LUMINA Study Group. LUpus in MInority populations: nature vs Nurture. Lupus. 2000;9(2):101–9.
3. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol. 1989;46(10):1121–3.
4. Burgos PI, Alarcon GS, McGwin Jr G, Crews KQ, Reveille JD, Vila LM. Disease activity and damage are not associated with increased levels of fatigue in systemic lupus erythematosus patients from a multiethnic cohort: LXVII. Arthritis Rheum. 2009;61(9):1179–86.
5. Jump RL, Robinson ME, Armstrong AE, Barnes EV, Kilbourn KM, Richards HB. Fatigue in systemic lupus erythematosus: contributions of disease activity, pain, depression, and perceived social support. J Rheumatol. 2005;32(9):1699–705.
6. Lombardi VC, Ruscetti FW, Das Gupta J, Pfost MA, Hagen KS, Peterson DL, et al. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. Science. 2009;326(5952):585–9.
7. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40(9):1725.
8. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum. 1992;35(6):630–40.
9. Urisman A, Molinaro RJ, Fischer N, Plummer SJ, Casey G, Klein EA, et al. Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. PLoS Pathog. 2006;2(3):e25.
10. Henrich TX, Li JX, Felsenstein D, Kotton CX, Plenge RX, Pereyra F, et al. Xenotropic murine leukemia virus-related virus prevalence in patients with chronic fatigue syndrome or chronic immunomodulatory conditions. J Infect Dis. 2010;202(10):1478–81.
11. Lo SC, Pripuzova N, Li B, Komaroff AL, Hung GC, Wang R, et al. Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. Proc Natl Acad Sci USA. 2010;107(36):15874–9.
12. Switzer WM, Jia H, Hohn O, Zheng H, Tang S, Shankar A, et al. Absence of evidence of xenotropic murine leukemia virus-related virus infection in persons with chronic fatigue syndrome and healthy controls in the United States. Retrovirology. 2010;7:57.
13. Erlwein O, Kaye S, McClure MO, Weber J, Wills G, Collier D, et al. Failure to detect the novel retrovirus XMRV in chronic fatigue syndrome. PLoS ONE. 2010;5(1):e8519.
14. van Kuppeveld FJ, de Jong AS, Lanke KH, Verhaegh GW, Melchers WJ, Swanink CM, et al. Prevalence of xenotropic murine leukaemia virus-related virus in patients with chronic fatigue syndrome in the Netherlands: retrospective analysis of samples from an established cohort. BMJ. 2010;340:c1018.
15. Groom HC, Boucherit VC, Makinson K, Randal E, Baptista S, Hagan S, et al. Absence of xenotropic murine leukaemia virus-related virus in UK patients with chronic fatigue syndrome. Retrovirology. 2010;7(1):10.
16. Luczkowiak J, Sierra O, Gonzalez-Martin JJ, Herrero-Beaumont G, Delgado R. No xenotropic murine leukemia virus-related virus detected in fibromyalgia patients. Emerg Infect Dis. 2011;17 (2):314–5.
17. Hong P, Li J, Li Y. Failure to detect Xenotropic murine leukaemia virus-related virus in Chinese patients with chronic fatigue syndrome. Virol J. 2010;7:224.
18. Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med. 1994;121(12):953–9.
19. Fischer N, Schulz C, Stieler K, Hohn O, Lange C, Drosten C, et al. Xenotropic murine leukemia virus-related gammaretrovirus in respiratory tract. Emerg Infect Dis. 2010;16(6):1000–2.