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IFN-α Confers Resistance of Systemic Lupus Erythematosus Nephritis to Therapy in NZB/W F1 Mice

Zheng Liu,* Ramalingam Bethunaickan,* Weiqing Huang,* Meera Ramanujam,* Michael P. Madaio,† and Anne Davidson*  

The critical role of IFN-α in the pathogenesis of human systemic lupus erythematosus has been highlighted in recent years. Exposure of young lupus-prone NZB/W F1 mice to IFN-α in vivo leads to an accelerated lupus phenotype that is dependent on T cells and is associated with elevated serum levels of BAFF, IL-6, and TNF-α, increased splenic expression of IL-6 and IL-21, formation of large germinal centers, and the generation of large numbers of short-lived plasma cells that produce IgG2a and IgG3 autoantibodies. In this study, we show that both IgG2a and IgG3 autoantibodies are pathogenic in IFN-α–accelerated lupus, and their production can be dissociated by using low-dose CTLA4-Ig. Only high-dose CTLA4-Ig attenuates both IgG2a and IgG3 autoantibody production and significantly delays death from lupus nephritis. In contrast, BAFF/APRIL blockade has no effect on germinal centers or the production of IgG anti-dsDNA Abs but, if given at the time of IFN-α challenge, delays the progression of lupus by attenuating systemic and renal inflammation. Temporary remission of nephritis induced by combination therapy with cyclophosphamide, anti-CD40L Ab, and CTLA4-Ig is associated with the abrogation of germinal centers and depletion of short-lived plasma cells, but relapse occurs more rapidly than in conventional NZB/W F1 mice. This study demonstrates that IFN-α renders NZB/W F1 relatively resistant to therapeutic intervention and suggests that the IFN signature should be considered when randomizing patients into groups and analyzing the results of human clinical trials in systemic lupus erythematosus. The Journal of Immunology, 2011, 187: 1506–1513.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the loss of tolerance to nucleic acids and their binding proteins and the production of autoantibodies that induce tissue damage (1). Nucleic acid–containing immune complexes are internalized into TLRs containing intracellular compartments in B cells and plasmacytoid dendritic cells (DCs) and amplify disease by enhancing cell activation and by inducing the production of type I IFNs (2). IFN-α induces maturation of myeloid DCs that provide costimulation for naïve CD4+ T cells and produce both IL-6 and BAFF, a cytokine that enhances selection, survival, and class switching of autoreactive B cells (3–5).

In young, lupus-prone NZB/W mice, but not in BALB/c mice, administration of adeno-virus-expressing IFN-α (Ad-IFNα) rapidly induces T cell activation and extensive germinal center (GC) formation with the generation of large numbers of short-lived plasma cells producing IgG2a and IgG3 autoantibodies that cause glomerulonephritis (6, 7). CD4 T cells are absolutely required for the production of pathogenic autoantibodies and the initiation of Ad-IFNα–induced disease (6). In addition, serum BAFF, IL-6, and TNF-α are elevated in Ad-IFNα–treated mice, and B cells in these mice express high levels of TLR7 (6).

Therapeutic agents that target T cell costimulatory pathways or that target BAFF and its homolog APRIL are being developed for the treatment of SLE. CTLA4Ig, a drug that inhibits CD28–B7 costimulation, prevents SLE onset in NZB/W mice but does not induce remission when used as a single agent (8). Remission of nephritis can be induced in NZB/W F1 mice by combination therapy with cyclophosphamide (CTX) and CTLA4Ig (8) or with CTX, CTLA4Ig, and anti-CD40L (triple therapy) (9). A clinical trial of abatacept (human CTLA4Ig) in combination with CTX for SLE nephritis is currently in progress (Clinicaltrials.gov identifier NCT00774852). Inhibition of BAFF can also prevent SLE onset in murine models and reverses disease in some of these models (10–14). An anti-BAFF Ab, belimumab, has shown efficacy in two recent phase III trials of moderately active SLE and is now Food and Drug Administration-approved for the treatment of SLE (15).

In this study, we show that both the B7–CD28 antagonist CTLA4Ig and the BAFF/APRIL inhibitor TACI-Ig delay disease onset in IFN-α–induced SLE but a higher dose of CTLA4Ig is required than in conventional NZB/W mice. Neither drug reverses or delays disease once high-titer autoantibodies are present in the serum. Triple therapy depletes autoantibody-producing plasma cells and induces remission in IFN-α–accelerated disease mice with a similar efficacy as it does in conventional NZB/W F1 mice. However, IFN-α accelerates relapse in a dose-dependent manner. We also show that the clinical effects of CTLA4Ig and TACI-Ig are achieved by different mechanisms. High-dose CTLA4-Ig attenuates both IgG3 and IgG2a autoantibody production and significantly decreases nephritis–associated mortality. In contrast, TACI-Ig treatment does not alter T cell activation or the production of pathogenic anti-dsDNA Abs, but it attenuates the renal inflammatory response to immune complex deposition.

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The online version of this article contains supplemental material.

Abbreviations used in this article: Ad-IFNα, adeno-virus-expressing IFN-α; CTX, cyclophosphamide; DC, dendritic cell; GC, germinal center; MMP, matrix metalloproteinase; MZ, marginal zone; SLE, systemic lupus erythematosus; T FH, follicular Th.
Materials and Methods

Prevention studies

NZB/W F1 females were purchased from Jackson Laboratory (Bar Harbor, ME) and were housed in a pathogen-free facility. Groups of mice were treated at 12 wk of age with a single i.v. injection of Ad-IFNα (3.3 × 10⁹ viral particles, as described previously) (6), and received either fully murine TACI-Ig (12) (500 μg, three times per week) or CTLA4-Ig (16) (100 μg or 200 μg, three times per week) or no treatment starting on the day of virus injection or 21 d thereafter. Mice were bled every other week, and urine was tested for proteinuria by dipstick weekly (Multistick; Fisher Scientific, Pittsburg, PA). Some mice were sacrificed and analyzed after 5 wk and some were followed until death.

Remission induction in Ad-IFNα treated NZB/W F1 mice

NZB/W mice were treated at 12 wk of age with a single i.v. injection of Ad-IFNα (3.3 × 10⁹ viral particles), a dose optimized to induce proteinuria starting at 22–25 d (6). Once fixed proteinuria of >300 mg/dl was detected on two occasions 24 h apart, the mice were randomized to treatment with a single i.p. injection of CTX (Cytoxan; Bristol-Myers Squibb, New York, NY) 50 mg/kg together with CTLA4-Ig (100 μg) and anti-CD40L Ab (250 μg) six doses each over 2 wk (double therapy), or with CTX together with six doses of CTLA4-Ig over 2 wk (double therapy), or with single agents alone (9). Controls received no treatment. Sera were collected and proteinuria was measured twice weekly. Six to 14 mice per group were sacrificed and analyzed 4 wk after treatment initiation. In a separate experiment, NZB/W F1 mice injected with Ad-IFNα (3.3 × 10⁹) [n = 15] or 1.0 × 10⁹ viral particles [n = 15] were treated with triple therapy at the onset of fixed proteinuria, and the survival rate of these mice was compared with that of untreated controls.

All experiments using animals were performed according to protocols that were reviewed and approved by the Institutional Animal Care and Use Committee of the Feinstein Medical Research Institute.

Serum Ig levels and anti-DNA Ab levels

Serum Ig levels and anti-DNA Ab levels were performed by ELISA as described previously (12, 16). Standard curves for Ig were established using serial dilutions of purified murine IgM, IgG2a, or IgG3 (Sigma-Aldrich, St. Louis, MO) and data expressed in micrograms per milliliter. Standard curves for dsDNA binding were obtained using sera from a high-titer mouse assigned an arbitrary level of 512 U and run in serial dilution on each plate.

ELISpot assay

ELISpot assays for total Ig-secreting cells and for anti-dsDNA–secreting cells were performed as described previously (16).

Flow cytometry analysis of spleen and peripheral blood

Spleen and PBMCs were analyzed for cell surface markers as described previously (17). B cells were gated using anti-CD19. T1 cells were CD23lo/IgMhi/CD21+, follicular cells were IgDhi/IgMint, and marginal zone (MZ) cells were CD23hi/IgM(lo)/CD21+, marginal zone (MZ) cells were CD23(lo)/IgM(lo)/CD21+ and class switched cells were IgD(lo)/IgM(lo). Plasma cells were B220(lo)/CD138(lo). CD4+ T cells were classified as memory (CD44hi/CD62Llo) or naive (CD44lo/CD62Lhi). Follicular Th (Tfh) cells were CD4+CXCR5+PD-1+PSGL1+ as described previously (18).

Immunohistochemistry and immunofluorescence

H&E sections were scored for glomerular damage and interstitial inflammation as described previously (12). Cyrosections (5 μm) of kidney and spleen were stained (17) with the following Abs: FITC-conjugated anti-mouse IgG2a, IgG3 (Southern Biotech, Birmingham, AL), peanut agglutinin (Vector Laboratories, Burlingame, CA), or PE-conjugated anti-mouse IgD (BD Pharmingen, San Diego, CA). Images were captured using a Zeiss AxioCam digital camera connected to a Zeiss Axioplan2 microscope.

Real-time PCR analysis of sorted splenic B cells, total spleen cells, and kidney cells

Real-time PCR was performed as described previously (6, 19). Data were first normalized to β-actin expression and then to the mean of naive controls, which was given an arbitrary value of 1.

Serum cytokine levels

Serum IFN-α levels were measured by commercial ELISA (PBL, Piscataway, NJ) at days 3 and 14 after adenosine virus injection in groups of four to five mice. Serum levels of IL-6, IL-17, IL-21, BAFF, IFN-γ, and TNF-α were measured in groups of four to eight mice using a commercial multiplex assay (Assaygate, Ijamsville, MD). Experiments were repeated once.

Statistics

Survival data were analyzed using Kaplan–Meier curves and log-rank test. Comparisons in the other figures and tables were performed using Mann–Whitney U test. The p values ≤ 0.05 were considered significant.

Results

Disease induction

We have previously reported that Ad-IFNα but not control Ad-LacZ induces the onset of SLE in NZB/W F1 mice starting 3–4 wk after virus administration and that this is associated with formation of large germinal centers, production of autoantibodies and activation of CD4 T cells and DCs (6). In contrast, no immune activation occurs in Ad-LacZ treated mice, and they do not develop accelerated disease (6). Increased serum levels of IFN-α were detected at day 3 (78 ± 52 pg/ml) and day 14 (212 ± 110 pg/ml), after Ad-IFNα administration compared with undetectable levels (<25 pg/ml) in naive or Ad-LacZ injected controls (either 3.3 × 10⁵ or 3 × 10⁶ particles), and markedly increased IFN-α mRNA expression was detected in the spleens of Ad-IFNα–treated mice for 2 wk followed by a slow decline over the subsequent 4 wk. This expression was accompanied by increased expression of IFN-inducible genes in the Ad-IFNα treated mice, but not in Ad-LacZ–treated controls (Supplemental Fig. 1). Because of the low dose administered, neither virus induced an acute cytokine response (20, 21) at 1 h (data not shown).

TACI-Ig treatment delays disease onset in Ad-IFNα–treated NZB/W F1 mice

Serum levels of BAFF increased in IFN-α–treated mice starting 2 wk after Ad-IFNα injection (6). To determine whether BAFF contributes to the accelerated disease in Ad-IFNα–treated mice, we administered continuous TACI-Ig treatment (500 μg) three times weekly starting on the same day as Ad-IFNα injection or 3 wk later. TACI-Ig treatment at day 0, but not at day 21 (Supplemental Fig. 2) delayed the onset of proteinuria (p = 0.0005; Fig.

FIGURE 1. Effects of treatments on proteinuria onset and survival. Proteinuria (A) and survival (B) of Ad-IFNα–treated NZB/W F1 mice. Data are pooled from two representative experiments with a total of 15–27 mice per group. The p values against Ad-IFNα–treated controls are: p = 0.0005 (proteinuria) and p = 0.0077 (survival), mice treated with TACI-Ig; p = 0.0555 (proteinuria) and p = 0.0483 (survival), mice treated with low-dose CTLA4-Ig; p < 0.0001 (proteinuria and survival), mice treated with high-dose CTLA4-Ig.
1A) and death ($p = 0.0077$; Fig. 1B) in Ad-IFNα–treated mice. TACI-Ig treatment significantly decreased the numbers of T2, marginal zone, and follicular B cells in the spleen (Table I). Despite the depletion of B cells, CD138+ plasma cells and plasmablasts were found in comparable numbers in the spleens of TACI-Ig treated and untreated mice (Table I). TACI-Ig treatment significantly lowered serum levels of both total IgM and IgM anti-dsDNA Abs, but it had no effect on IgG Abs (Fig. 2). ELISpot assays confirmed that TACI-Ig–treated mice had significantly fewer total IgM secreting cells in the spleens (Fig. 3A), but no decrease in IgG secreting cells. Immunofluorescence staining of spleens and kidneys indicated that TACI-Ig treatment had no effect on the formation of GCs, the generation of IgG producing cells in spleen, or glomerular IgG deposition (Figs. 3B, 4B). Despite the presence of renal IgG deposits, TACI-Ig–treated mice showed significantly less glomerular and interstitial damage than the mice demonstrated similar levels of glomerular and interstitial damage compared with the untreated controls (Fig. 4A). Interestingly, some of the mice in the high-dose CTLA4-Ig group had glomerular Ab deposition in the absence of a serum anti-dsDNA response, indicating that non-DNA binding autoantibodies had arisen (data not shown). Nevertheless, survival was markedly prolonged in this group of mice (Fig. 1B), and they had a significantly lower interstitial renal score when they were sacrificed at 35 wk of age than were IFN-α controls sacrificed at 19 wk (Fig. 4A).

Delayed low-dose CTLA4-Ig treatment had a similar effect on proteinuria and survival as did treatment beginning at day 0 (Supplemental Fig. 2).

Effects of TACI-Ig and CTLA4-Ig treatment on inflammatory mediators in Ad-IFNα treated NZB/W F1 mice

Using real-time PCR, we detected elevated levels of IL-6, IL-10, and IL-21 transcripts in the spleen cells of Ad-IFNα–treated mice. Upregulation of AID, IL-21, and IL-10, but not IL-6, was inhibited by both low- and high-dose CTLA4-Ig (Fig. 5). The expression of AID and IL-21 remained low in the spleens of mice treated with high-dose CTLA4-Ig, even after 23 wk of treatment (data not shown), indicating that differentiation of Tfh cells was inhibited. In contrast, TACI-Ig treatment did not significantly affect the expression of any of these molecules in the spleen (Fig. 5). However, TACI-Ig treatment resulted in significantly decreased serum levels of TNF-α (TACI-Ig–treated $n = 4$) versus Ad-IFNα controls $n = 5$, mean ± SD, 5.3 ± 4.7 versus 25.9 ± 14.1 pg/ml; $p = 0.0159$). No changes were observed in the serum levels of the

### Table I. Number of spleen cell subsets

<table>
<thead>
<tr>
<th></th>
<th>No. of Cells per Spleen</th>
<th>TACI-Ig (n = 8)</th>
<th>CTLA4-Ig Low (n = 7)</th>
<th>CTLA4-Ig High (n = 5)</th>
<th>Untreated (n = 7)</th>
<th>Naive (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell no. × 10⁷</td>
<td>9.5 ± 2.5***</td>
<td>11.1 ± 2.2</td>
<td>11.8 ± 4.7</td>
<td>13.0 ± 3.1</td>
<td>8.1 ± 1.5***</td>
<td></td>
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<tr>
<td>CD19 × 10⁷</td>
<td>1.9 ± 0.8*</td>
<td>4.2 ± 1.1</td>
<td>3.4 ± 1.5</td>
<td>5.8 ± 1.6</td>
<td>3.6 ± 1.1**</td>
<td></td>
</tr>
<tr>
<td>CD19/CD69 × 10⁶</td>
<td>2.4 ± 1.5†</td>
<td>1.6 ± 0.7†***</td>
<td>1.1 ± 0.6*</td>
<td>4.0 ± 2.3</td>
<td>1.2 ± 0.5***</td>
<td></td>
</tr>
<tr>
<td>Follicular × 10⁷</td>
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<td>2.5 ± 0.9</td>
<td>ND</td>
<td>3.0 ± 0.7</td>
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<tr>
<td>T1 × 10⁶</td>
<td>2.2 ± 1.1***</td>
<td>2.7 ± 0.8</td>
<td>ND</td>
<td>5.7 ± 2.1</td>
<td>2.2 ± 0.4**</td>
<td></td>
</tr>
<tr>
<td>T2 × 10⁶</td>
<td>1.3 ± 0.8*</td>
<td>7.8 ± 3.5</td>
<td>ND</td>
<td>7.7 ± 2.4</td>
<td>5.2 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Ti/T2</td>
<td>2.1 ± 1.0*</td>
<td>0.4 ± 0.2</td>
<td>ND</td>
<td>0.8 ± 0.3</td>
<td>0.4 ± 0.1*</td>
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<tr>
<td>MZ × 10⁶</td>
<td>0.7 ± 0.5*</td>
<td>4.9 ± 1.7</td>
<td>ND</td>
<td>6.7 ± 1.9</td>
<td>3.7 ± 0.9***</td>
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</tr>
<tr>
<td>IgM/IgD× 10⁶ (switched)</td>
<td>5.8 ± 3.3</td>
<td>2.0 ± 1.2*</td>
<td>2.0 ± 1.0*</td>
<td>8.2 ± 5.1</td>
<td>1.7 ± 0.8**</td>
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<tr>
<td>CD138* × 10⁶</td>
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<td>0.9 ± 0.4*</td>
<td>0.5 ± 0.3*</td>
<td>3.0 ± 2.3</td>
<td>0.7 ± 0.3***</td>
<td></td>
</tr>
<tr>
<td>CD4 × 10⁷</td>
<td>3.8 ± 1.0</td>
<td>3.6 ± 0.8</td>
<td>3.1 ± 1.3</td>
<td>3.7 ± 0.8</td>
<td>2.4 ± 0.5***</td>
<td></td>
</tr>
<tr>
<td>CD4/CD4+CD62L- (memory) × 10⁷</td>
<td>1.0 ± 0.4</td>
<td>0.6 ± 0.3***</td>
<td>0.4 ± 0.2*</td>
<td>1.1 ± 0.4</td>
<td>0.6 ± 0.1***</td>
<td></td>
</tr>
<tr>
<td>CD4/CD4+CD62L- (naive) × 10⁷</td>
<td>2.4 ± 0.7</td>
<td>2.7 ± 0.4*</td>
<td>2.5 ± 1.1</td>
<td>2.2 ± 0.7</td>
<td>1.6 ± 0.3*</td>
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<td>CD8 × 10⁷</td>
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<td>2.0 ± 0.3***</td>
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<td>1.2 ± 0.2</td>
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</tr>
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<td>CD11b/CD11c× 10⁶</td>
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<td>0.6 ± 0.4*</td>
<td>0.6 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>0.3 ± 0.1***</td>
<td></td>
</tr>
</tbody>
</table>

The p values are compared with untreated control Ad-IFNα mice. Only significant p values are shown.

*p < 0.01, **p < 0.02, ***p < 0.05.
other cytokines measured (data not shown), and neither treatment altered the splenic expression of IFN-\(\alpha\)-inducible genes (Supplemental Fig. 1).

We have shown previously that the kidneys of NZB/W mice express a number of inflammatory mediators at the onset of proteinuria and that some of these decrease to prenephritic levels after remission induction, suggesting that they are biomarkers for active renal inflammation (19). Some of these mediators are elaborated by renal mononuclear phagocytes that become activated at the onset of disease (19, 23), and a subset of these was tested by real-time PCR. We detected elevated expression of matrix metalloproteinase (MMP)-14, CCL2, CCL5, osteopontin, CXCL1, ITGAM, CXCL13, IKBKE, CCL20, LCN2, and VCAM-1 by real-time PCR. We detected elevated expression of matrix metalloproteinase (MMP)-14, CCL2, CCL5, osteopontin, CXCL1, ITGAM, CXCL13, IKBKE, and MMP14 after 23 wk of treatment compared with age matched naive controls. Of these, TACI-Ig treatment significantly inhibited the upregulation of MMP-14, CCL5, IKBKE, CCL20, and LCN2 with a trend toward downregulation of CXCL13. Similarly, in long-term survivors of high-dose CTLA4-Ig treatment, we observed decreased renal expression of ITGAM, LCN2, IKBKE, and MMP14 after 23 wk of treatment compared with IFN-\(\alpha\)-treated mice harvested at the age of 19 wk (Supplemental Fig. 3). These findings show a decrease in renal inflammation that is consistent with the lower renal histologic scores.

**Triple therapy induces remission in Ad-IFN-\(\alpha\)–treated NZB/W F1 mice**

We have shown previously that therapy with a single dose of CTX, together with a 2-wk course of CTLA4-Ig and anti-CD40L Abs, induces prolonged remission of established kidney disease in NZB/W F1 mice (9). In Ad-IFN-\(\alpha\)–treated mice, triple therapy induced complete remission of proteinuria (defined as proteinuria \(\leq 30\) mg/dl measured at least twice, 1 wk apart) in 86.7% of mice treated with \(3.3 \times 10^8\) viral particles and in 66.7% of mice treated with \(1.0 \times 10^9\) viral particles. Triple therapy significantly prolonged the life span of Ad-IFN-\(\alpha\)–treated mice (Fig. 6A), but the mice that had received the higher dose of Ad-IFN-\(\alpha\) relapsed more rapidly than their counterparts treated with a low dose (mean remission duration of high-dose Ad-IFN-\(\alpha\) treatment versus low-dose Ad-IFN-\(\alpha\) treatment = 15.9 \pm 14.0 d versus 73.4 \pm 52.5 d; \(p = 0.0097\)). Control studies were performed in mice receiving the higher virus dose. CTLA4-Ig alone did not induce remission in these mice, whereas single therapy with either CTX or anti-CD40L achieved 50% and 22.2% remission rates, respectively (Fig. 6B). Combined therapy with CTX and CTLA4-Ig induced remission in half of the mice (Fig. 6B). Even when this combination was started at day 21, prior to the onset of proteinuria, the benefit was no different to that of CTX alone (Supplemental Fig. 2). Mice treated with triple therapy showed significantly less glomerular (\(p = 0.0033\)) and interstitial damage (\(p = 0.0129\)) than did the untreated controls (Fig. 6C), whereas mice that received control single or double therapy showed similar levels of renal damage compared with the untreated mice (data not shown).

Spleens were harvested for phenotypic analysis 3–4 wk after treatment initiation (Table II). Triple therapy reduced the number

**FIGURE 3.** Effects of treatments on germinal centers and plasma cells in the spleen. A. The numbers of IgG or IgM plasma cells per spleen from different groups of mice were determined by ELISpot assay. The \(p\) values are compared with Ad-IFN-\(\alpha\)–treated controls. Median plus interquartile range is shown. Data are representative of two experiments with 5–10 mice per group. \(*p < 0.05, **p < 0.02, †p < 0.01.\) B. Immunofluorescence staining of spleens (original magnification \(\times 5\)) with anti-IgD (red), peanut agglutinin (green; upper panels), and anti-IgG2a (green; lower panels). Data are representative of two experiments of five to eight mice per group. Results are similar between the spleens from the mice treated with high-dose or low-dose CTLA4-Ig. IgG3-producing B cells were present even in naive mice, and their numbers were variable in treated mice and Ad-IFN-\(\alpha\) controls (not shown).
of follicular (p = 0.0008, versus untreated controls), activated (p = 0.0429), and class-switched B cells (p = 0.0012) but not MZ B cells in the spleens. We have shown previously that the IFN-α–induced expansion of MZ B cells in the spleen is T cell independent. The total number of splenic CD4 T cells was not affected by triple therapy; however, fewer CD69+ activated (p = 0.0012) and CD44+/CD62L+ effector memory CD4 T cells (p = 0.0012) were found in spleens of mice treated with triple therapy compared with those of untreated controls. Splenocytes from mice treated with double therapy showed a similar phenotype to that of mice treated with triple therapy. Mice treated with anti-CD40L Ab showed fewer class-switched B cells and effector memory T cells in the spleens than did the untreated controls. A decrease in follicular B cells was observed 4 wk after a single dose of CTX. CTLA4-Ig treatment alone had no effect on the phenotype of splenocytes in Ad-IFNα–treated mice, which is consistent with its lack of therapeutic effects (Table II).

Triple therapy eliminates GCs and reduces the numbers of Ab-secreting cells in the spleens of Ad-IFNα–treated NZB/W F1 mice

By ELISpot assay, mice treated with triple therapy had a significant reduction in the number of total and anti-dsDNA IgM- and IgG-secreting cells in the spleen (Fig. 7A, 7B). Similar effects were observed in the mice receiving double therapy (Fig. 7A, 7B). The number of Ab-secreting cells in the spleens of Ad-IFNα–treated mice treated with anti-CD40L, CTLA4-Ig, or CTX single therapy was not altered by the time that spleens were harvested 4 wk after therapy (Fig. 7A, 7B).

The decrease of IgG-secreting cells in the spleens from the mice treated with triple or double therapy was confirmed by immuno-
tolerance in conventional NZB/W F1 mice, because prophylactic GC formation, production of autoantibodies, or deposition of IFN-α accelerated lupus onset and protected the mice from interstitial inflammation. Nevertheless, CTLA4-Ig treatment markedly delayed protection was not complete and kidney administration of high-dose CTLA4-Ig. Even in mice treated with pathogenic IgG3 autoantibodies that were attenuated only after production of pathogenic IgG2a anti-dsDNA Abs. Resistance to despite preventing T and B cell activation, GC formation, and the overexpression accelerates disease progression in several murine SLE models (7, 25, 26). We have shown previously that IFN-α–accelerated lupus is accompanied by T and B cell activation and GC formation, elevated serum levels of IgG2a and IgG3 autoantibodies, increased production of BAFF, IL-6, and TNF-α, and upregulation of TLR7 in splenic B cells. Nevertheless, T cells are absolutely required for initiating disease in the IFN-α–accelerated model. In this study, we assessed the importance of B7-CD28 costimulation and BAFF/APRIL signaling in the pathogenesis of IFN-α–accelerated lupus using CTLA4-Ig and TACI-Ig treatment, respectively. We show that low-dose CTLA4-Ig treatment did not prevent or delay the onset of nephritis in Ad-IFNα–treated mice despite preventing T and B cell activation, GC formation, and the production of pathogenic IgG2a anti-dsDNA Abs. Resistance to low-dose CTLA4-Ig was likely caused by the persistence of pathogenic IgG3 autoantibodies that were attenuated only after administration of high-dose CTLA4-Ig. Even in mice treated with high-dose CTLA4-Ig, protection was not complete and kidney deposition of IgG eventually occurred despite continued treatment. Nevertheless, CTLA4-Ig treatment markedly delayed proteinuria onset and protected the mice from interstitial inflammation. In contrast, TACI-Ig treatment significantly ameliorated IFN-α–accelerated lupus without affecting lymphocyte activation, GC formation, production of autoantibodies, or deposition of IgG2a and IgG3 in the kidneys.

B7-CD28 costimulation is absolutely required for the break of tolerance in conventional NZB/W F1 mice, because prophylactic CTLA4-Ig treatment decreases both class switching and somatic mutation and prevents the production of pathogenic IgG anti-dsDNA Abs (16, 22). In Ad-IFNα–treated mice, we have observed that IgG2a autoantibodies derive predominantly from fluorescence staining and was associated with abolished development of germinal centers in the spleens. In contrast, the mice receiving single therapy with CTLA4-Ig or CTX had comparable GCs and IgG-producing cells in their spleens versus those of the untreated controls (Fig. 7C). The mice treated with anti-CD40L Ab showed variable results that correlated with the low rate of remission induced by this therapy (data not shown).

The decrease in serum autoantibodies and the lack of renal damage in the mice treated with triple therapy was in parallel with greatly diminished glomerular IgG deposition (Fig. 7B). Double therapy also reduced glomerular IgG deposits, although to a lesser extent (Fig. 7B). In contrast, single CTX or CTLA4-Ig treatment did not reduce glomerular IgG deposition (Fig. 7B). Therefore, remission induced by triple therapy was a result of the downstream effects on the kidneys (9) and clearance of renal immune complex deposits.

**Discussion**

IFN-α is a key cytokine in the pathogenesis of SLE (24), and its overexpression accelerates disease progression in several murine SLE models (7, 25, 26). We have shown previously that IFN-α–accelerated lupus is accompanied by T and B cell activation and GC formation, elevated serum levels of IgG2a and IgG3 autoantibodies, increased production of BAFF, IL-6, and TNF-α, and upregulation of TLR7 in splenic B cells. Nevertheless, T cells are absolutely required for initiating disease in the IFN-α–accelerated model. In this study, we assessed the importance of B7-CD28 costimulation and BAFF/APRIL signaling in the pathogenesis of IFN-α–accelerated lupus using CTLA4-Ig and TACI-Ig treatment, respectively. We show that low-dose CTLA4-Ig treatment did not prevent or delay the onset of nephritis in Ad-IFNα–treated mice despite preventing T and B cell activation, GC formation, and the production of pathogenic IgG2a anti-dsDNA Abs. Resistance to low-dose CTLA4-Ig was likely caused by the persistence of pathogenic IgG3 autoantibodies that were attenuated only after administration of high-dose CTLA4-Ig. Even in mice treated with high-dose CTLA4-Ig, protection was not complete and kidney deposition of IgG eventually occurred despite continued treatment. Nevertheless, CTLA4-Ig treatment markedly delayed proteinuria onset and protected the mice from interstitial inflammation. In contrast, TACI-Ig treatment significantly ameliorated IFN-α–accelerated lupus without affecting lymphocyte activation, GC formation, production of autoantibodies, or deposition of IgG2a and IgG3 in the kidneys.

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germinal centers, whereas IgG3 anti-dsDNA Abs are derived predominantly from extracellular sources (6). The pathogenicity of the anti-dsDNA IgG3 Abs that arise in Ad-IFNα–treated mice may be the result of either T cell-driven clonal expansion of high-affinity B cells or T cell-dependent affinity maturation that occurs in extracellular foci (27). Recent studies have identified an expanded extracellular Th cell population in SLE-prone mice (28–30) that mediates IgG production through IL-21 and CD40L. Our data suggest that T cells supporting the extracellular response are less dependent on B7-CD28 costimulation than are germinal center T FH cells.

We have shown previously that the expression of IL-21 and IL-6 is elevated in the spleens of Ad-IFNα–treated mice (6). CTLA4-Ig treatment inhibited the upregulation of IL-21, but did not prevent the elevated expression of IL-6 in the spleens of Ad-IFNα-treated mice. The ability of IL-6 to promote the production of anti-dsDNA Abs has been implicated previously in the pathogenesis of lupus (31–33). Although both IL-6 and elevated serum levels of BAFF can contribute to the relative resistance of IFNα–treated mice to CTLA4-Ig, they are not sufficient to induce autoantibodies if T cells are completely absent (6).

Ad-IFNα–treated NZB/W F1 mice treated with TACI-Ig manifested a delay in onset of proteinuria and prolonged survival despite the robust production of pathogenic autoantibodies. Although TACI-Ig treatment reduced the number of splenic IgM plasma cells, the short-lived IgG plasma cells that produce pathogenic autoantibodies in the IFNα–induced model (6, 34) were totally unaffected by TACI-Ig treatment. It is not entirely clear why IgM-producing plasma cells are more sensitive to BAFF/APRIL blockade than are IgG-producing plasma cells. One explanation involves the strength of BCR signaling itself because IgG expressing cells have different rates of BCR clustering (35), an exaggerated calcium flux, and different gene expression compared with IgM-bearing cells (36). The resistance of splenic IgG plasma cells to TACI-Ig treatment in IFNα–treated NZB/W mice is different from findings previously reported in NZM2410 and MRL/lpr mice (37–39). This discrepancy suggests that support for IgG plasma cell survival after IFNα acceleration is due to extrinsic factors that render these cells independent of BAFF/APRIL signaling for their survival.

It has been shown previously that immune complex deposition in the kidneys does not lead to renal pathology unless renal effector cells are also activated (9, 40, 41). In conventional NZB/W F1 mice, TACI-Ig–mediated B cell depletion results in significantly decreased numbers of activated T cells and DCs in the spleens, which is associated with an overall decrease in circulating inflammatory cytokines and dampened endothelial activation, thus decreasing inflammatory cell infiltration into target organs (12, 13, 17). In Ad-IFNα–treated NZB/W F1 mice however, TACI-Ig–mediated B cell depletion did not result in significant decreases in spleen size or impairment of T cell activation, nor did it affect the splenic expression of IL-21 and the T cell-dependent production of pathogenic IgG anti-dsDNA Abs. Finally, although B cells have been shown to produce IL-6 in lupus animal models and in patients with SLE (42, 43), the expression of IL-6 in the spleens of Ad-IFNα–treated mice was not inhibited by TACI-Ig treatment. These observations suggest that TACI-Ig treatment does not protect Ad-IFNα–treated mice entirely by depleting B cells.

An alternate explanation is that TACI-Ig treatment directly targets the kidney. Ad-IFNα–treated NZB/W F1 mice develop interstitial infiltrates of macrophages at the onset of proteinuria (6). We found that TACI-Ig treatment decreased renal infiltration with macrophages, and the infiltrating cells failed to upregulate CD11b, which is a hallmark of renal inflammation (11, 19). In addition, our study shows that despite substantial renal immune complex deposition, the upregulation of some inflammatory markers (MMP-14, CCL5, CCL20, IKKβ, and LCN2) in the kidneys of Ad-IFNα–treated NZB/W F1 mice was significantly inhibited by TACI-Ig treatment. Many of these inflammatory mediators are produced by infiltrating mononuclear phagocytes that have encountered immune complexes (19, 23, 44). Furthermore, TACI-Ig prevented the increase of serum levels of TNFα that occurs at nephritis onset. In NZM2410 mice that typically have few renal infiltrating cells, TACI-Ig treatment similarly reduced renal damage and activation of renal macrophages and endothelial cells (11). These findings suggest that TACI-Ig treatment exerts its protective role by inhibiting the initial activation of intrinsic renal cells upon encountering immune complexes, leading to less production of inflammatory mediators in the kidney. A direct effect of BAFF on DCs has been reported by Lai Kwan Lam et al., who showed that silencing of BAFF in the inflamed synovium decreases local DC activation (45). Further study is required to identify the cells bearing BAFF/APRIL receptors in the inflamed kidneys of Ad-IFNα–treated NZB/W F1 mice.

We demonstrated previously that triple therapy with CTX, anti-CD40L, and CTLA4Ig induced remission in 84% of NZB/W F1 mice with established nephritis (9). In this study, we show that a similar percentage of Ad-IFNα–treated mice entered remission after triple therapy but relapsed rapidly. Mice treated with high-dose IFNα virus relapsed more quickly than mice treated with a lower dose of virus, and the mice treated with low-dose virus relapsed more quickly than conventional NZB/W mice (9). Triple therapy markedly reduced the production of pathogenic anti-dsDNA Abs in Ad-IFNα–treated NZB/W F1 mice and reversed both glomerular and interstitial damage, as it does in conventional NZB/W mice (19), but this was only temporary. Therefore, although the short-lived plasma cells induced by IFNα (6) are susceptible to cytotoxic reagents and costimulatory blockade, they rapidly return after treatment ends. We have shown previously in NZB/W F1 mice that IFNα promotes renal infiltration of activated macrophages that produce matrix metalloproteinases and growth factors, resulting in early fibrosis and glomerular cell proliferation (44). Based on these studies, we hypothesize that IFNα also promotes the return of activated macrophages to the kidneys once immune complexes deposit again, leading to relapse.

Our findings show that IFNα does not merely accelerate the progression of lupus; it also alters important aspects of the disease and renders mice more resistant to immune modulation. Similarly, IFNα has been shown to prevent anti-CD40L Abs from establishing tolerance in an animal model of skin transplantation by enhancing the expression of costimulatory molecules on DCs and consequently promoting CD8 T cell priming (46, 47). These findings are clinically relevant, because patients with SLE with the IFNα signature may have clinical features that are distinct from the general population of patients with SLE (48–51) and may respond differently to therapies. This finding calls for special consideration when designing clinical studies or developing therapeutics for SLE.

Disclosures
The authors have no financial conflicts of interest.

References