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22. Tyrosine phosphorylated human wild-type STAT-1α was produced as described (*9*). Mutated STAT-1α (W37A) was expressed from pAcSG2 in baculovirus-infected insect cells. PCR was used to exchange codon 37 [TGG] with [GCA] in the Nco I/Spe I fragment of human STAT-1 cDNA. Additionally, a 6-His tag was added to the COOH-terminus. Modifications were confirmed by sequencing. Insect cells were lysed (dounce homogenizer), and mutated STAT-1α was purified under native conditions on Ni^{2+} nitrilotriacetic acid (Qiagen) and eluted with 200 mM imidazole in 20 mM tris HCl (pH 8.0), 10 mM $MgCl_2$, 50 mM KCl, and 5 mM DTT. In vitro phosphorylation was done as described (*9*).
23. Gel-shift experiments and determination of tetramer stability were done as described (*9*) with an oligonucleotide containing two copies of the STAT recognition element from the c-fos gene (*26*) spaced by 10 base pairs (5′-GCCAGTCAGTTCCCGTCAATG-CATCAGGTTCCCGTCAATGCAT-3′, binding sites underlined). Both protein preparations (Tyr-phosphorylated wild-type STAT-1α and W37A mutant) were titrated in gel-shift experiments with an oligonucleotide containing a single M67 (*26*) site (5′-GCCGAT-TTCCCGTAAATCAT-3′) to assure similar loading of active protein.
24. Transient transfections were done on six-well plates with 50% confluent U3A cells using the calcium phosphate method as instructed by the manufacturer (Stratagene) with the following modifications. Transfection reactions contained 4.5 μg per well of either wild-type STAT-1α or the W37A mutant in plasmid pcDNA3 (Invitrogen), 4 μg of luciferase reporter plasmid pLuc (C. M. Horvath), and 0.4 μg of β-galactosidase reporter plasmid (Stratagene). The luciferase reporter contained in its Bam HI site as an enhancer element two tandemly arranged weak STAT-1 binding sites (5′-GATCAGTTCCCGTCAAT-CATGATCCAGTTCCCGTCAATGATCCCCGGGA-TC-3′) from the human c-fos promoter. Thirty-six hours after transfection, cells were treated with interferon-γ (5 ng/ml, Amgen) for 10 hours or left untreated. Luciferase assays (Promega) and β-galactosidase assays (Stratagene) were done according to the manufacturer's protocol. Protein expression and Tyr-phosphorylation were checked in gel-shift experiments with whole-cell extracts for both wild-type and mutant protein and were comparable. All results shown are luciferase activities normalized to β-galactosidase activity.
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Uncoupling of Immune Complex Formation and Kidney Damage in Autoimmune Glomerulonephritis

Raphael Clynes, Calin Dumitru, Jeffrey V. Ravetch*

The generation of autoantibody and subsequent tissue deposition of immune complexes (IC) is thought to trigger the pathogenic consequences of systemic autoimmune disease. Modulation of the autoantibody response disrupts pathogenesis by preventing the formation of ICs; however, uncoupling IC formation from subsequent inflammatory responses seems unlikely because of the apparent complexity of the IC-triggered inflammatory cascade. However, the disruption of a single gene, which encodes the γ chain of the Fc receptor, was found to achieve this uncoupling in a spontaneous model of lupus nephritis, the New Zealand Black/New Zealand White (NZB/NZW) mouse. Gamma chain–deficient NZB/NZW mice generated and deposited IC and activated complement, but were protected from severe nephritis, thus defining another potential pathway for therapeutic intervention in autoimmune disease.

NZB mice develop autoantibodies and autoimmune hemolytic anemia, but show no signs of glomerular disease until crossed to the NZW background to generate NZB/NZW (B/W F_1) mice (*1*). A minimum of three distinct genetic loci are required for the manifestation of autoimmune glomerulonephritis in the B/W F_1, two derived from NZB and one from NZW mice (*2*, *3*). Several features of this model are consistent with lupus in humans. Females develop disease at a frequency 10 times that of males, and IC and complement deposition in glomeruli are observed. Significant proteinuria is seen concomitant with the serological appearance of antibodies to DNA as well as ICs of the immunoglobulin G1 (IgG1), IgG2a, and IgG2b subclasses beginning at 4 months (*1*). Median survival is 6 months, with mortality resulting from renal failure. Several studies have demonstrated the essential role of B cells (*4*) and autoantibodies (*5*, *6*) in disease development. Agents that interfere with autoantibody production have been shown to attenuate disease (*7*–*12*). Disruption of the subsequent inflammatory response triggered by glomerular IC deposition represents an alternative therapeutic approach, but success may be complicated by the large number of possible proinflammatory molecules presumed to be activated by ICs, including complement components and the cellular receptors for IgG. Complement depletion attenuates disease in several induced models of glomerulonephritis (*13*, *14*), and anti-C5a treatment modulates glomerular injury in a spontaneous murine model (*15*). However, the primacy of complement activation in IC-triggered inflammation has been questioned by several recent genetic studies (*16*–*20*).

In the classical model of IC-triggered inflammation, the Arthus reaction, the demonstration that FcγRs are essential whereas complement is not (*16*–*20*) suggests that the fundamental assumption of the pathogenesis of autoimmune glomerulonephritis as being mediated by complement activation (*13*, *21*–*27*) requires reevaluation. Such studies have been facilitated by the availability of defined murine strains deficient in components of this pathway. Mouse strain $\gamma^{-/-}$, which is deficient in the Fc receptor (FcR) γ chain, does not express the activation receptors FcγRI and FcγRIII, but still bears the inhibitory receptor FcγRIIB. To determine if the spontaneous autoimmune glomerulonephritis in the B/W F_1 required FcγRs for disease development, NZB and NZW mice were backcrossed for eight generations to the $\gamma^{-/-}$ mouse strain, and animals homozygous or heterozygous for the disruption in the γ chain were identified. We obtained $\gamma^{+/-}$ and $\gamma^{-/-}$ B/W F_1 animals and observed them for evidence of autoimmune glomerulonephritis. As reported (*1*), B/W F_1 animals have a median survival of 200 days, succumbing to the sequelae of renal failure (Fig. 1A). In contrast, $\gamma^{-/-}$ B/W mice had a prolonged survival, with 82% (20 of 22) alive at 9 months. In B/W mice, the appearance of proteinuria presages the onset of

Laboratory of Molecular Genetics and Immunology, The Rockefeller University, New York, NY 10021, USA.

*To whom correspondence should be addressed. E-mail: ravetch@rockvax.rockefeller.edu

clinically severe disease, with mortality following within weeks. In contrast, $\gamma^{-/-}$ B/W animals have a delayed onset and a reduced incidence of proteinuria ($P < 0.00012$) compared to their heterozygous littermates (Fig. 1B). In addition, the onset of proteinuria in the $\gamma^{-/-}$ B/W animals did not correlate with mortality; $\gamma^{-/-}$ B/W animals with significant proteinuria did not progress to the sequelae of glomerulonephritis and renal failure (*28*).

To determine the molecular basis for protection, $\gamma^{-/-}$ and $\gamma^{+/-}$ B/W mice were assayed for the presence of antibodies to double-stranded DNA (dsDNA) and circulating ICs (Table 1). No significant differences were seen in isotype, specificity, or total immunoglobulin between these animals, indicating that the backcrossing had transferred the B/W loci responsible for autoantibody production. In addition, lack of FcγRI and FcγRIII did not affect the clearance of soluble ICs, with similar steady-state levels of circulating complexes seen in both genotypes. In contrast, clearance of insoluble ICs—such as antibody-coated red blood cells, platelets, or microbial pathogens—is FcR-dependent (*20*, *29*).

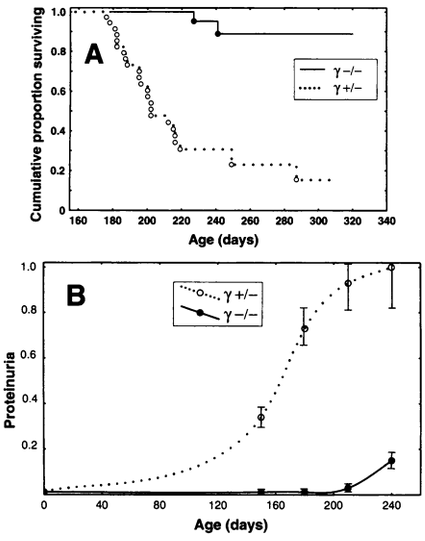

Fig. 1. (**A**) $\gamma^{-/-}$ enhances B/W survival. Kaplan-Meier cumulative proportion survival of $\gamma^{+/-}$ and $\gamma^{-/-}$ B/W F_1 female mice (*45*). A total of 31 $\gamma^{-/-}$ and 39 $\gamma^{+/-}$ B/W F_1 mice were followed. Median survival of $\gamma^{+/-}$ B/W mice was 200 ± 7 days; median survival of $\gamma^{-/-}$ B/W mice was 400 days. Premorbid $\gamma^{+/-}$ B/W animals were uremic, anemic, cachectic, and edematous. (**B**) Delayed onset and diminished severity of proteinuria in $\gamma^{-/-}$ B/W mice. Severe proteinuria (>5 mg/ml) (*46*) developed in all $\gamma^{+/-}$ B/W mice, with a median age of onset of 188 ± 11 days. In contrast, the disease incidence, severity, and time of onset were attenuated in $\gamma^{-/-}$ B/W mice. Normalized data are expressed as: total proteinuria = (incidence) × (mean 24-hour urinary protein).

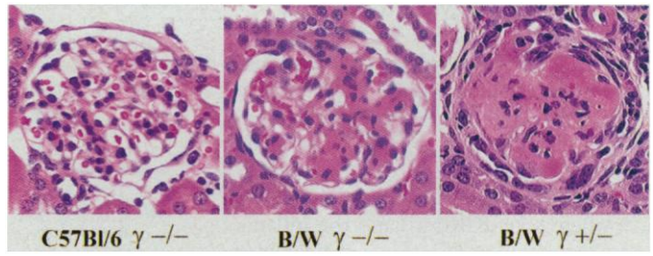

Fig. 2. Glomerulonephritis is blocked in $\gamma^{-/-}$ B/W mice. Representative renal glomeruli (*47*) of C57Bl/6 $\gamma^{-/-}$, B/W $\gamma^{-/-}$, and B/W $\gamma^{+/-}$ 7-month-old female mice (magnification ×400). Pathological features of B/W $\gamma^{+/-}$ glomeruli include mesangial thickening and hypercellularity evolving into end-stage sclerotic and crescentic changes. C57Bl/6 $\gamma^{-/-}$ glomeruli demonstrate normal glomerular architecture, and B/W $\gamma^{-/-}$ glomeruli show relatively mild mesangial thickening without concomitant inflammatory changes. Despite the development of mesangial thickening in B/W $\gamma^{-/-}$ mice, there is little hypercellularity and no evidence of end-stage glomerular changes.

Production of ICs in $\gamma^{-/-}$ B/W mice were not expected to differ, because B and T cell responses are normal in $\gamma^{-/-}$ mice (*30*, *32*).

Glomerular samples taken from $\gamma^{-/-}$ and $\gamma^{+/-}$ B/W mice were examined histologically and by immunofluorescence. Active glomerular disease was seen in the $\gamma^{+/-}$ B/W animals, including mesangial thickening and hypercellularity evolving into end-stage crescent formation and sclerosis (Fig. 2). IC deposition was observed in both genotypes, along with deposition of complement C3. However, age-matched $\gamma^{-/-}$ B/W showed no evidence of inflammatory disease; only mesangial thickening was seen, indicative of IC deposition revealed by immunofluorescence (Fig. 3). Such deposition is seen only in the B/W background, because $\gamma^{-/-}$ animals on a variety of backgrounds do not generate or deposit IC or C3 in their kidneys (*33*). Thus, despite the deposition of ICs and C3 in the glomeruli of $\gamma^{-/-}$ B/W, the inflammatory response was uncoupled, indicating that the presence of FcγRs was required, and complement activation by ICs is not sufficient to initiate an inflammatory cascade.

An induced model of IC-mediated glomerulonephritis done in $\gamma^{-/-}$-deficient mice (*34*) confirmed our results. Rabbit antibodies to glomerular basement membrane, when passively transferred into wild-type mice sensitized to rabbit IgG, developed an acute glomerulonephritis. In the absence of γ chain, reduction in disease was seen. Complement depletion with cobra venom factor did not attenuate disease, supporting the data shown in Fig. 3.

The likely receptor involved in IC triggering is FcγRIII, whose expression on mesangial cells may be essential for disease initiation (*35*, *36*). The similarities in serum IC levels and deposition in $\gamma^{-/-}$ and wild-type mice suggest that these receptors have a minimal contribution to the clearance of ICs, indicating that IC clearance is

Table 1. Titers of ICs and antibodies to dsDNA for $\gamma^{+/-}$ and $\gamma^{-/-}$ mice. Results are expressed as a percent of a pooled serum from 7-month-old B/W female mice used to create a standard curve (*48*). Means ± SEM of two separate experiments of 21 heterozygous and 29 homozygous deficient female animals 6 to 7 months of age are shown.

	Mouse strain		
	$\gamma^{+/-}$	$\gamma^{-/-}$	C57Bl/6 $\gamma^{-/-}$
Immune complexes*	84 ± 30	66 ± 18	<1
Anti-dsDNA			
Total IgG	109 ± 24	98 ± 12	<1
IgG1	92 ± 21	165 ± 27	1
IgG2a	77 ± 16	106 ± 14	<1
IgG2b	101 ± 40	86 ± 16	<1
IgG3	121 ± 24	146 ± 23	1
IgM	143 ± 19	114 ± 11	<1

*Aggregated IgG was used as a positive control for C1q binding.

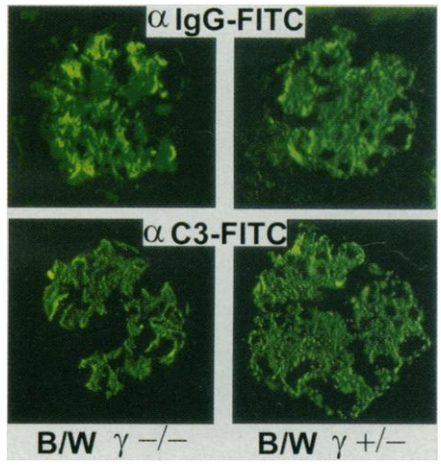

Fig. 3. IC and complement deposition are similar in $\gamma^{+/-}$ and $\gamma^{-/-}$ B/W mice. Immunohistochemical analysis (*47*) of kidneys from the animals shown in Fig. 2. Both mice had comparable concentrations of circulating ICs and autoantibodies to dsDNA; however, proteinuria was evident in the $\gamma^{+/-}$, but not the $\gamma^{-/-}$ B/W mouse. Prominent mesangial IgG and C3 staining is evident in B/W mice of both genotypes.

either mediated by FcγRII, complement receptors, or both. These divergent roles for FcRs and complement in autoimmune disease offer an explanation for the apparent paradox that deficiencies in complement increase the risk of lupus (*37*, *38*). The complement system may regulate autoreactive B cells (*39*) as well as contribute to IC clearance, whereas FcγRIII mediates the inflammatory activation by ICs. Thus, deficiencies in complement would result in an increase in autoantibodies and a reduction of IC clearance with a corresponding increase in IC deposition, thereby increasing FcR-mediated activation. These studies and ours indicate that complement and Fc receptors have evolved for distinctly different roles in their interaction with ICs. Complement has been shown to be essential for innate immunity against microbial pathogens, requiring natural antibodies to mediate their protective effect (*40*, *42*), whereas FcγRs have emerged as the principal system for coupling antigen-specific IgG antibodies to cellular effector responses and play a minor role in host innate immunity (*29*, *43*, *44*). Therefore, this distinction argues for the development of new therapeutic strategies based on FcR blockade for the treatment of autoimmune glomerulonephritis.

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45. C57Bl/6/129 $\gamma^{-/-}$ mice (*23*) were successively backcrossed to NZB/BINJ and NZW/LacJ (Jackson Laboratories, Bar Harbor, ME) for eight generations before self-mating to generate $\gamma^{-/-}$ and $\gamma^{+/-}$ mice. The eighth generation NZB $\gamma^{-/-}$ males and NZW $\gamma^{+/-}$ females were bred to generate a group of 31 $\gamma^{-/-}$ and 39 $\gamma^{+/-}$ B/W female offspring mice. Mice were genotyped by polymerase chain reaction of tail-tip DNA samples. (Primer sequences: neo: CTCGTGCTTTACGGTATCGCC; gamma 1: ACCCTACTCTACTGTCGACTCAAG; gamma 2:TCACGGCTGGCTATAGCTGCCTT. Annealing temperature was 62°C. Knockout and wild-type amplified products were 260 and 224 base pairs, respectively).
46. Urine was collected at 2-week intervals and read by dipstick (2GP Chemstrip, Boehringer-Mannheim, Indianapolis, IN). We scored 3+ determinations (5 mg/ml) as positive for proteinuria. Using metabolic cages (Fisher, Pittsburgh, PA), we obtained 24-hour urine collections from all mice with 3+ proteinuria. Statistical analysis of proteinuria and survival data were done with the StatSoft software package (Tulsa, OK).
47. For histological analysis, formalin-fixed sections were stained with hematoxylin and eosin. For immunofluorescence studies, acetone-fixed cryosections were stained with (1:1000 diluted) fluorescein isothiocyanate (FITC) goat anti-mouse C3 and IgG [Cappel (ICN), Aurora, OH] and were washed extensively. We used $\gamma^{-/-}$ and $\gamma^{+/+}$ C57Bl/6 kidneys as negative controls (*28*).
48. Diluted serum (1:100) from 6- to 7-month-old B/W $\gamma^{-/-}$ and $\gamma^{+/-}$ mice were added to enzyme-linked immunosorbent assay (ELISA) plates coated with C1q (Sigma, St. Louis, MO) for detection of ICs and to dsDNA-coated plates (United Biotech, Mountain View, CA) for detection of antibodies to chromatin. After washing away unbound serum, the appropriate rat anti-mouse IgG, IgG1, IgG2a, IgG2b, IgG3, or IgM (Pharmingen, San Diego, CA) was added. Alkaline-phosphatase–conjugated AKP polyclonal anti-rat IgG (Pharmingen, San Diego, CA) was used as secondary antibody. After incubation with *para*-nitrophenyl phosphate substrate, the samples were read spectrophotometrically at 405 nm with an ELISA reader (Molecular Devices, Sunnyvale, CA). Pooled serum from 7-month-old wild-type B/W female mice was used to generate a reference standard curve.

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The Minor Histocompatibility Antigen HA-1: A Diallelic Gene with a Single Amino Acid Polymorphism

Joke M. M. den Haan,* Leslie M. Meadows,* Wei Wang,* Jos Pool,* Els Blokland, Tracie L. Bishop, Carla Reinhardus, Jeffrey Shabanowitz, Rienk Offringa, Donald F. Hunt, Victor H. Engelhard, Els Goulmy†

The minor histocompatibility antigen (mHag) HA-1 is the only known mHag for which mismatching is correlated with the development of severe graft versus host disease (GvHD) after human leukocyte antigen–identical bone marrow transplantation. HA-1 was found to be a nonapeptide derived from an allele of the *KIAA0223* gene. The HA-1–negative allelic counterpart encoded by *KIAA0223* had one amino acid difference from HA-1. Family analysis with HA-1 allele-specific polymerase chain reaction showed an exact correlation between this allelic polymorphism and the HA-1 phenotype. HA-1 allele typing of donor and recipient should improve donor selection and allow the determination of bone marrow transplantation recipients with high risk for HA-1–induced GvHD development.

Bone marrow transplantation (BMT) is the current treatment for hematologic malignancies, severe aplastic anemia, and immune deficiency disease. A frequent and life-threatening complication after allogenic human leukocyte antigen (HLA)–identical BMT is GvHD (*1*). Disparities in genes other than the major histocompatibility complex (MHC), referred to as minor histocompatibility antigens (mHags), are clearly involved in the development of GvHD. The mHags are recognized by T