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IgG reactive with the p40 protein encoded by the LINE-1 retroelement: comment on the article by Carter et al

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To the Editor:

The nature and specificity of the self-antigens targeted by autoantibodies characteristic of systemic autoimmune diseases can suggest clues to mechanisms initiating disease. In systemic lupus erythematosus (SLE), anti-double stranded DNA autoantibodies are features of active disease and are particularly associated with lupus nephritis, and the identity of the self or foreign antigens that “break tolerance”, leading to anti-dsDNA and other autoantibody specificities, has been a topic of extensive study. In that context, the studies published by Carter et al (1) identifying reactivity of sera from SLE patients with the 40 kD protein product of the first open-reading frame (ORF) of the human LINE-1 (L1) retroelement are of great interest.

The authors studied the reactivity of sera from SLE patients, disease control patients with systemic sclerosis and healthy donors with purified L1 p40 using both immunoblot and ELISA techniques. Several striking observations were noted. Not only sera from SLE patients but also samples from healthy donors reacted with p40 by immunoblot. A large number of patient and control samples were then tested by ELISA, using the same purified p40 protein as antigen. The ELISA data largely supported the immunoblot results, with SLE sera showing significantly higher levels of IgG reactive with L1 p40 than sera from healthy donors.

Data from my laboratory support those results. A His-tagged L1 ORF1 p40 protein was expressed and purified and used in immunoblot and ELISA studies. The immunoblot shown in the Figure panel A demonstrates analysis of sera from 7 healthy donors and 7 SLE patients. Six of the 7 SLE sera showed bands, some strong, corresponding to 42 kD, shown in previous studies to identify our p40 fusion protein. Surprisingly, all of the sera from healthy donors also reacted with the electrophoresed p40 protein, although in most cases the bands were less strong than those identified by SLE sera. We developed an ELISA using the recombinant L1 p40 protein in an antigen-antibody capture assay. Like the results from Carter et al, we found significantly higher expression of IgG reactive with L1 p40 in SLE sera than in sera from healthy donors (Figure, panel B). Together, our immunoblot and ELISA results are highly similar to those of Carter et al, showing reactivity of both SLE and

healthy donor sera with purified L1 p40 protein by immunoblot and the levels of IgG anti-p40 in SLE patients' sera significantly higher than those of healthy donors.

What is particularly striking in the study by Carter et al and not addressed in our experiments is the relationship between presence of anti-p40 antibodies in patient sera and clinical and serologic features of active disease. High levels of anti-p40 were associated with SLEDAI score and lupus nephritis. Most notable was the highly significant association of p40 level with presence of anti-DNA antibodies as well as anti-C1q autoantibodies and low C3. These significant associations raise the question of whether the detected anti-p40 reactivity in fact reflects reactivity with DNA. We demonstrated that both RNA and DNA are associated with ribonucleoprotein (RNP) particles containing L1 p40, and the p40 protein is released from the particles following 30 minute incubation with RNase or DNase (data not shown). Detailed analyses by others have demonstrated that the L1 p40 monomer forms a coiled coil-mediated trimer that binds DNA non-specifically with high affinity (2). Carter et al considered the potential for IgG in their test sera to bind DNA and supported the specificity of SLE IgG for L1 p40 by inhibiting reactivity in their ELISA with a 10-fold excess of soluble p40 (we noted a similar result taking the same approach). They also included DNase in the ELISA procedure without reducing the level of IgG reactivity. However, the DNase was added to the adsorbed p40 at the same time as patient serum. Further investigation of the contribution of DNA to the p40 reactivity seems worthwhile in order to fully interpret the presented results. As noted in their paper, anti-DNA antibodies are frequently detected in sera from healthy subjects, again raising the possibility that DNA reactivity could be contributing to the reactivity of IgG from healthy donors and SLE patients with L1 p40 in the studies by Carter et al and those from our laboratory.

The potential for genomic retroelements to contribute to the induction or amplification of systemic autoimmune disease has been raised by many investigators. Those elements, with sequence and genomic structure similar to retroviruses, are abundant in our genome. LINE-1 is of particular interest in SLE (3), with a number of "active" transposable L1s in the human genome. L1 transcription and translation are usually rigorously controlled through genome methylation and mechanisms implemented by members of the APOBEC family, endogenous nucleases and other enzymes that edit potentially stimulatory nucleic acids, yet our previous studies demonstrated that L1 RNA transcripts and L1 p40 protein can be expressed in the involved renal and salivary gland tissue of patients with SLE or Sjogren's syndrome, respectively (4). In addition, L1 nucleic acid can induce type I interferon, a key pathogenic mediator in SLE (4). In that regard, the data from Carter et al provide support for a relationship between high levels of IgG anti-p40 and a high type I interferon score (1). The RNP particles that contain L1 p40, associated L1 RNA, DNA as well as a multitude of proteins that have been implicated as autoantigens in systemic autoimmune diseases (5) should be priority targets for detailed study of the mechanisms through which the viral remnants in our genomes might act as initiators of autoimmunity.

Acknowledgments

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Reactivity of SLE and healthy donor IgG with LINE-1 p40 fusion protein.

An L1 ORF1 sequence subcloned from the embryonal carcinoma NTERA-2 clone D1 cell line was cloned into a pQE-30 expression vector. E.coli were transformed and p40 protein expressed and purified with a histidine tag. A. 700 ng p40 fusion protein per lane was electrophoresed and transferred to PVDF membranes, cut into strips, immunoblotted with 1/100 sera from SLE patients or healthy donors and developed with anti-human IgG. B. Microwells were coated with 500 ng/well of L1 p40 fusion protein, blocked with 5% milk, and incubated overnight with a 1/50 dilution of SLE or healthy donor sera. In addition, 5 samples from patients with other autoimmune rheumatic diseases (3 polymyositis, 1 Sjogren's syndrome, 1 psoriatic arthritis) were assayed. After washing, alkaline phosphatase-labeled goat anti-human IgG was added and the color reaction measured at 405 nm absorbance. The presented experiments were performed by Jun Liang Zhou. This work

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