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High Prevalence and Disease Correlation of Autoantibodies Against p40 Encoded by Long Interspersed Nuclear Elements in Systemic Lupus Erythematosus

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Objective. Long interspersed nuclear element 1 (LINE-1) encodes 2 proteins, the RNA binding protein p40 and endonuclease and reverse transcriptase (open-reading frame 2p [ORF2p]), which are both required for LINE-1 to retrotranspose. In cells expressing LINE-1, these proteins assemble with LINE-1 RNA and additional RNA binding proteins, some of which are well-known autoantigens in patients with systemic lupus erythematosus (SLE). This study was undertaken to investigate whether SLE patients also produce autoantibodies against LINE-1 p40.

Methods. Highly purified p40 protein was used to quantitate IgG autoantibodies in serum from 172 SLE patients and from disease controls and age-matched healthy subjects by immunoblotting and enzyme-linked immunosorbent assay (ELISA). Preparations of p40 that also contained associated proteins were analyzed by immunoblotting with patient sera.

Results. Antibodies reactive with p40 were detected in the majority of patients and many healthy controls. Their levels were higher in patients with SLE, but not those with systemic sclerosis, compared to healthy subjects (P = 0.01). Anti-p40 reactivity was higher in patients during a flare than in patients with disease in remission (P = 0.03); correlated with the SLE Disease Activity Index score (P = 0.0002), type I interferon score (P = 0.006), decrease in complement C3 level (P = 0.0001), the presence of anti-DNA antibodies (P < 0.0001) and anti-C1q antibodies (P = 0.004), and current or past history of nephritis (P = 0.02 and P = 0.003, respectively); and correlated inversely with age (r = –0.49, P < 0.0001). SLE patient sera also reacted with p40-associated proteins.

Conclusion. Autoantibodies reacting with LINE-1 p40 characterize a population of SLE patients with severe and active disease. These autoantibodies may represent an early immune response against LINE-1 p40 that does not yet by itself imply clinically significant autoimmunity, but may represent an early, and still reversible, step toward SLE pathogenesis.

INTRODUCTION

Long interspersed nuclear element 1 (LINE-1; also known as L1) constitutes 17% of the human genome (1–4). While most of the ~500,000 LINE-1 copies are mutated and truncated, some ~180 are seemingly intact and a handful of them remain "hot" today (5), i.e., they continue to retrotranspose by a copy-and-paste mechanism, occasionally disrupting genes or regulatory regions by novel insertions (6). To counteract this threat, an elaborate set of defense mechanisms has evolved against retroelements and retroviruses (7–12), and it has been proposed that many human diseases, including cancer and immune-mediated diseases, are connected with LINE-1 biology (13,14).

Indeed, loss-of-function mutations in several genes for these defense mechanisms cause a severe developmental disease known as Aicardi-Goutières syndrome (15,16), which is characterized by constitutively high production of type I interferons (IFNs), neurologic deficits due to IFN toxicity, and autoimmunity with all

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the hallmarks of systemic lupus erythematosus (SLE). In patients with Aicardi-Goutières syndrome with mutations in the cytosolic DNase *TREX1* gene (17,18), type I IFNs are produced in response to aberrantly present intracellular DNA (which TREX1 normally degrades). Further, in patients with Aicardi-Goutières syndrome with mutations in *RNASEH2* (17), which degrades DNA–RNA heteroduplexes, or *SAMHD1* (19,20), which removes deoxynucleotides required for reverse transcription, the IFN-driving aberrant DNA apparently results from reverse transcription of cellular RNAs. The cellular enzyme most likely responsible for this reverse transcription is the second open-reading frame (ORF2p) of LINE-1, which encodes a highly efficient reverse transcriptase (21,22) that can use many cellular RNA templates, including its own messenger RNA (mRNA) (3,4) or Alu transcripts, to generate DNA species that may trigger IFN production.

There are additional reasons to suspect that LINE-1 could potentially be involved in SLE development, perpetuation, and/or disease flares: 1) the first ORF of LINE-1 encodes a 40-kd RNA binding protein (p40), which is physically associated with Ro, La, small nuclear RNP 70, and other well-known SLE autoantigens (23–26) together with RNA in heterogenous macromolecular assemblies (possibly stress granules); and 2) while LINE-1 loci are largely silent in healthy subjects, LINE-1 transcripts and p40 protein have been detected in patients with SLE and Sjögren's syndrome (27–29). Furthermore, LINE-1 transcription can be induced by many conditions known to precipitate SLE flares, such as reduced genomic methylation (29), low DNA methyltransferase (DNMT) expression (30), DNMT1 polymorphisms, demethylating drugs (e.g., hydralazine and procainamide [31]), and ultraviolet light (32). LINE-1 loci are also transcriptionally active in patients with Aicardi-Goutières syndrome (33), suggesting that LINE-1 ORF2p is indeed the reverse transcriptase responsible for the aberrant DNA (34) that drives type I IFN production and the disease in patients with Aicardi-Goutières syndrome (35). Inhibitors of the reverse transcriptase can reduce the IFN gene signature in patients with Aicardi-Goutières syndrome (36).

In this study, we demonstrate that a majority of SLE patients have IgG autoantibodies against LINE-1 p40 protein and that the reactivity against this autoantigen correlates with disease activity and serologic measures of disease. We also show that patients have autoantibodies against some p40-associated proteins.

MATERIALS AND METHODS

SLE patients. A first cohort of patients with SLE (n = 10) was recruited through the University of Washington Division of Rheumatology Biorepository to participate in research studies at the University of Washington Medical Center. The study was approved by regional ethics boards (STUDY00006196), and written informed consent was obtained from all participants. A second cohort of SLE patients with disease in remission (n = 83), SLE patients experiencing a flare (n = 79), disease controls (with systemic sclerosis; n = 20), and healthy individuals (n = 78) was recruited in the Department of Medicine, Skåne University Hospital (Lund, Sweden). The study was approved by the Lund University local ethics board (LU06014520 and LU 378-02). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. The Swedish patient cohorts have been described in great detail previously (37–39).

Purification of LINE-1 ORF1p p40 protein. ORF1p was expressed in *Escherichia coli* LOBSTR pLysS pRare2 (DE3) (40) from plasmid pMT538, containing full-length synthetic human ORF1p (ORFeusHS) with an N-terminal HIS6-TEV sequence in a pETM11 backbone such that cleavage leaves only an N-glycine scar. Protein was purified using nickel–nitrilotriacetic acid affinity, cleaved from the column overnight using excess TEV protease and RNAse A, and then further purified by size exclusion in a buffer containing 50 m*M* HEPES pH 7.8, 500 m*M* NaCl, 10 m*M* $MgCl_2$, and 0.5 m*M* tris(2-carboxyethyl)phosphine. Peak fractions corresponding to monomeric ORF1p were pooled and concentrated at ~8 mg/ml. The purity of this preparation is illustrated in Figure 1A.

Separate p40 preparations were generated to include p40-associated proteins (26). Anti-FLAG affinity capture of C-terminal, FLAG-tagged ORF1p was conducted as previously described (41,42). Briefly, HEK $293T_{LD}$ cells expressing either doxycycline-inducible, intact LINE-1 (*ORF1::FLAG*; pLD288); ORF1p alone (*ΔORF2*; pLD603); or, as a control, empty vector (pCEP-puro), were all subjected to anti-FLAG affinity capture. At the point of elution, ORF1p-containing macromolecules were released either by native elution in 3× FLAG peptide (1 mg/ml) or by the application of lithium dodecyl sulfate–containing NuPAGE sample buffer. For each sample type: 50 mg cell powder per experiment, extracted at 25% (weight/volume) in 20 m*M* HEPES, pH7.4, 1% (volume/volume) Triton X-100, 500 m*M* NaCl, supplemented with protease inhibitors. Centrifugally clarified extracts were combined with 50 µl of anti-FLAG magnetic medium.

Immunoblotting. One microgram of p40 protein per sodium dodecyl sulfate (SDS) gel was resolved by electrophoresis and transferred onto PVDF membranes, which were cut into 12–15 strips, and immunoblotted with patient or healthy control serum, each diluted 1:100, and developed by horseradish peroxidase–conjugated anti-human IgG and enhanced chemiluminescence. Anti–LINE-1 ORF1p antibody, clone 4H1, was from MilliporeSigma.

Enzyme-linked immunosorbent assays (ELISAs). Purified p40 protein was adsorbed onto 96-well polystyrene plates at 330 ng/well in 0.1*M* carbonate (pH 9.6) buffer overnight, washed in phosphate buffered saline (PBS)–Tween, and blocked in 1% bovine serum albumin in PBS for 2 hours. Patient or healthy control serum was added at 0.5%

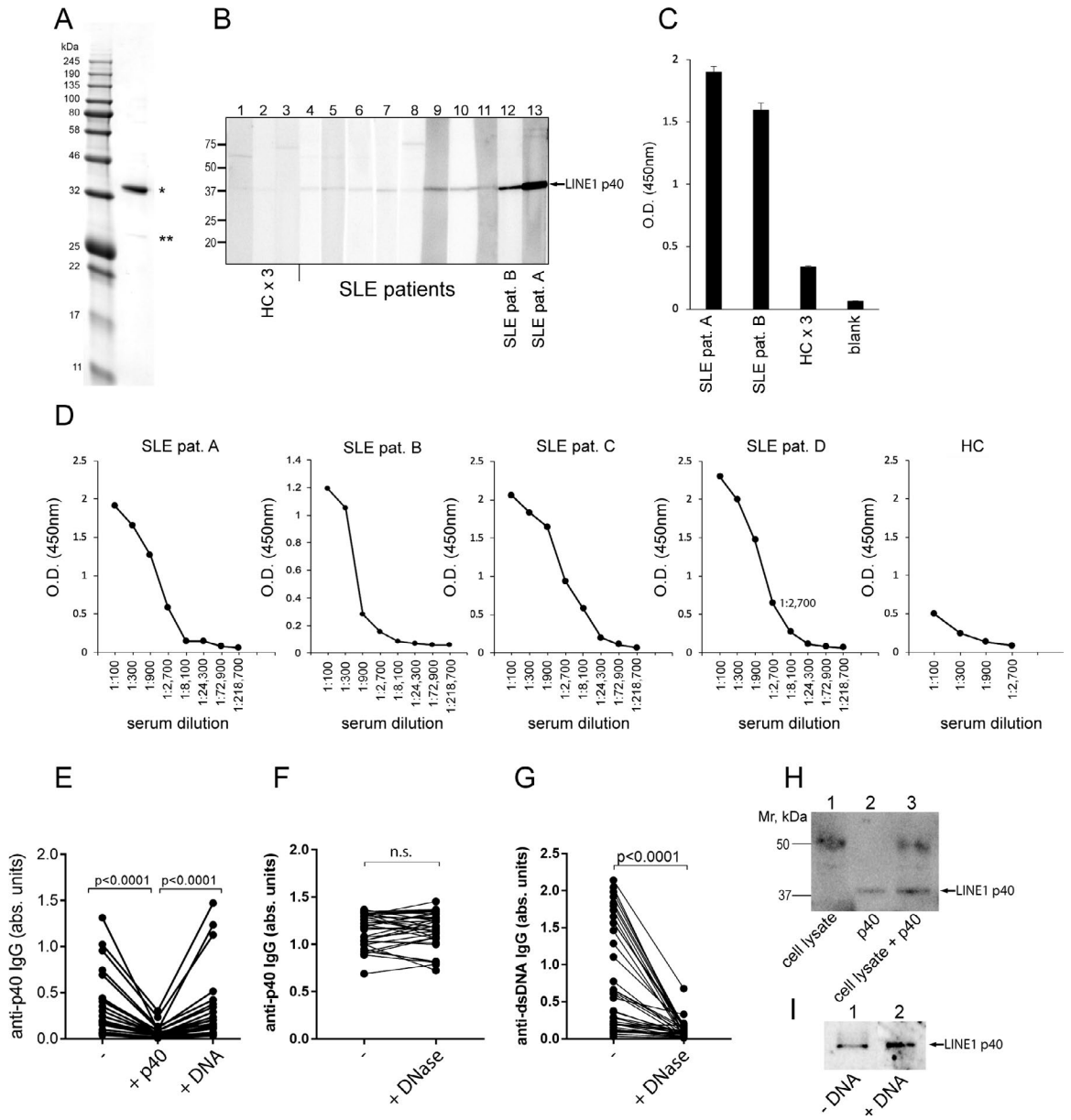

Figure 1. Systemic lupus erythematosus (SLE) sera recognize long interspersed nuclear element 1 (LINE-1) open-reading frame 1 p40 protein. **A**, Coomassie brilliant blue staining of the purified p40 preparation. The **asterisk** denotes p40 and the **double asterisks** indicate a minor amount of cleaved p40. **B**, Immunoblot showing levels of anti-p40 antibodies in sera from 3 healthy controls (HCs; lanes 1–3) and 10 patients with SLE (lanes 4–13). **C**, Enzyme-linked immunosorbent assay (ELISA) for anti-p40 antibodies in the samples from SLE patient A (SLE pat. A; lane 13 in **B**), SLE patient B (lane 12 in **B**), and the 3 healthy controls combined. Bars show the mean ± SD (n = 9 wells per group). **D**, ELISA for anti-p40 antibodies with the indicated dilutions of sera from 4 SLE patients and 1 healthy control, including the same patients (SLE patients A and B) as in **B**. **E**, ELISA for anti-p40 antibodies in SLE patient samples without additions to the assay (–), with a 10-fold excess of soluble p40, and with an equal amount of soluble DNA. **F**, ELISA for anti-p40 antibodies in SLE patient samples without additions to the assay and with DNase. **G**, ELISA for anti-double-stranded DNA (anti-dsDNA) antibodies in SLE patient samples without additions to the assay and with DNase. In **E–G**, lines represent individual patients. **H**, Immunoblot showing anti-p40 reactivity with a neutrophil lysate from SLE patient serum, 300 ng of p40, and a mixture of neutrophil lysate and p40. **I**, Immunoblot showing anti-p40 reactivity in SLE serum without additional treatment and in the presence of 1 μg soluble DNA. abs = absorbance; NS = not significant.

in blocking buffer for overnight incubation at 4°C, washed extensively, and then incubated with a 1:20,000 dilution of horseradish peroxidase–conjugated anti-human IgG. The reaction was then washed and developed with tetramethylbenzidine, the color reaction was terminated with 2*N* sulfuric acid, and absorbance was measured at 450 nm using a plate reader.

In competition ELISAs, 3 μg of soluble p40 or 3 μg of salmon sperm DNA was added to the wells at the same time as patient serum. DNase treatment (to prevent DNA from potentially associating

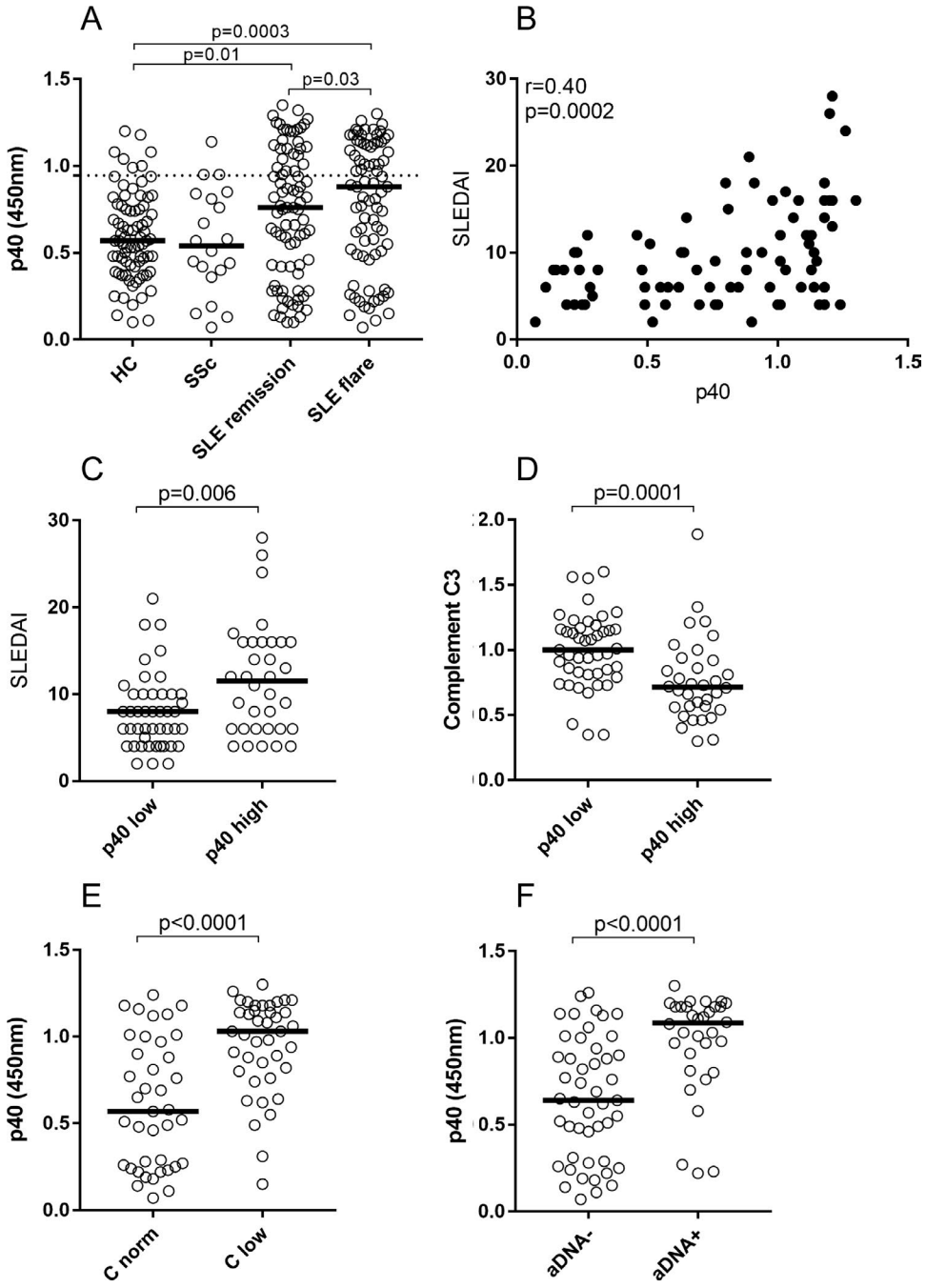

Figure 2. Correlation of levels of autoantibodies against LINE-1 p40 with SLE disease activity. **A**, Quantitation of autoantibodies reactive with LINE-1 p40 in serum from healthy control subjects (n = 78), patients with systemic sclerosis (SSc; n = 20), patients with SLE in remission (n = 83), and patients with SLE during a flare (n = 79). The broken line indicates the 90th percentile in healthy controls. **B**, Correlation between anti-p40 autoantibody levels and SLE Disease Activity Index (SLEDAI) score in the 79 SLE patients experiencing a flare. **C**, SLEDAI score in the SLE patients with anti-p40 reactivity below the 90th percentile in healthy controls (p40 low) and those with anti-p40 reactivity above the 90th percentile in healthy controls (p40 high). **D**, Levels of complement C3 in SLE patients categorized as in **C**. **E**, Anti-p40 reactivity in SLE patients with normal complement levels as defined by the SLEDAI and those with low complement levels as defined by the SLEDAI. **F**, Anti-p40 reactivity in SLE patients without anti-dsDNA antibodies and those with anti-dsDNA antibodies. Symbols represent individual subjects; horizontal lines show the median. *P* values were determined by Mann-Whitney U test in **A** and **C**–**F** and by Spearman's correlation test in **B**. See Figure 1 for other definitions.

with p40) was carried out by adding 1 μg/ml of DNase in buffer with Mg^{2+} and Ca^{2+} to wells with either adsorbed p40 or DNA at the same time patient serum was added.

Type I IFN assay. Type I IFN activity was measured as previously described (43–45). Briefly, endothelial WISH cells were cultured with patient serum and analyzed for the induction of 6

IFN-regulated genes and 3 housekeeping genes using a QuantiGene Plex 2.0 assay according to the recommendations of the manufacturer (Panomics).

Statistical analysis. For unpaired sample sets with non-Gaussian distribution, Mann-Whitney U test and Spearman's correlation test were used, as applicable. For paired sample sets, Wilcoxon matched pairs signed rank test was used. In some analyses, logistic regression analysis was used for dichotomized variables. As a cutoff for positivity, the 90th percentile in the healthy controls was used. GraphPad Prism and IBM SPSS software were used for statistical analyses. *P* values less than 0.05 were considered significant.

RESULTS

Autoantibodies against LINE-1 p40. To determine if SLE patients have autoantibodies of the IgG class against LINE-1 proteins, 1 μg purified p40 was resolved on SDS gels, transferred onto PVDF membranes, which were cut into 15–20 vertical strips, and immunoblotted with 1:100 diluted sera from SLE patients or healthy subjects. As shown in Figure 1B, all 10 SLE patients had antibodies against p40, some strong, some weaker, while healthy subjects showed a very faint band. The intensity of the p40 band was strongest in the 2 SLE patients with the highest SLE Disease Activity Index (SLEDAI) scores (46).

Quantitation of anti-p40 autoantibodies. To better quantitate the anti-p40 reactivity, and to be able to screen a larger number of SLE patients, healthy controls, and other disease controls, we developed an ELISA using the highly purified p40 protein. Reactivity in these assays correlated closely with the intensity of the bands on the p40 immunoblots with sera from the same patients (Figure 1C). As shown in Figure 2A, autoantibodies reactive with p40 were detected in the majority of patients and healthy controls, but their levels were considerably higher in patients with SLE, but not those with systemic sclerosis, than in healthy subjects ($P = 0.01$). Reactivity was also higher in SLE patients experiencing a flare (n = 79) compared to those whose disease was in remission (n = 83) ($P = 0.03$). Using the sera at a higher dilution (1:1,000) resulted in similar data, but with significant loss of resolution for the lower and medium values, while gaining a somewhat better resolution for the highest values. Strongly reactive sera still gave a positive signal at dilutions down to 1:8,100 or 1:24,300 (Figure 1D).

Specificity of anti-p40 autoantibodies. Because p40 can bind nucleic acids, we wanted to exclude the possibility that patient autoantibodies may react with double-stranded DNA (dsDNA) in complex with p40. ELISAs performed in the presence of a 10-fold excess of soluble p40 resulted in a marked decrease in IgG binding to the plate-bound p40, while an equal amount of soluble DNA had no effect (Figure 1E). Similarly, when DNase was included in the ELISA, no change in p40 reactivity was observed (Figure 1F), while binding of autoantibodies to a DNA-coated plate was greatly reduced (Figure 1G). Furthermore, patient sera still recognized p40 when it was mixed with total cell lysates of blood neutrophils (Figure 1H), and the addition of DNA had no effect on anti-p40 reactivity in immunoblot analysis (Figure 1I). These

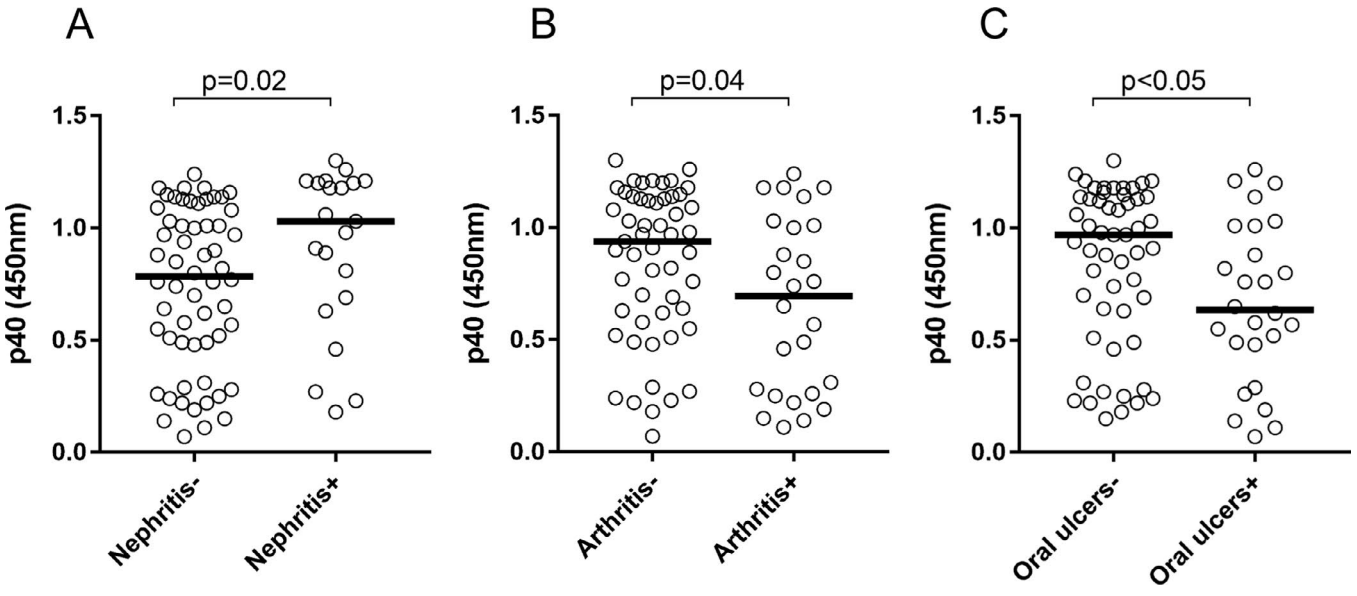

Figure 3. Correlation between anti-p40 autoantibody levels and systemic lupus erythematosus (SLE) organ manifestations. **A**, Reactivity with p40 in SLE patients without established kidney involvement and those with established kidney involvement. **B**, Reactivity with p40 in SLE patients without arthritis and those with arthritis. **C**, Reactivity with p40 in SLE patients without a history of oral ulcers and those with a history of oral ulcers. Symbols represent individual patients; horizontal lines show the median. *P* values were determined by Mann-Whitney U test. See Results for a discussion of the impact of Bonferroni correction on the *P* values.

experiments demonstrate that SLE patient autoantibodies directly recognize LINE-1 p40 protein.

Association of anti-p40 autoantibody levels with higher disease activity. As already suggested by the immunoblot in Figure 1B, anti-p40 reactivity correlated with the SLEDAI score ($P = 0.0002$) in the patients with an SLE flare (Figure 2B). Patients with high titers (above the 90th percentile in healthy controls) had higher SLEDAI scores than those with levels below this cutoff (Figure 2C). Anti-p40 antibody levels were also associated with complement consumption ($P = 0.0001$) (Figures 2D and E) and the presence of anti-dsDNA antibodies ($P < 0.0001$) (Figure 2F). Taken together, these data indicate that higher anti-p40 levels tend to accompany active disease.

Associations of anti-p40 antibody levels with organ manifestations and with other autoantibodies. Higher anti-p40 antibody levels also characterized SLE patients with active lupus nephritis ($P = 0.02$) (Figure 3A), and a history of nephritis ($P = 0.003$) but were inversely correlated with active arthritis ($P = 0.04$) (Figure 3B) and a history of oral ulcers ($P < 0.05$) (Figure 3C). While these correlations were significant, applying a Bonferroni correction for multiple correlates rendered the correlations with both arthritis and oral ulcers nonsignificant.

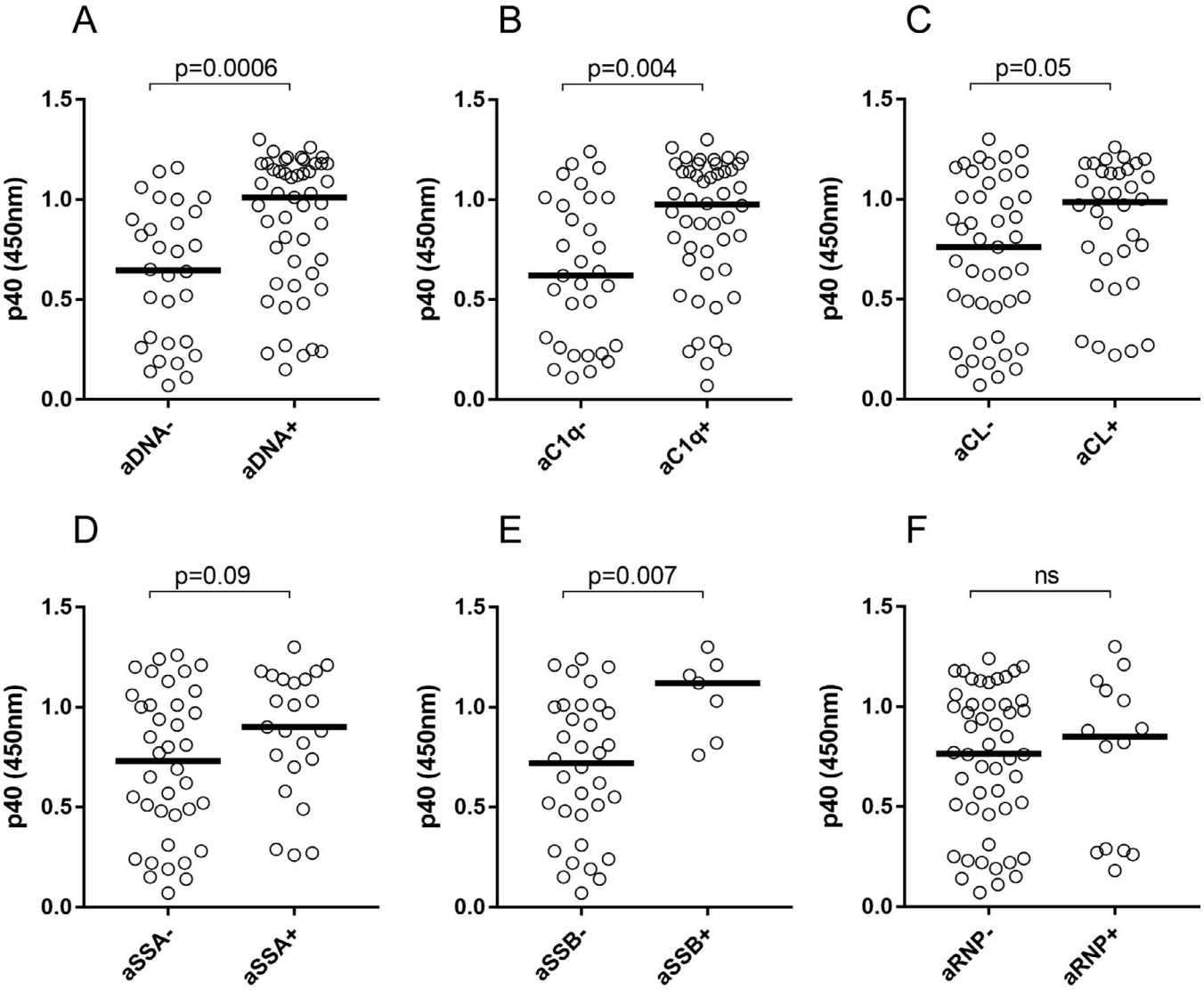

Figure 4. Correlation of levels of autoantibodies against LINE-1 p40 with the presence of other autoantibodies. **A**, Reactivity with p40 in SLE patients without a history of anti-dsDNA positivity and those with a history of anti-dsDNA positivity. **B**, Reactivity with p40 in SLE patients without anti-C1q antibodies and those with anti-C1q antibodies. **C**, Reactivity with p40 in SLE patients without anticardiolipin antibodies (aCLs) and those with aCLs. **D**, Reactivity with p40 in SLE patients without anti-Ro/SSA antibodies and those with anti-Ro/SSA antibodies. **E**, Reactivity with p40 in SLE patients without anti-La/SSB antibodies and those with anti-La/SSB antibodies. **F**, Reactivity with p40 in SLE patients without anti-RNP antibodies and those with anti-RNP antibodies. Symbols represent individual patients; horizontal lines show the median. *P* values were determined by Mann-Whitney U test. See Figure 1 for other definitions.

Whether significant or not, these inverse correlations were unexpected. Since SLE is a heterogenous disease that may include several molecularly distinct endotypes, it is possible that arthritis and oral ulcers arise by molecular mechanisms that do not include LINE-1 biology or p40 autoantibodies.

We next investigated whether anti-p40 antibodies are associated with other common lupus autoantibodies, including those against dsDNA, complement C1q, Sm, RNP, Ro/SSA, La/SSB, and cardiolipin. Briefly, anti-p40 antibody levels were strongly associated with anti-dsDNA levels (P = 0.0006) (Figure 4A) and anti-C1q antibodies (P = 0.004) (Figure 4B), consistent with their association with nephritis, as well as anticardiolipin antibodies (P = 0.05) (Figure 4C). Further, anti-p40 antibodies were correlated with Ro/SSA positivity (P = 0.09) (Figure 4D) and La/SSB positivity (P = 0.007) (Figure 4E), although the correlation with Ro/SSA did not reach statistical significance. There was no significant association with Sm (not shown) or anti-RNP antibodies (Figure 4F).

Increased anti-p40 antibody levels in patients with elevated type I IFN levels. The sera from this cohort of SLE patients were previously analyzed for type I IFN levels using a

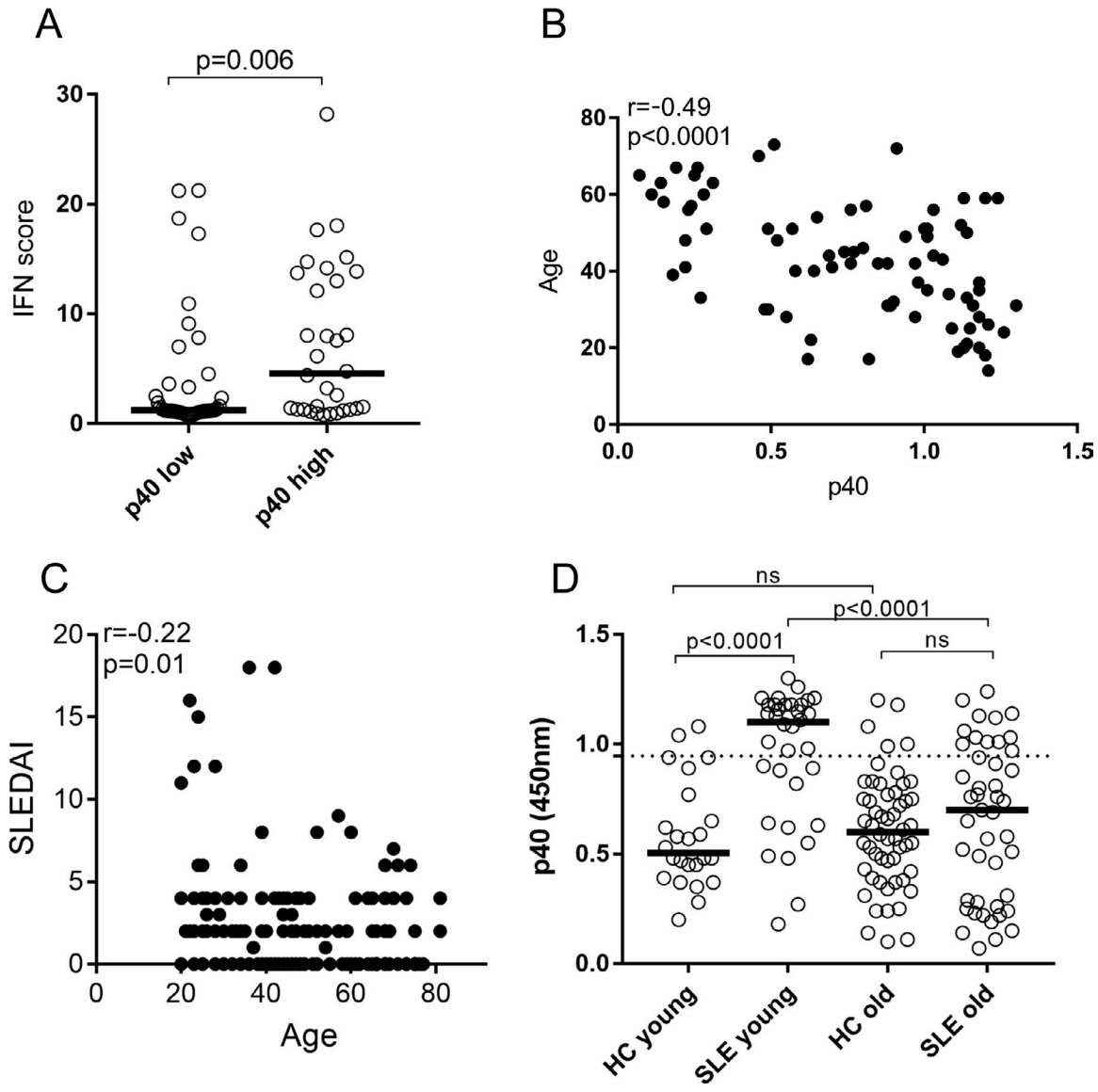

Figure 5. Correlation of type I interferon (IFN) score and of age with anti-p40 autoantibody levels. **A**, Induction of type I IFN–inducible genes by serum from SLE patients with anti-p40 reactivity below the 90th percentile in healthy controls (p40 low) and those with anti-p40 reactivity above the 90th percentile in healthy controls (p40 high). **B**, Inverse correlation between anti-p40 reactivity and age in the SLE patients. **C**, Inverse correlation between SLE Disease Activity Index (SLEDAI) score and age in the SLE patients. **D**, Anti-p40 reactivity in healthy controls and SLE patients grouped by age, where subjects <40 years old were classified as young and subjects ≥40 years old were classified as old. The broken line indicates the 90th percentile in healthy controls. In **A** and **D**, symbols represent individual subjects; horizontal lines show the median. P values were determined by Mann-Whitney U test and Spearman's correlation test. See Figure 1 for other definitions.

reporter cell, and measuring the induction of type I IFN–regulated genes (43–45). Patients with levels of anti-p40 antibodies above the 90th percentile in the healthy subjects also had elevated levels of type I IFNs ($P = 0.006$) (Figure 5A). There was also a direct correlation between autoantibody level and type I IFN activity ($r = 0.36$, $P < 0.0001$). In logistic regression analysis, patients with anti-p40 antibodies more often had high levels of type I IFNs (odds ratio 3.26 [1.25–8.53]; $P = 0.02$).

Inverse correlation of anti-p40 autoantibody levels with age. Unexpectedly, our data set also revealed a highly significant inverse correlation of anti-p40 reactivity with the age of the SLE patients ($r = –0.49$; $P < 0.0001$) (Figure 5B). This association may be, at least in part, explained by the higher SLEDAI in younger patients ($r = –0.22$; $P = 0.01$) (Figure 5C). Nevertheless, when the entire cohort of SLE patients and healthy controls was divided into 2 groups based on age with a cut-off at 40 years, the association of anti-p40 reactivity with SLE became even more marked in the younger group ($P < 0.0001$) (Figure 5D) compared to the total cohort (Figure 2A), while it became statistically insignificant in the older patient group. There was a trend toward increased anti-p40 reactivity in the older group of healthy controls.

Autoantibodies against p40-associated proteins in SLE patients. Since LINE-1 p40, together with its cognate mRNA, is located in cellular stress granules in complex with several other RNA binding proteins, we wanted to see if any of these associated proteins are also targets of the immune response in SLE. To this end, epitope-tagged p40 was purified from overexpressing cells under conditions that allowed associated proteins to co-purify with p40. These preparations were immunoblotted with the sera of SLE patients who had strong reactivity with p40. As shown in Figure 6, weaker bands at ~23, 27, 34, 60, 100, 145, and a smear at ~200 kd were discernible in these blots. Although p40-associated proteins of these sizes have been reported (e.g.,

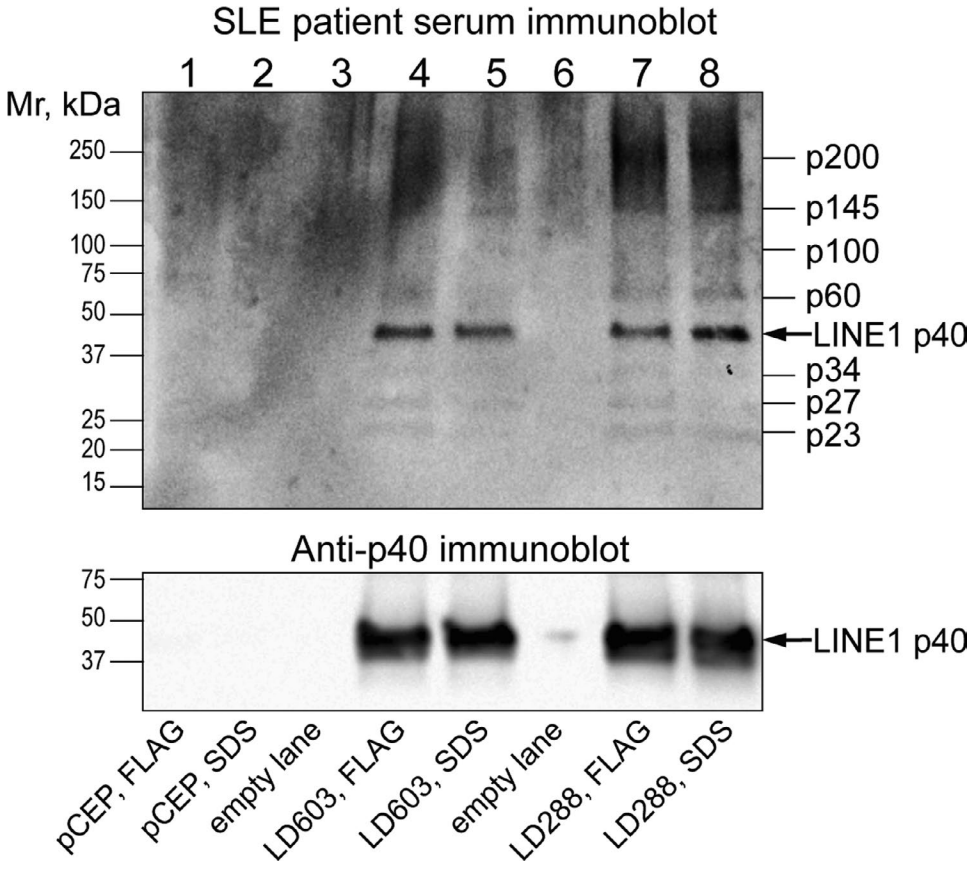

Figure 6. Sera from patients with systemic lupus erythematosus (SLE) contain autoantibodies against proteins that co-purify with p40. Top, Immunoblotting of p40 with sera from patients with SLE with strong reactivity with p40. Bottom, Immunoblotting of the same samples as in the top panel with anti-p40 monoclonal antibody. Similar results were obtained on 2 additional immunoblots. Lane 1, Anti-FLAG immunoprecipitate from cells transfected with empty vector (pCEP) and eluted with FLAG peptide. Lane 2, Anti-FLAG immunoprecipitate from cells transfected with empty vector (pCEP) and eluted with sodium dodecyl sulfate (SDS). Lane 3, Empty. Lane 4, Anti-FLAG immunoprecipitate from cells transfected with the p40 expression vector LD603 and eluted with FLAG peptide. Lane 5, Anti-FLAG immunoprecipitate from cells transfected with the p40 expression vector LD603 and eluted with SDS. Lane 6, Empty. Lane 7, Anti-FLAG immunoprecipitate from cells transfected with the p40 expression vector LD288 and eluted with FLAG peptide. Lane 8, Anti-FLAG immunoprecipitate from cells transfected with the p40 expression vector LD288 and eluted with SDS.

Ro/SSA at 60 kd), the identities of the proteins recognized by SLE sera in Figure 6 remain to be established in future studies.

DISCUSSION

Our findings reveal a previously unrecognized autoantigen in SLE, the LINE-1 ORF1-encoded p40 protein. Unlike most of the well-characterized autoantigens in this disease, p40 is recognized by IgG in a majority of SLE patients (depending on how one defines the threshold for positivity), as well as in many healthy control subjects, albeit mostly with much lower titers. In this respect, anti-p40 autoantibodies resemble anti-dsDNA antibodies, which are also present in a subset of healthy subjects, yet correlate with active SLE. Importantly, we excluded the possibility that anti-p40 autoantibodies represent anti-dsDNA antibodies recognizing p40-bound DNA.

Clearly, anti-p40 antibodies do not by themselves herald clinically relevant autoimmunity, but more likely represent an early phase of self-reactivity that may, or may not, progress toward SLE. In healthy individuals, LINE-1 transcription is typically undetectable, being largely suppressed by DNA methylation. However, expression can be induced by environmental or genetic factors that reduce this methylation, such as certain drugs, reduced expression of methyltransferases, ultraviolet light, and perhaps viral infections. LINE-1 expression is also elevated in malignant cells. Hence, it may be that healthy subjects occasionally express enough p40 to provoke a low level humoral immune response to it. Although we have not studied LINE-1 expression in the thymus, we surmise that these elements may remain transcriptionally silent during T cell selection in the thymus, as well as during B cell maturation in the bone marrow. If so, humans may have a weak, or even absent, central tolerance against LINE-1 p40.

Over the past 25 years, many investigators have suggested that endogenous retroviruses or retroelements may play a role in the pathogenesis of SLE (47–50), proposing various mechanisms for the induction of autoimmunity, such as molecular mimicry, superantigen properties of retroelement proteins, or the perturbation of the transcription of nearby genes. In comparison, only a few studies focused on LINE-1 and, to the best of our knowledge, never tested SLE patients for direct humoral immunity against LINE-1 proteins. Taken together, our findings that nearly all SLE patients have autoantibodies against the LINE-1 p40 protein and that these antibodies are associated with disease activity, specific disease manifestations, low complement levels, other autoantibodies, and type I IFNs, suggest that LINE-1 biology is coupled in some way to SLE pathogenesis.

First, it should be noted that LINE-1 may lack any causative role and perhaps is targeted by the immune response as an innocent bystander. The physical interaction of p40 with well-known SLE autoantigens would be compatible with such a role, at least if one assumes that Ro and La are the intended antigens for the immune response. However, it is equally plausible that the reverse is true, namely, that the LINE-1 proteins, by virtue of their biologic functions, are responsible for the immune attack on cells that express LINE-1 and that other associated proteins are the innocent bystanders. The recognition of p40-associated proteins by SLE autoantibodies (Figure 6) would support this notion. We speculate that individuals who express more LINE-1, either in an episodic or a chronic manner, boost their humoral and cellular immunity against p40 over time and eventually reach levels of response that may be pathogenic.

Cells that express the LINE-1–encoded proteins may display features of virally infected cells. In addition to the immunogenicity of p40, these cells may have sufficient amounts of the ORF2 protein, which has reverse transcriptase activity, to generate DNA copies of available RNA species, such as its own cognate mRNA, Alu element transcripts, and others. Such DNA copies can presumably trigger the cyclic GMP-AMP synthase (cGAS)/stimulator of IFN genes pathway to induce expression of IFNβ (35), which appears to play an important role in driving SLE (51–54). Indeed, transfection of LINE-1 into cells induces production of IFNβ (55). Also, a recent study (56) showed that blood mononuclear cells from ~17% of SLE patients have detectable cyclic GMP-AMP, the second messenger exclusively made by cGAS when it is activated by aberrant intracellular DNA (18,57). Given the minute quantities and rapid turnover of this second messenger, these data likely represent an underestimate. Further, cells that contain active LINE-1 proteins may also up-regulate major histocompatibility complex expression, and other surface markers induced either directly by cGAS through IRF3 activation, or indirectly by IFNβ signaling (58), resulting in a chronic, but perhaps episodic, (auto)immune response against such cells.

An unexpected feature of our data set was the inverse correlation of anti-p40 reactivity with the age of the SLE patients. This inverse correlation can be partly explained by the presence of many young patients with high SLEDAI scores. There was also a trend toward increasing anti-p40 reactivity in healthy controls with age, similar to how anti-dsDNA antibodies tend to increase slowly with age. The difference between younger SLE patients and age-matched controls was more striking than in the total population. Although this age correlation does not have any immediately obvious explanation, it may be related to the decline in general humoral immunity with age (59), the group of young SLE patients with very active disease, or the typical presentation of SLE earlier in life and its slow decline in activity over time. As anti-p40 reactivity was strongly increased in young SLE patients compared to young control subjects, this correlation is compatible with an early role of p40 immunogenicity in the pathogenesis of the disease.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. T. Mustelin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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