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doi: 10.4049/jimmunol.174.5.2499

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IFN-α Induces Early Lethal Lupus in Preautoimmune (New Zealand Black × New Zealand White)F₁ but Not in BALB/c Mice

Alexis Mathian,* Arthur Weinberg,† Mike Gallegos,* Jacques Banchereau,2,3* and Sophie Koutouzov2,4*

Recent studies indicate that IFN-α is involved in pathogenesis of systemic lupus erythematosus. However, direct proof that IFN-α is not only necessary, but also sufficient to induce lupus pathogenicity is lacking. In this study, we show that in vivo adenovector-mediated delivery of murine IFN-α results in preautoimmune (New Zealand Black (NZB) × New Zealand White (NZW))F₁, but not in normal, mice, in a rapid and severe disease with all characteristics of systemic lupus erythematosus. Anti-dsDNA Abs appeared as soon as day 10 after initiation of IFN-α treatment. Proteinuria and death caused by glomerulonephritis occurred in all treated mice within, respectively, ~9 and ~18 wk, at a time when all untreated (NZB × NZW)F₁ did not show any sign of disease. IFN-α in vivo induced an overexpression of B lymphocyte stimulator in circulation at similar levels in both the preautoimmune and the normal mouse strains. All effects elicited by IFN-α were dose dependent. (NZB × NZW)F₁ infused with purified murine IFN-α also showed acceleration of lupus. Thus, prolonged expression of IFN-α in vivo induces early lethal lupus in susceptible animals. The Journal of Immunology, 2005, 174: 2499–2506.
injections, or severity of mouse lupus models is questioned. Therefore, the direct importance of type I IFNs in lupus pathogenicity still remains an open question. In the present study, we re-evaluated the effects of IFN-α in vivo on the course of lupus response and asked whether IFN-α can break tolerance and initiate a de novo autoimmune reaction, but also enhance an existing, but silent autoimmune process and precipitate a clinically overt disease. Our data show that continuous in vivo expression of IFN-α precipitates in preautoimmune (New Zealand Black (NZB) × New Zealand White (NZW))F1 mice, but not in normal BALB/c mice, the autoimmune process, and kidney damage, leading to premature death from severe immune complex glomerulonephritis. Our data support the notion that sustained IFN-α in vivo results, in susceptible individuals, in a brisk disease that has all characteristics of SLE.

Materials and Methods

Generation of IFN-α adenovirus (Adv)

The recombinant Adv vector containing the mIFN-α subtype 5 cDNA under the control of the CMV promoter/enhancer (AdCMVmIFN5) was obtained from Qiobiogen. The virus was cloned by plaque assay on 293 cells and functionally tested for mIFN-α protein expression by ELISA (PBL Biomedical Laboratories) before being amplified. AdCMVpLpA lacking a cDNA insert was used as a negative control. Viruses were propagated on 293 cells and purified by two rounds of cesium chloride density gradient centrifugations. The recombinant virus titer was determined by OD at 260 nm (1 A260 = 1.1 × 1013 particles/ml), and aliquots were stored at −80°C in a buffer containing 10 mM Tris (pH 8.0), 2 mM MgCl2, and 5% sucrose. For all in vivo studies, aliquots of virus preparations were diluted in saline before injection.

Mice and IFN-α treatment

Female NZB and male NZW were purchased from Harlan, and a colony of (NZB × NZW)F1 mice (NZB/W) was developed in specific pathogen-free barrier facility. In some instances, experiments were conducted on NZB/W purchased from Harlan. BALB/c mice were used as nonautoimmune controls. Female mice (8–16 mice/group) were treated at 9–11 wk of age with a single i.v. injection in the retro-orbital plexus of 1 × 1010 IFN-α Adv particles. Controls received the same amount of control Adv particles. In some experiments, mice were injected with gradual doses of IFN-α Adv ranging from 0.03 × 1010 to 1 × 1010 viral particles (vp). Alternatively, mice were treated with successive 14-day periods of continuous infusion of purified mouse IFN-α (PBL) administered by micro-osmotic pump (model 1002; ALZET). Previous report showed that delivery of IFN-α/EU/per pump), as assessed using the

Anti-ssDNA and anti-dsDNA Abs.

Serum autoantibody levels were assessed by ELISA, as previously described (30). Briefly, ELISA plates pretreated with poly(L-lysine) (100 μg/ml) were coated with λ phage dsDNA (5 μg/ml in PBS, pH 7.2) (Roche Diagnostics). ssDNA was prepared by boiling λ phage dsDNA (250 μg/ml) for 10 min and immediately chilling on ice, and was plated at 5 μg/ml PBS. After overnight incubation at 4°C, plates were blocked with PBS/10% FCS or, alternatively, a PBS/20% solution of blocker casein (Pierce) to block samples from the ALZET experiments (i.e., serum samples might contain Abs developed against BSA added to stabilize IFN-α). sera (starting from 1/100 up to 1/1500 dilution) or kidney eluates (1/4–1/264) were incubated for 2 h at room temperature. Bound IFN-α was detected using anti-IFN-α mAb (Fc specific) (1/1000) (Sigma-Aldrich) added to the plates for 90 min. Binding was measured by adding ABTS substrate solution, and the OD was read at 405 nm. A positive control NZB/W serum was run in serial dilutions on each plate to allow standardization. For kidney eluates, raw OD was converted to arbitrary units (AU)/ml by setting the OD of the 1/100 dilution of the standard serum to 1000 AU/ml.

Results

In vivo experiments, sera diluted 1/10 were assessed for anti-dsDNA Abs using Crithidia luciae immunofluorescence detection (Immunoconcepts)

Evaluation of renal disease

Proteinuria.

Urine was tested for proteinuria using dipstick (Chemiwipe 2 GP; Roche Diagnostics). Proteinuria scored as + for levels of 30 mg/dl, ++ for 100 mg/dl, and +++ for levels ≥500 mg/dl. Mice were considered to have proteinuria if two consecutive urine samples scored 2+.

Renal eluates.

Four individual IFN-α-treated NZB/W premortem mice (harvested, respectively, at 52, 60, 66, and 90 days after initiation of IFN-α treatment), four age-matched control Adv NZB/W, and four proteinuric, unmanipulated NZB/W (43 wk of age) were used for elution of Igs from the kidneys. Renal elution was performed, as described (31), with slight modifications. Briefly, blood vessels were perfused via the left ventricle with 20 ml of ice-cold PBS. Kidneys were harvested, the medulla removed, and cortex was weighed. The cortex was washed three times with PBS/BSA. Kidneys were resuspended into 5 M urea/0.15 M glycine, pH 2.8, and the suspensions were sonicated and slowly stirred at 4°C overnight. Kidney eluates were collected after centrifugation (20,000 × g × 30 min) and filtered first through a 70-μm cell strainer (BD Discovery Labware), followed by a passage through an Acrodisc syringe filter (PALL). Eluates were then neutralized with 1/10 (v/v) 2 M Tris, pH 8.0, and dialyzed extensively against PBS. Renal eluates were aliquoted and stored at −80°C until use.

Renal histology.

Kidneys fixed with Formalin were embedded in paraffin, and sections were stained with periodic acid Schiff (PAS) and read blind by a pathologist (A. Weinberg). For IgG and C3 staining, frozen sections (4–5 μm) were fixed with acetone, washed with PBS, and blocked with PBS/10% normal rat serum/5% FCS. Sections were stained with a mix of FITC-conjugated rat anti-mouse IgG1, IgG2a, IgG2b, and IgG3 mAbs (each at 1/100 dilution) (BD Pharmingen) for 1 h at RT. For complement staining, sections were blocked with PBS/10% normal rat serum/0.5% FCS, incubated with FITC-conjugated goat F(ab')2 to mouse C3 (1/100) (Cappel, MP Biomedicalals). Sections were mounted with fluoromount (Vector Laboratories) and analyzed by microscopy.

Statistics

Results were compared using Mann-Whitney U test or the log-rank test for survival. A p value of <0.05 was considered significant.

Results

Long-lasting IFN-α delivery in vivo precipitates clinical signs of lupus in susceptible mice

We investigated the effects of prolonged mouse IFN-α administration in NZB/W and BALB/c mice using Adv-mediated gene transfer technology. A replication-deficient murine rIFN-α Adv
was administered once at $10^{10}$ vp/mouse to young, preautoimmune NZB/W and age-matched control BALB/c mice. Control groups for each strain consisted of mice treated with PBS or with $10^{10}$ vp of control Adv. Mice were bled at days 3, 10, and 42 after Adv delivery. As shown in Fig. 1A, blood concentrations of IFN-α at day 3 reached median levels of 714 and 614 pg/ml in NZB/W (n = 33) and BALB/c (n = 21) mice, respectively (NS). On day 10, serum IFN-α levels dropped to 195 pg/ml in NZB/W and 258 pg/ml in BALB/c mice (NS). IFN-α was still present in blood 42 days after administration of IFN-α Adv (133 pg/ml in NZB/W and 76 pg/ml in BALB/c (NS). No IFN-α was found in circulation of mice injected with control Adv. Thus, IFN-α Adv treatment provides sustained IFN-α concentrations in blood of both lupus-prone and nonautoimmune mice.

Yet, the clinical outcome was strikingly different (Fig. 1B). BALB/c mice injected with IFN-α Adv showed no sign of proteinuria or premature death. In sharp contrast, young NZB/W mice became rapidly proteinuric. As early as 28 days after IFN-α treatment, 4 of 16 mice already had heavy proteinuria (>$500$ mg/dl; +++) and 3 of 16 had mild proteinuria ($<100$ mg/dl; +). At day 35, >80% of IFN-α-treated NZB/W mice were proteinuric; 9 of 16 (56%) had levels $>500$ mg/dl (++;+) and 4 of 16 (25%) had 100 mg/dl (++;). All mice were severely proteinuric 63 days after initiation of IFN-α treatment (i.e., mice were then 19 wk old), while the first mice that became proteinuric in the NZB/W control groups were $>25$ wk old. Thus, prolonged expression of IFN-α in vivo induces early proteinuria in NZB/W lupus-prone mice.

IFN-α precipitated the death of NZB/W mice. The first death occurred at day 40 after initiation of IFN-α treatment. Fifty percent (8 of 16) of IFN-α-treated NZB/W mice died by day 66, and all mice were dead by day 130 (i.e., at $>29$ wk of age). At this age, all control NZB/W (32 of 32) were alive. Thus, sustained IFN-α in vivo accelerates renal damage and death in genetically susceptible animals.

**IFN-α induces glomerulonephritis in NZB/W mice**

We next characterized the kidney alterations elicited by IFN-α in NZB/W mice. Kidneys of IFN-α-treated NZB/W mice with heavy proteinuria (>500 mg/dl; 40 days posttreatment) looked pale and enlarged, features not observed in NZB/W control groups. Histological examination revealed a severe diffuse proliferative glomerulonephritis with either segmental or global involvement of all glomeruli. Mesangial hypercellularity and sclerosis were associated with glomerular enlargement and segmental or global thickening of capillary basement membranes. Wire loop lesions and segmental glomerulosclerosis were easily identified (Fig. 2A). Many capillary lumens were filled with monocytes, lymphocytes, and rare neutrophils. Trichrome stain demonstrated large numbers of deposits in the mesangium and subendothelial regions as well as intramembranous and epimembranous deposits (data not shown). These features were qualitatively and quantitatively similar to the lesions that developed spontaneously in NZB/W mice (our results not shown) (32). Kidneys from untreated and control Adv-injected NZB/W looked normal (Fig. 2A). Immunofluorescence staining of glomeruli from IFN-α-treated NZB/W showed that IgG and C3 intensely decorated the mesangium and peripheral capillary loops in a granular pattern. Again, NZB/W control groups showed neither IgG nor C3 deposits. Histological and immunofluorescence analysis of BALB/c kidneys demonstrated complete lack of kidney lesions whether mice were treated with IFN-α or control Adv.

Lupus nephritis might be due to deposition of immune complexes composed of anti-dsDNA Abs and DNA (see for review Ref. 33). We therefore eluted Igs from kidneys of heavily proteinuric, IFN-α-treated NZB/W and tested them for autoantibody specificity. Kidneys of IFN-α-treated NZB/W mice contained amounts of total IgG (5.5 ± 4.5 μg per 100 mg of cortex (mean ± SD)) comparable to those found in 43-wk-old, spontaneously proteinuric NZB/W (7.4 ± 4.1; NS) (Fig. 2B). Kidney eluates from IFN-α-treated NZB/W contained anti-ssDNA and anti-dsDNA IgG autoantibodies at levels comparable to those found in spontaneously proteinuric NZB/W. For instance, anti-dsDNA IgG were 13.8 ± 9.9 (mean ± SD) in IFN-α-treated NZB/W vs 14.5 ± 15.9 AU/μg IgG in NZB/W (NS). Thus, histology, deposition in glomeruli of IgG and complement C3, along with the presence of kidney-associated IgG with anti-nuclear specificity, suggest that IFN-α promotes a typical lupus glomerulonephritis in susceptible animals.

**Anti-dsDNA IgG develop specifically in IFN-α-treated NZB/W mice**

Treatment of NZB/W and BALB/c mice with control Adv did not affect the levels of circulating IgG when compared with untreated animals (Fig. 3). In contrast, administration of IFN-α Adv induced in both strains a sharp increase of circulating IgG. In BALB/c mice, total IgG increased by $>3$-fold at day 10 ($p < 0.0001$ vs control Adv) and by $>1.6$-fold at day 42 ($p = 0.02$). Likewise,
there was a ~2.5-fold increase in IgG in NZB/W, at day 10 (p < 0.00005), that returned to basal values at day 42 (Fig. 3A). Note that basal IgG levels in control NZB/W are ~3 times as high as in

FIGURE 2. In vivo Adv-mediated delivery of mIFN-α induces the rapid development of immune complex glomerulonephritis. A, Kidney sections prepared from proteinuric NZB/W mice 40 days post-IFN-αAdv treatment, or from age-matched unmanipulated and control Adv mice, were stained with PAS (upper panel), a mixture of FITC-conjugated anti-IgG1, anti-IgG2a, anti-IgG2b, and anti-IgG3 Abs (middle panel), or with FITC-conjugated anti-C3 Abs (lower panel). Results shown are from one mouse in each experimental group, and staining is representative of three mice/group, run in parallel. Note the deposits along the capillaries and in the mesangium (arrows). B, Eluates from kidneys of four control Adv mice (○) (negative control); four sick, unmanipulated, 43-wk-old, NZB/W (●) (positive control); and four individual proteinuric IFN-α-Adv-treated NZB/W mice (harvested at 52, 60, 66, and 90 days posttreatment) (●) were tested for total IgG and for anti-nuclear IgG autoantibody specificity. Thick horizontal lines represent mean values. Vertical lines represent positive SD.

FIGURE 3. In vivo Adv-mediated delivery of mIFN-α induces production of anti-dsDNA Abs specifically in NZB/W mice. BALB/c and NZB/W mice, either unmanipulated (□) or injected with 1 × 10¹⁰ control Adv vp (●) or 1 × 10¹⁰ IFN-αAdv vp (●), were bled at days 10 and 42 after initiation of treatment. A, Total serum IgG was measured by ELISA. Values represent the mean ± SEM from 8–10 mice in each group. *, p < 0.05; **, p < 0.01; *** p < 0.001; ****, p < 0.0001. B and C, Sera from mice 21 days posttreatment were tested for anti-nuclear IgG autoantibodies. Each dot represents an individual mouse. The middle panel (purple) shows anti-ssDNA and anti-dsDNA IgG values in sera of unmanipulated 25–28-wk-old, proteinuric (+/+ + + +) NZB/W mice (n = 9–11). Thin black horizontal lines represent mean values. Gray horizontal lines represent the threshold values set as the mean OD ± 3 SD in untreated mice.
treatment of NZB/W and BALB/c mice with control Adv did not affect the levels of circulating BLyS when compared with untreated animals. In contrast, administration of IFN-α Adv induced in both strains increased levels of BLyS in circulation as soon as day 24 posttreatment. Circulating BLyS further increased at day 48 to reach in NZB/W mice as well as in BALB/c mice levels observed in sick 10-mo-old NZB/W. Thus, IFN-α treatment induces sustained BLyS concentrations in blood of both lupus-prone and nonautoimmune mice.

Very low doses of IFN-α can induce rapid lethal lupus

Young NZB/W mice were administered progressively lower doses of IFN-α Adv down to 0.03 × 10^10 vp. As shown in Fig. 5A, the levels of circulating IFN-α detected at days 3 and 10 after initiation of treatment indeed gradually decreased with decreasing doses of IFN-α Adv. Overall, treated mice can be divided into two groups: a high IFN-α Adv group (e.g., 0.3–1 × 10^10 Adv vp range) and a low IFN-α Adv group (e.g., 0.03–0.1 × 10^10 Adv vp range). Indeed, there was no statistical difference in the levels of circulating IFN-α at day 10 elicited by the different IFN-α Adv doses within each of the two groups.

Remarkably, progressively lower doses of IFN-α Adv progressively delayed occurrence of proteinuria and death (Fig. 5B and C). Occurrence of proteinuria (Fig. 5B) apparently reflected the differences in IFN-α levels observed between the high and low IFN-α groups. Fifty percent of the mice (16 of 32) treated with high IFN-α became proteinuric within 27 days after initiation of treatment, while 50% of the mice (12 of 24) treated with low IFN-α became proteinuric within 34 days (p < 0.004). Survival was even a more sensitive index of in vivo IFN-α levels as decreases in IFN-α Adv doses significantly delayed occurrence of death (Fig. 5C). Overall, all mice treated with high IFN-α were dead by day 90–103, while mice treated with low IFN-α were dead by day 135–154 (p < 0.0001).

Remarkably, the lowest dose of IFN-α Adv (0.03 × 10^10 vp), which elicited levels of circulating IFN-α that were undetectable 10 days after initiation of treatment (Fig. 5A), yet induced proteinuria and death in all treated animals (Fig. 5, B and C). Thus, severity of lupus depends upon the dose of IFN-α, although minute amounts of IFN-α precipitate a lethal lupus.

Occurrence and levels of circulating anti-dsDNA Abs are dependent upon the dose of IFN-α Adv (Fig. 5, D and E). Anti-dsDNA IgG appeared as soon as day 10 after initiation of IFN-α treatment in the high IFN-α group; 9 of 16 mice were positive (e.g., above threshold; mean ± 3 SD in controls), while only 1 of 16 mice was positive in the low IFN-α group (p < 0.05). Later on, between days 10 and 38, 15 of 16 mice in the high IFN-α group and 16 of 16 in the low IFN-α group became positive, although at lower levels than that observed in sick, unmanipulated NZB × NZW mice (see Fig. 3C). Notably, anti-dsDNA reached high titers between days 52 and 94 in animals that survived in the low IFN-α group (Fig. 5E).

Sustained release of low doses of purified IFN-α induces lupus disease

Although the above data demonstrate that occurrence of disease is related to the dose of IFN-α in vivo, it is possible that the adenovector plays a role of adjuvant for IFN-α. Thus, young NZB/W mice were infused with a low dose of purified IFN-α using microosmotic pumps. Continuous delivery of IFN-α (10^5 U/per pump) did not yield measurable levels of IFN-α in blood (data not shown). Yet, this low dose of IFN-α induced substantial levels of anti-dsDNA IgG Abs in seven to eight of nine mice, as illustrated...
by positive *Crithidia* immunofluorescence staining and ELISA detection (Fig. 6A and Table I). Remarkably, all mice, except one (eight of nine), became proteinuric within 39–78 days after initiation of treatment with purified IFN-α (Table I), while all controls at that time were devoid of proteinuria. These proteinuric mice showed glomerulonephritis with IgG and C3 deposits in the mesangium and along the basement membrane (Fig. 6B). There was no difference in the occurrence of anti-dsDNA IgG or of proteinuria whether or not mice had also received the control adenovector. Thus, IFN-α alone induces a dramatic acceleration of lupus in susceptible individuals.

**Discussion**

The present study demonstrates that the prolonged expression of IFN-α in vivo leads to dramatic acceleration of lupus manifestations in young lupus-prone mice. Using Adv-mediated gene transfer of mouse IFN-α5, elevated serum levels of IFN-α were rapidly obtained, which persisted for at least 42 days.

This prolonged expression of IFN-α in vivo results, in genetically susceptible preautoimmune lupus mice, in a brisk disease that has all characteristics of SLE. Anti-dsDNA Abs appeared in circulation as soon as day 10 after initiation of IFN-α treatment and mice rapidly died of lupus glomerulonephritis, as illustrated by glomerular hypercellularity, heavy capillary and mesangial IgG and C3 deposits (32, 39–41), and the presence of anti-nuclear autoantibodies in kidney eluates (31, 42). Disease acceleration mediated by IFN-α is striking as proteinuria and death occurred ~12 and ~21 wk earlier, respectively, than in untreated NZB/W mice (our colony) (32, 39).

Systemic administration of purified preparations of mIFN-αβ (three times per week from birth/1 mo to 33–37 wk of age) has been shown to induce only a slight acceleration of disease in NZB/W and NZB lupus-prone strains (27, 28, 43). In particular, IFN-αβ did not accelerate the development of proteinuria, and acceleration of death was modest. These studies using semipurified preparations of type I IFN (e.g., containing both IFN-α and IFN-β) did not directly attribute to IFN-α the key role in the acceleration of the disease. Furthermore, the effect of a prolonged expression of IFN-αβ in a normal genetic background was not evaluated. Our study addresses both points. It is very likely that the difference with our results holds in both the duration of, and persistence in, study addresses both points. It is very likely that the difference in the occurrence of anti-dsDNA IgG or of proteinuria whether or not mice had also received the control adenovector. Thus, IFN-α alone induces a dramatic acceleration of lupus in susceptible individuals.

**FIGURE 6.** Continuous infusion of IFN-α in vivo precipitates lupus disease. Ten-week-old NZB/W mice were implanted s.c. with micro-osmotic pumps containing either PBS (n = 9) or mIFN-α-A (n = 9). Fourteen-day calibrated pumps were replaced three to four times to deliver constant amounts of IFN-α-A in vivo for a total period of 8–10 wk. A, Immunofluorescence in *Crithidia luciliae* (sera diluted 1/10). B, Kidney sections of mice infused with IFN-α-A for 56 days (8 wk) and harvested at day 60. Upper panel, PAS staining. Middle panel, Sections from control or IFN-α-A-treated mice were stained with a mixture of FITC-conjugated anti-IgG1, anti-IgG2a, anti-IgG2b, and anti-IgG3. Lower panel, Staining with anti-C3.

Impregnation in vivo, might represent a model of IFN-α dysregulation seen in human lupus (10–13, 23, 24).

IFN-α in vivo enhances total IgG Abs both in BALB/c and NZB/W mice, a result in accordance with recent report (44). Despite the induction of anti-ssDNA IgG autoantibodies in BALB/c mice, IFN-α-failed, however, to induce the appearance of antidual DNA IgG and glomerulonephritis, suggesting that IFN-α is not sufficient to induce a full SLE-like phenotype in a normal genetic background. We cannot, however, rule out that if followed for several months after initiation of IFN-α treatment, BALB/c mice would not have shown signs of SLE-like disease. The human studies have also suggested that IFN-α treatment of patients with cancer or infectious diseases can induce full-blown SLE in certain susceptible individuals (15, 16). Several predisposing loci have been identified for several lupus strains (reviewed in Refs. 3, 5). However, genetic basis conferring to certain animals the susceptibility for IFN-α to trigger autoimmunity has yet to be determined. Recently described IFN-inducible gene, *ig202*, might be, among other susceptibility genes, a potential candidate that accounts for

**Table I. Continuous delivery of low doses of purified IFN-α induces precipitation of lupus**

<table>
<thead>
<tr>
<th>Micro-osmotic Pump</th>
<th>Control Adv (10^10 vp)</th>
<th>Experiment No.</th>
<th>Anti-dsDNA IgG&lt;sup&gt;a&lt;/sup&gt; C. *luciliae&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Number of &gt;0 Mice</th>
<th>Occurrence (day posttreatment)</th>
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<tr>
<td>PBS</td>
<td>–</td>
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<td>ND</td>
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<tr>
<td>IFN-αA</td>
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<td>1</td>
<td>2/2</td>
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<td>2</td>
<td>1/2</td>
<td>1/2</td>
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<td>74</td>
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<sup>a</sup> Serum samples were analyzed at day 52 (Experiment no. 1) and day 65 (Experiment no. 2) after initiation of treatment. ELISA was considered positive if OD obtained with samples run at 1/100 dilution was >OD given by a positive control NZB/W serum run at 1/3200. Presence of anti-dsDNA IgG Abs was confirmed in two ELISA blocking systems (e.g., FCS and casein) as serum samples might contain Abs developed against BSA added to stabilize IFN-α-A.

<sup>a</sup> Serum samples were analyzed at day 52 (Experiment no. 1) and day 66 (Experiment no. 2) and run at 1/100 dilution.

<sup>a</sup> Mice were sacrificed at day 62 (Experiment no. 1), and day 79 (Experiment no. 2), and glomerulonephritis was confirmed by kidney histology, ND, not detectable.
the observed IFN-α-mediated disease (45). The influence of the genetic background appears to be crucial as it has been very recently reported that type I IFNs suppress autoimmunity in MR-Lpr/lpr mice (46).

IFN-α in vivo induced the expression of BLyS to a similar level in both the autoimmune and the normal strains. BLyS is a member of the TNF ligand family that is a potent coactivator of B cells in vitro and in vivo (37). Mice transgenic for BLyS develop symptoms characteristic of SLE, such as the presence of high levels of rheumatoid factors, circulating immune complexes, anti-DNA autoantibodies, and Ig deposition in the kidneys, and suggest that dysregulation of BLyS expression may be a critical element in the chain of events leading to autoimmunity (34–38). Consistent with a possible role for BLyS in Ab-mediated autoimmune diseases, in patients with SLE, rheumatoid arthritis, and Sjögren’s syndrome, an association was found between elevated levels of BLyS in the blood and the severity of disease (38, 47, 48). Given our finding that mice treated with IFN-α develop sustained levels of BLyS in circulation, it is tempting to suggest that high BLyS levels observed in human SLE are a consequence of an overproduction of IFN-α in vivo. However, overexpression of both BLyS and IFN-α does not lead to the development of a lupus-like phenotype in BALB/c mice. Several explanations can be put forward to explain such an absence of effect. Among those, the genetic background (e.g., most of BLyS transgenic mice are on BL/6 background (35, 37, 38), time for development of autoimmunity (e.g., several months in BLyS Tg mice), and duration and levels of BLyS expression in blood of Tg mice may explain why BALB/c behave as a BLyS-resistant mouse strain in our experimental conditions. It is also possible that the association of IFN-α and BLyS precipitates autoimmunity in lupus-prone mice, while, on the contrary, IFN-α would counteract BLyS-mediated autoimmunity in normal mice. These hypotheses are currently under investigation.

In regard with the severity of IFN-α-induced glomerulonephritis in NZB/W mice, it is intriguing that these mice injected with high IFN-α Adv had low levels of serum anti-dsDNA Abs. A first explanation is that IFN-α induces glomerulonephritis independently of anti-dsDNA autoantibodies, as it has been recently reported in different autoimmune models (49, 50) and in glomerular lesions induced by IFN at birth in normal mice (51). In this regard, it is possible that IFN-α might have a direct deleterious effect on NZB/W kidneys, as proposed previously to explain IFN-α-mediated glomerular lesions in newborn normal mice (52). A second explanation is that in the brisk IFN-α-driven lupus process, nephritogenic Abs rapidly deposit into the kidneys or are lost in the urine during renal flare, and might therefore become barely detectable in circulation (53–55). The presence of anti-dsDNA Abs in substantial amounts in kidneys of IFN-α-treated NZB/W mice would rather favor the second hypothesis, although the two mechanisms may not be mutually exclusive.

If Adv gene transfer technology has major advantages (long-term protein expression, low cost), it, however, may induce the generation of cellular immune responses, inflammation (56), and, possibly, tissue damage (57). The Adv infection might indeed provide an adjuvant role in the acceleration of SLE observed upon IFN-α delivery, and this has implications for the role of infection in triggering lupus disease. However, our data provide several lines of evidence to assign little role of the virus in the brisk lupus disease we see upon prolonged in vivo expression of IFN-α. First, it is worth mentioning that serum IFN-α elicited by the highest dose of IFN-α Adv did not induce any premature death or hepatic toxicity (data not shown). Second, none of BALB/c or NZB/W mice injected with $1 \times 10^{10}$ vp of empty Adv developed any biological nor clinical sign of lupus. Third, we could not detect the presence of anti-Adv Abs (as measured by ELISA) (58) in kidney eluates from $1 \times 10^{10}$ IFN-α Adv-treated NZB/W mice (data not shown). These Abs, complexed or not with adenoviral particles, could very well have participated in the IFN-α-driven immune complex glomerulonephritis (59). Finally, a strong line of evidence is provided by our observation that continuous infusion of rmIFN-α also accelerated lupus autoimmunity. Altogether, this suggests that IFN-α per se is the driving force leading to the early expression of lupus manifestations.

It is intriguing that, at variance with SLE patients, lupus-prone mice, of the several strains tested, do not have circulating IFN-α (our unpublished results) (26). It is possible, however, that serum IFN-α levels are below the threshold of detection and/or IFN-α is preferentially expressed into (inflamed) target tissues, as it has been recently shown in cutaneous lupus erythematosus lesions (60). Nonetheless, it is important to stress that SLE patients show a generalized up-regulation of IFN-related genes regardless of the presence or absence of IFN-α in their sera (23, 24).

Recent work has suggested a role for type I IFNs in murine lupus. Indeed, NZB congeneric lupus-prone mice and C57BL/6 lpr/lpr mice lacking IFNAR-1, the α-chain of the common receptor for type I IFNs, have reduced lupus-like disease (25, 26). Our present work not only adds evidence to the concept that type I IFNs are major effectors in the pathogenesis of lupus, but also directly demonstrates a major role of the genetic background conferring to IFN-α its pathogenic role.

In conclusion, the present study provides the direct demonstration that prolonged in vivo expression of IFN-α induces, in susceptible individuals, a brisk disease that has all characteristics of SLE. Moreover, we have established a new experimental model in which lupus disease is amenable in a few weeks and which will permit to dissect further the etiology and the cellular and molecular mechanisms that initiate and sustain the lupus autoimmunity response.

Acknowledgments

We thank Dr. R. Gerard (University of Texas Southwestern, Dallas, TX) for his precious advice on adenovectors. We also thank C. Joseph, S. Clayton, and F. Marches for their expert technical help. We are also grateful to Dr. C. Mohan for his help in kidney elution technique and for providing us with lupus mouse sera. We thank Drs. V. Pascual, K. Palucka, I. Gresser, A. Dalloiu, and D. Emilie for stimulating discussions and critical reading of the manuscript.

Disclosures

The authors have no financial conflict of interest.

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