

Supporting Information

Zagury et al. 10.1073/pnas.0900615106

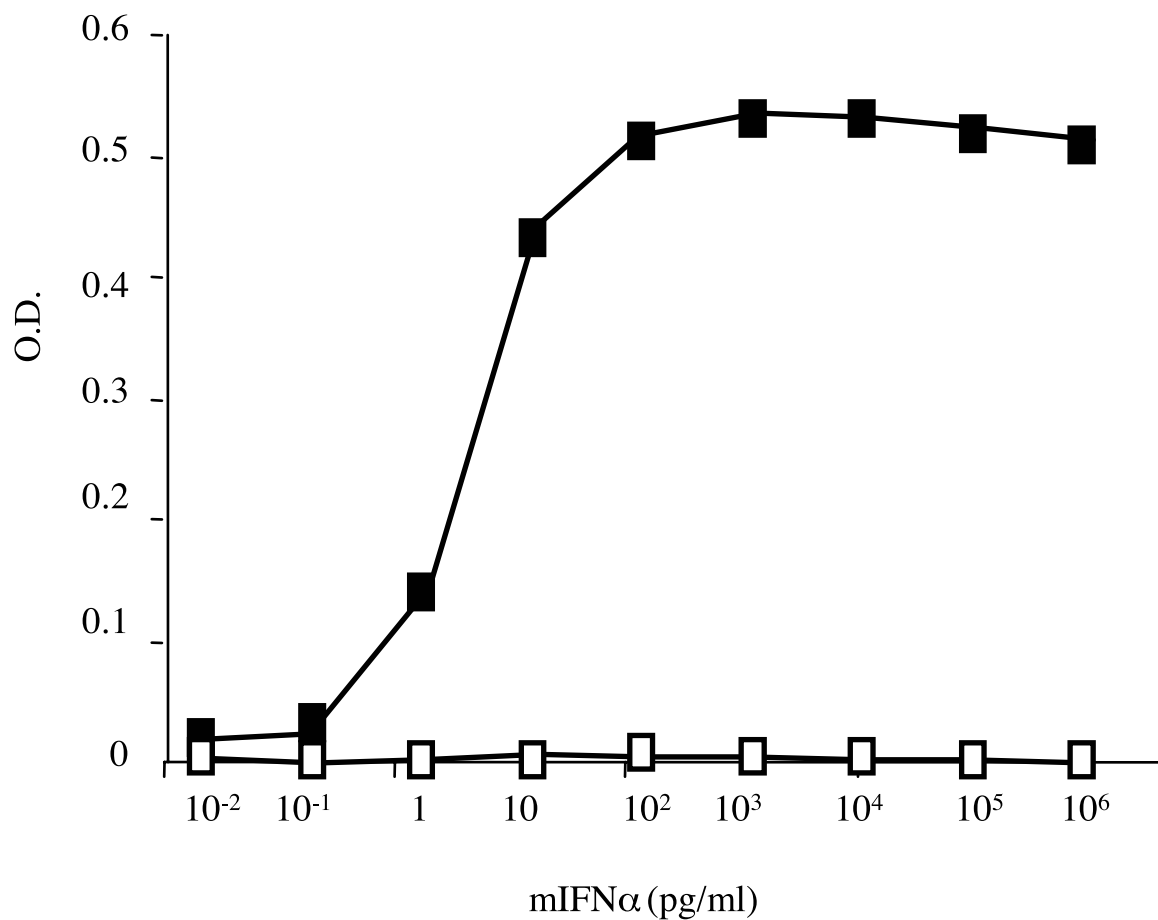


Fig. S1. Comparative evaluation of biological activity of native murine IFN α (mIFN α ; filled symbols) versus mIFN α kinoid (open symbols) on encephalomyocarditis virus (EMCV)-infected L929 cells. Inhibition of viral-induced cytopathic effect (CPE) by mIFN α was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays (see *Materials and Methods*).

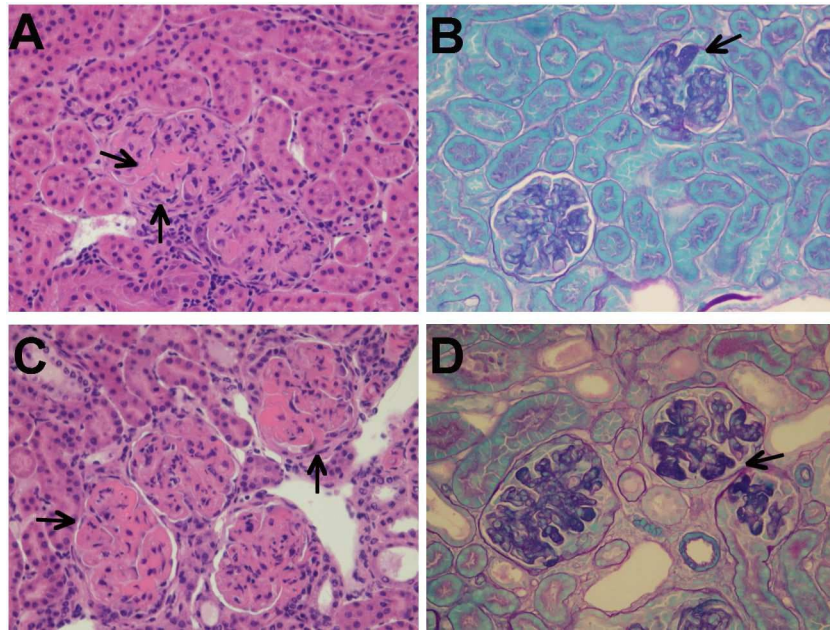


Fig. S3. Representative tissue sections of kidneys from NZB/W mice stained with H&E (*Left*) or periodic acid/Schiff light green (*Right*), illustrating the extensive lesions as reported in the main text. (Original magnification, 40 \times .) (*A and B*) Animal no. 1: severe glomerular deposits (indicated by black arrows). These were seen in the majority of glomeruli; the overall grading was 4. Some sclerosis is present in the H&E photograph (upward arrow). (*C and D*) Animal no. 2: extensive deposits were noted in all glomeruli, with sclerosis seen in the H&E and thickening of basement membranes in tubules; the overall grading was 5.

Table S1. Pool of sera from positive control (group A), kinoid-immunized (group B), and *KLH-immunized (group C) animals collected 7 days after last boosting, were tested as to their content in anti-IFN α Ab classes by ELISA

Group	Immunization	IgG1	IgG2a	IgG2b	IgG3	IgA	IgM
A	PBS control	<100	<100	<100	<100	<100	100
B	IFN α kinoid	$>2 \times 10^5$	$>2 \times 10^5$	$>2 \times 10^5$	3×10^3	<100	3×10^3
C	*KLH	100	<100	100	<100	<100	300

Results are expressed as the reciprocal of the serum dilution at an OD ≥ 0.300 . Only sera from group B animals exhibit significant high titers of anti-IFN α Abs.

Table S2. Circulating mIFN α measured by ELISA (PBL Biomedical Laboratories) and expressed as IU/mL of mouse serum

Groups	D-15	D0	D7	D28	D49	D77	D109
A Positive control (n mice)	3.8 \pm 1.1 (n=12)	Adv challenge	4.8 \pm 2.9 (n=12)	5.2 \pm 2.1 (n=11)	5.1 \pm 1.0 (n=6)	4.9 (n=1)	- (n=0)
B Kinoid-immunized mice (n mice)	3.4 \pm 1.05 (n=12)		5.5 \pm 3.2 (n=12)	6.1 \pm 3.5 (n=12)	5.9 \pm 1.1 (n=10)	6.0 \pm 0.6 (n=7)	0.9 \pm 2.2 (n=7)
C KLH-immunized mice (n mice)	3.7 \pm 0.4 (n=12)		5.5 \pm 2.2 (n=12)	4.6 \pm 1.1 (n=12)	5.0 \pm 1.0 (n=12)	5.3 \pm 0.6 (n=5)	0.3 (n=1)
D Reference (n mice)	3.7 \pm 0.4 (n=7)		2.8 \pm 0.3 (n=7)	3.1 \pm 0.3 (n=7)	6.0 \pm 1.4 (n=7)	5.5 \pm 0.8 (n=6)	0.1 \pm 0.3 (n=6)

Data are expressed by the mean \pm SD of tested serum samples correspond to estimated values, given the sensitivity threshold of the kit (PBL Biomedical Laboratories).

Table S3. Comparative evolution of clinical end points in the different groups of mice

Groups	Anti-mIFN α serum IC at peak	Proteinuria, onset day after challenge*	Death, onset day after challenge*
A: Positive control	Bg [†]	d32	d47
B: Kinoid-immunized	1,550	d95	d121
Responders (B1–B6)	2,750	d116	d123
Non/low responders (B7–B12)	500	d55	d74
C: KLH-immunized	Bg	d53	d74
D: Negative reference (without IFN α Adv treatment)	Bg	d95	d112

*Data correspond to the median in each group.

[†]Bg, serum IC background (≤ 400 /mL).

Table S4. Histological lesions of kidneys collected from surviving mice at autopsy (day 123 after challenge)

Microscopic findings*, kidneys	Kinoid-immunized/IFN α Adv (group B)												KLH- immunized/ IFN α Adv (group C)		PBS/CT Adv reference (group D)							
	B1		B2		B3		B4		B5		B6		C1		D1		D2		D3		D4	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Glomerular deposition					Min	Slight			Min	Min	Slight	Slight	Mod	Mod	Mod	Mod			Slight	Slight	Min	Min
Glomerular sclerosis					2	2			2	2	2	3	5	4	5	5			4	4	2	1
Tubular basophilia																						
Tubular degeneration																						
Protein droplets in tubules					1	1																
Protein (hyaline) casts									1	2	2	2	3	2	2	2			1	1	1	1
Inflammation interstitial																						
Lymphoid deposits, pelvic			1F	2F	3F	1F			1F	1F	1F		3F				1F	1F	3F	1F		2F
Overall score [†]	0	1			1	2			2			2	5			4	1		4			2

*Both H&E slides and tissue sections stained with periodic acid/Schiff light green were blindly evaluated.

[†]Gradings used. All gradings of severity are indicative of total effect, e.g., estimated percentage of tubules with hyaline cast in the section examined. Grade 1, <20%; 2, 20–40%; 3, 40–60%; 4, 60–80%; 5, 80–100%. For glomeruli, the minimal (Min), Slight, Mod, and Sev are used to indicate the general level of alteration and the grade the extent as a percentage, e.g., minimal 2 implies that 20–40% are affected but at a minimal level. Findings limited to a particular area, e.g. lymphoid deposits, were designated with a F for focal or D for diffuse. The overall score for a kidney is based on an evaluation of the overall condition of the kidney, using an arbitrary scale of 1–5, 1 being the most normal and 5 being severely affected.