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Non-H-2 genes alter the H-2 determined susceptibilities in immune complex nephritis

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Non-H-2 genes alter the H-2 determined susceptibilities in immune complex nephritis. These experiments examined the effects of genes outside of the H-2 region on disease susceptibility and pathogenesis. Four strains of mice with the susceptible H-2 type, H-2^d, but different non-H-2 genes were studied. B10.D2, Balb/c, NZB, and DBA/2J mice were injected with 4 mg of apoferritin i.p. q.d. for 28 days. B10.D2 and Balb/c mice developed proliferative and crescentic glomerulonephritis. NZB mice developed proliferative and crescentic glomerulonephritis with wire loop lesions suggestive of lupus. DBA/2J mice developed only minimal mesangial proliferation without crescents or necrosis. Electron microscopy showed subepithelial and mesangial deposits in B10.D2, moderate subepithelial and mesangial deposits in Balb/c, and marked mesangial, subendothelial and subepithelial deposits in NZB. Immunofluorescence demonstrated the presence of IgG, IgM, C3 and apoferritin in these deposits. The DBA/2J mice had only minimal mesangial deposits by immunofluorescence and electron microscopy. These experiments demonstrate that non-H-2 genes alter the H-2^d determined disease susceptibility seen in H-2 congenic mice. NZB genes can alter the disease so that lupus-like lesions develop and DBA/2J genes can substantially ameliorate the disease.

Certain types of human immune complex glomerulonephritis occur more commonly in patients with particular human leukocyte antigens (HLA). Examples include membranoproliferative glomerulonephritis [1], membranous glomerulonephritis [2] and Berger's IgA nephropathy [3]. The association between genes in the major histocompatibility complex and the development of human glomerulonephritis is not absolute, suggesting that other factors may also be important.

The work of several investigators suggested a role for genes in the H-2 region in an animal model of immune complex glomerulonephritis (Apo-ICGN) which is induced in mice with horse spleen apoferritin [4, 5]. The importance of the H-2 region in determining susceptibility was definitively demonstrated using the H-2 congenic strains B10 (H-2^b), B10.BR (H-2^k) and B10.D2 (H-2^d) which share all the same genes except in the H-2 region where they are divergent. These experiments clearly demonstrated a role for the H-2 genes because the B10 mice were essentially normal, the B10.BR mice had mesangial proliferation and the B10.D2 mice had dramatic necrotizing and proliferative glomerulonephritis [6].

In addition, a role for non-H-2 genes in the pathogenesis of Apo-ICGN was suggested by two studies. Apo-ICGN was studied in a series of H-2^k mice with the moderately susceptible H-2 type (H-2^k) and different non-H-2 genes. Differences in disease susceptibility were observed with CBA and B10.BR being susceptible and C3H being resistant [7]. It would be beneficial to look at the effects of non-H-2 genes in the more susceptible H-2^d strains. The gene for C5, which is not coded for in the H-2 region, is important in the pathogenesis of Apo-ICGN because C5 deficient mice with a highly susceptible H-2 type were less susceptible to the glomerular injury induced by the injection of horse spleen apoferritin than were C5 sufficient mice [4].

The purpose of these studies was to further examine the role of genes outside of the H-2 region in mice with the most susceptible H-2 type, H-2^d. For these experiments four different H-2^d strains were chosen; Balb/c, DBA/2J, NZB and B10.D2.

Methods

Mice

Four strains of H-2^d mice with different non-H-2 genes were studied. Five- to six-week-old male B10.D2/nsnJ, Balb/cByJ, NZB/BlnJ and DBA/2J mice were purchased from the Jackson Laboratory, Bar Harbor, Maine, USA. The mice were fed standard chow, had unlimited access to water and were maintained in the animal care facility of the SUNY Health Sciences Center at Syracuse. Portions of the data on the B10.D2 mice have been previously published [6].

Protocol

Mice were allowed to acclimate to the animal care facility for one to two weeks and were seven to eight weeks old at the beginning of the study. Animals in the apoferritin group received 4 mg of horse spleen apoferritin (Sigma Chem. Co., St. Louis, Missouri, USA) in 0.2 cc of normal saline i.p. q.d. for twenty-eight days. Animals in the control group received 0.2 cc of normal saline containing small amounts of cadmium chloride to adjust for cadmium contained in the apoferritin. On day 32, the mice were sacrificed by cervical dislocation. If a mouse became moribund prior to day 32, it was sacrificed and the data utilized if adequate tissues could be obtained.

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On days 0 and 14 blood was obtained from the tail vein. At the time of sacrifice, blood was obtained by cardiac puncture. Serum was separated and stored at −70°C. On days 0, 14, and 28, twenty-four hour urine collections were obtained by placing the animals in metabolic cages with unlimited access to water but no access to food. Urine was centrifuged to remove sediment and frozen at −70°C until the assays were performed.

Assays

Blood urea nitrogen was assayed by the urease technique [8] and urinary protein was measured by the Coomassie blue dye binding technique [9]. Anti-apoferritin IgG levels were determined by an enzyme-linked immunosorbent assay (ELISA) technique as previously described [6].

Anti-double stranded DNA antibodies were measured by ELISA [10]. Single stranded DNA (ssDNA) and double stranded DNA (dsDNA) was prepared as described [11]. Immulon II plates (Dynatech, Santa Monica, California, USA) were coated with 50 μl/well of poly-l-lysine at 50 μg/ml (Sigma), incubated at room temperature for 30 minutes, and washed three times in Tris-buffered saline (TBS). The wells were then filled with 50 μl/well of ssDNA or dsDNA at a concentration of 2.5 μg/ml and incubated for two hours at room temperature. The plates were washed twice with TBS and incubated with 50 μg/ml of poly-l-glutamate (Sigma) for one hour at room temperature. The wells were washed and left filled with TBS at 4°C until ready for use. At the time of the assay, wells were washed, filled with 50 μl of phosphate buffered saline (PBS) with 3% BSA for one hour and then washed twice with PBS-Tween and twice with PBS. Serum was diluted 1/800 in PBS containing 1% BSA and 0.05% Tween and renal eluates were adjusted to 10 μg of protein per ml. Forty microliters were added in duplicate to the ssDNA or dsDNA coated plates. Positive controls included sera and renal eluate from NZB X SWR F_1 mice which produce high titers of anti-double stranded DNA antibodies. Negative controls included Balb/c and AKR serum. After a two hour incubation at room temperature the plates were washed with PBS-Tween and PBS. An alkaline phosphatase conjugated anti-mouse IgG and IgM (Boehringer-Manheim, Indianapolis, Indiana, USA) was added at 1/2000 and incubated overnight at 4°C. The plates were washed twice with PBS and 1% Tween, twice with PBS and the reaction was developed with para-nitrophenyl phosphate at 5 mg/ml (Sigma). The color reaction was read after 30 minutes on an Microelisa autoreader (Dynatech, Santa Monica, California, USA) at 405 nm.

Immunoglobulin elution

Immunoglobulin was eluted from the kidneys by a variation of the technique of Woodroffe and Wilson [12]. At the time of sacrifice kidneys were snap frozen in OCT compound (Lab Tek Products, Naperville, Illinois, USA) and stored at −70°C. At the time of extraction, the kidneys were thawed and washed with PBS, then homogenized for 15 seconds in a tissue homogenizer (Tekmar, Cincinnati, Ohio, USA), and extensively washed in PBS at 4°C until the supernatant had an $O.D._{280}$ of less than 0.050. The homogenate was suspended in 0.5 cc of 0.02 M citrate buffer (pH = 3.2) at a ratio of at least 10 mls per mg of tissue, and incubated at 37°C with shaking for three hours. The suspension was centrifuged at 1500 g for 20 minutes and the supernatant was dialyzed against 0.02 M PBS with 0.1% NaN_3.

Pathology

The kidneys were removed at the time of sacrifice after cardiac puncture. For light microscopy tissue was fixed in buffered formalin, embedded in paraffin, sectioned at 3 to 5 μm, and stained with periodic acid-Schiff as well as hematoxylin and eosin.

For electron microscopy, tissue was fixed in buffered 2.5% glutaraldehyde, stained with osmium tetroxide, embedded in Araldite 502 (Electron Microscopy Sciences, Fort Washington, Pennsylvania, USA), sectioned at 60 nm on a Nova microtome (LKB, Gaithersburg, Maryland, USA) and stained with uranyl acetate and lead citrate.

Fresh tissue for immunofluorescence microscopy was snap frozen in OCT compound (Lab Tek Products) and stored at −70°C. Three micrometer sections were prepared with a Damon/IEC microtome (International Equipment Corp., Needham Hts., Massachusetts, USA) and stained with fluorescein isothiocyanate conjugated goat anti-mouse IgG, IgM, IgA, C3 and albumin (Cooper Biomedical Inc., Malvern, Pennsylvania, USA) as described previously [6]. To detect the presence of apoferritin, kidney sections were stained with rhodamine conjugated mouse anti-horse ferritin (Jackson Immunoresearch Lab., Inc., Avondale, Pennsylvania, USA) which reacts with apoferritin by ELISA assay.

All biopsies were interpreted and scored in a blinded fashion. Light microscopy was graded as normal, 0; mild proliferation, 1; marked proliferation without crescents, 2; marked proliferation with less than 25% crescents, 3; and marked proliferation with greater than 25% crescents, 4.

Statistics

All data are recorded as the mean ± SEM. For continuous variables with comparisons between two groups, Student's two-tailed *t*-test was used. For continuous variables with comparisons between more than two groups analysis of variance and Scheffe's F-test were used. Discontinuous data such as the pathologic scores were compared by the Wilcoxon rank sum test [13].

Results

Pathology

There were marked differences in the glomerular pathology between the four strains. All twelve of the apoferritin injected Balb/c mice were abnormal with 50% of the mice having crescents. These crescents were present in <25% of glomeruli giving a pathology score of 3. Eleven of the twelve had marked mesangial proliferation (Fig. 1A) and the twelfth had mild mesangial proliferation. Casts of protein and red cells were also seen within the renal tubules. There were eight saline-injected controls, all of which survived the protocol and had no significant lesions.

The NZB mice also had very dramatic pathologic abnormalities. Of the twelve apoferritin injected mice that started the protocol, only seven survived for the full 32 days. Material was available for pathologic examination in eleven of these mice. Eight of eleven mice had crescents, and four of these had

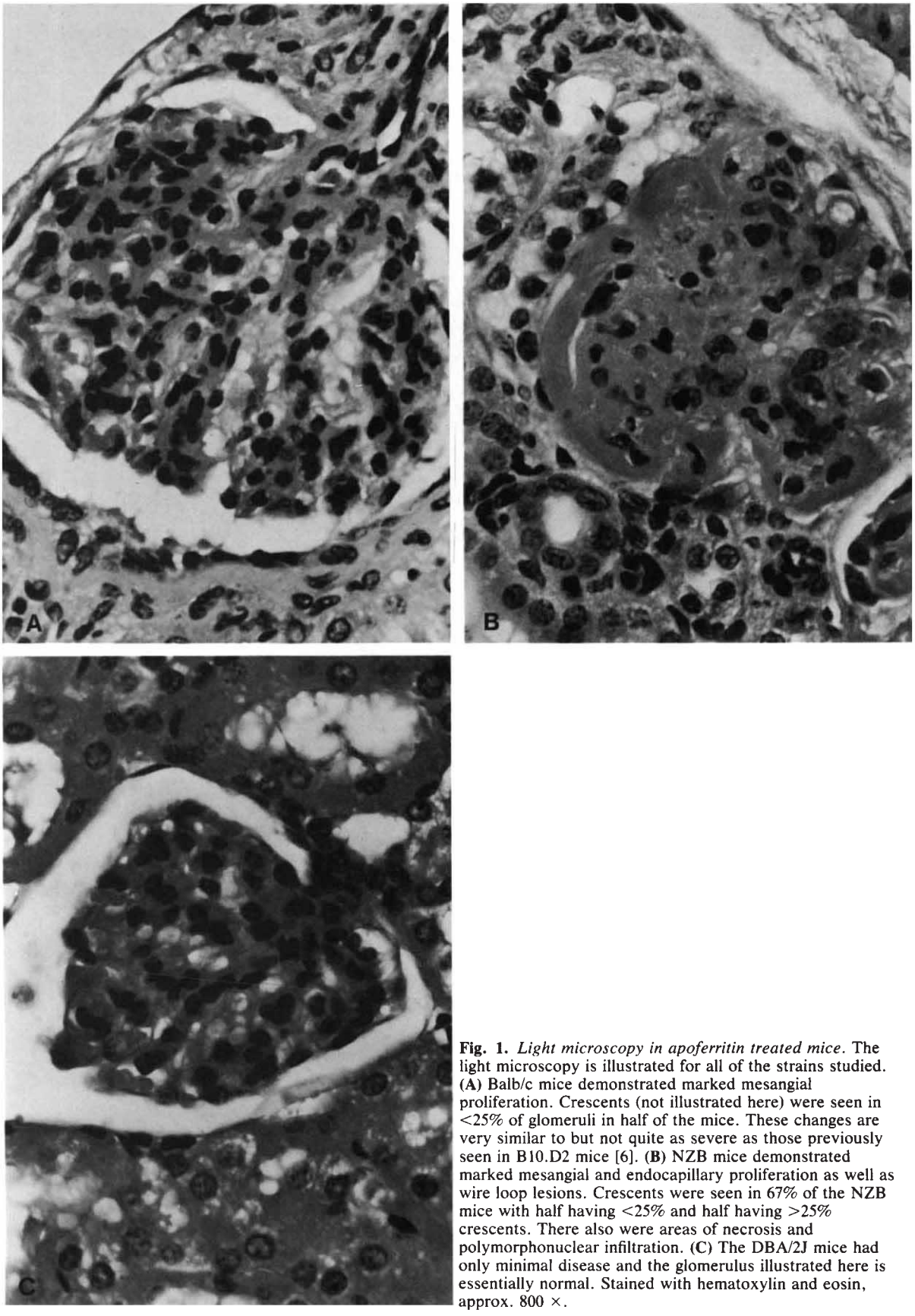

Fig. 1. *Light microscopy in apoferritin treated mice.* The light microscopy is illustrated for all of the strains studied. **(A)** Balb/c mice demonstrated marked mesangial proliferation. Crescents (not illustrated here) were seen in <25% of glomeruli in half of the mice. These changes are very similar to but not quite as severe as those previously seen in B10.D2 mice [6]. **(B)** NZB mice demonstrated marked mesangial and endocapillary proliferation as well as wire loop lesions. Crescents were seen in 67% of the NZB mice with half having <25% and half having >25% crescents. There also were areas of necrosis and polymorphonuclear infiltration. **(C)** The DBA/2J mice had only minimal disease and the glomerulus illustrated here is essentially normal. Stained with hematoxylin and eosin, approx. 800 ×.

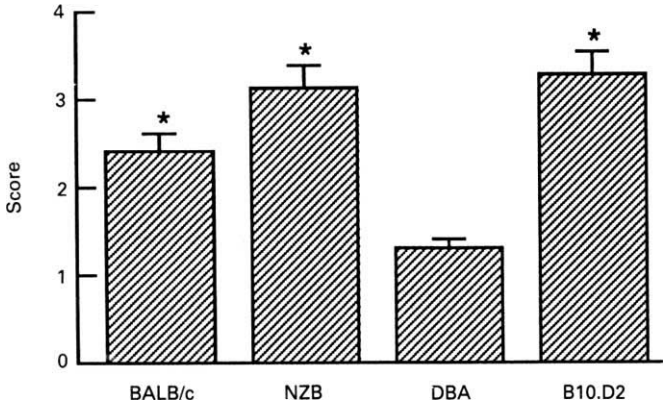

Fig. 2. *Light microscopy scores.* The light microscopy scores for the three strains studied are illustrated. The B10.D2 mice [6] are included for comparison. Balb/c, NZB and B10.D2 mice all had significantly higher pathology scores (*$P < 0.001$) than the DBA/2J mice. Normal saline injected controls score 0 or 1.

extensive crescents in >25% of glomeruli giving a pathology score of 4. In addition there was tubular atrophy and occasional casts. There was also mesangial and endocapillary proliferation, extensive areas of necrosis and infiltration with polymorphonuclear leukocytes. The other three mice had marked proliferation but no crescents. The most intriguing finding, however, was the presence of massive deposits within the glomerular basement membrane suggestive of "wire loop" lesions as might be seen in systemic lupus erythematosus (Fig 1B). There were eight saline-injected control NZB mice, of which seven survived the protocol and had only minimal mesangial proliferation.

Apoferritin injected DBA/2J mice had very modest lesions (Fig 1C). Only one of twelve had marked mesangial proliferation and none of the mice had any crescents. All of the other mice had only minimal mesangial proliferation. Pathologic scores for the apoferritin-injected DBA/2J mice did not differ significantly from the seven saline-injected DBA/2J mice who survived the protocol.

Eight of the ten apoferritin injected B10.D2 mice who survived also had severe lesions with diffuse endocapillary and mesangial proliferative glomerulonephritis [6]. The B10.D2 mice had somewhat more crescents, necrosis, interstitial infiltrate and casts than the Balb/c. All ten of the saline injected B10.D2 mice survived and had no significant lesions [6].

Mean pathologic scores of apoferritin treated mice were 2.4 ± 0.19 for the Balb/c, 3.1 ± 0.25 for the NZB and 1.33 ± 0.10 for the DBA/2J. The B10.D2 mice had a score of 3.25 ± 0.25 [6]. Balb/c, NZB and B10.D2 mice all had significantly higher pathology scores ($P < 0.001$) than the DBA/2J (Fig. 2) and their saline injected controls ($P < 0.01$). Apoferritin-injected DBA/2J mice were not significantly different than their saline injected controls. Since the scoring system does not account for the wire loops seen in the NZB mice, they scored similarly to the B10.D2 and Balb/c strains. However, the pathology in apoferritin-treated NZB kidneys was qualitatively quite different than the other groups.

Immunofluorescence microscopy revealed the presence of heavy deposits of IgG, IgM and C3 in the glomeruli of the Balb/c and NZB mice with severe disease (Fig. 3. A-B) as was previously described in B10.D2 mice [6]. In Balb/c the deposits were seen in the mesangial and capillary loop regions. In NZB mice the deposits were also present in the capillaries and the mesangium. Additionally, staining with rhodamine-conjugated anti-horse spleen ferritin which reacts with apoferritin revealed the presence of apoferritin within the deposits in the Balb/c strain as was described in the B10.D2 [6]. In the NZB mice, apoferritin localized to the wire loops (Fig. 3C).

The precise location of these deposits was determined by electron microscopy. The Balb/c mice had deposits primarily in the mesangial, perimesangial, and subepithelial regions (Fig. 4A). The NZB mice had many large deposits in the mesangium, subepithelial and subendothelial positions (Fig. 4B). The DBA/2J mice had only small mesangial deposits (Fig. 4C). In the B10.D2 mice the deposits were in the mesangial and subepithelial position [6].

IgG antiapoferritin antibody

The IgG antiapoferritin antibody responses were measured by ELISA (Fig. 5). Prior to injections, NZB mice had significantly elevated anti-apoferritin IgG levels compared to the other strains ($P < 0.05$, vs. all other strains, Scheffe F-test), although the magnitude of this elevation was not great. By the fourteenth day, the antibody levels were rising in all strains but with marked elevations in the B10.D2 mice ($P < 0.05$ vs. all other strains, Scheffe F-test). At sacrifice the antibody levels had actually decreased in the B10.D2 mice and to some degree in the DBA/2J. However, the levels in the Balb/c and NZB mice continued to rise. The levels at day 32 were significantly elevated for the NZB ($P < 0.05$, vs. all other strains, Scheffe F-test).

In addition, the anti-apoferritin IgG levels in apoferritin versus saline injected animals were significant for all the strains studied ($P < 0.05$, Student's t-test) and the anti-apoferritin IgG levels in the saline-injected NZB mice exceeded the levels in the apoferritin injected DBA/2J mice at day 32 ($P < 0.025$, Student's t-test).

Anti-nuclear antibodies

Anti-ssDNA and anti-dsDNA antibodies were also measured by ELISA in the NZB mice (Fig. 6A). There was a rise in the level of the serum anti-dsDNA antibodies in seven of nine apoferritin-injected mice. Two mice did not have elevated levels of anti-double stranded DNA antibodies. One was moribund and had a markedly elevated blood urea nitrogen. If that mouse is excluded, the data is significant ($P < 0.02$). The other mouse without elevated anti-double stranded DNA antibodies in the serum had the least glomerulonephritis of the apoferritin injected group with a pathology score of two. Serum anti-ssDNA antibody levels were not significantly elevated in NZB (data not shown). Serum from the other strains was also tested for anti-ssDNA and anti-dsDNA antibodies. Balb/c had no elevations of either antibody. For B10.D2 and DBA/2J the serum anti-ss or anti-ds DNA antibodies in the serum were both elevated relative to saline injected controls.

Renal eluate anti-dsDNA from NZB is illustrated in Figure 6B and the renal eluate anti-ssDNA from NZB is shown in Figure 6C. For both autoantibodies the data is significant ($P < 0.01$). No significant elevations could be found in the renal

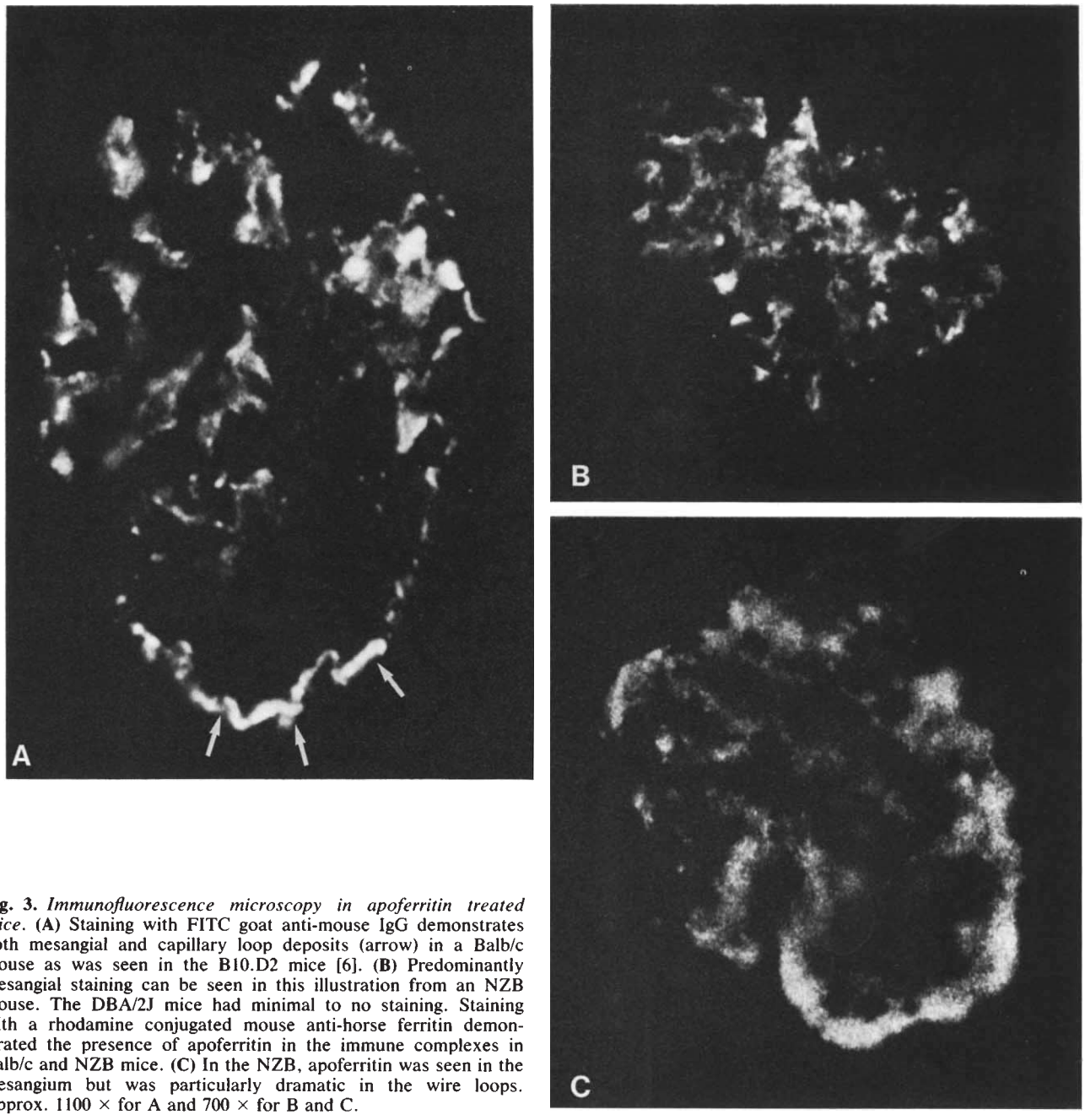

Fig. 3. *Immunofluorescence microscopy in apoferritin treated mice.* **(A)** Staining with FITC goat anti-mouse IgG demonstrates both mesangial and capillary loop deposits (arrow) in a Balb/c mouse as was seen in the B10.D2 mice [6]. **(B)** Predominantly mesangial staining can be seen in this illustration from an NZB mouse. The DBA/2J mice had minimal to no staining. Staining with a rhodamine conjugated mouse anti-horse ferritin demonstrated the presence of apoferritin in the immune complexes in Balb/c and NZB mice. **(C)** In the NZB, apoferritin was seen in the mesangium but was particularly dramatic in the wire loops. Approx. 1100 × for A and 700 × for B and C.

eluate anti-ss or anti-dsDNA antibodies from B10.D2, Balb/c or DBA/2J (data not shown).

Blood urea nitrogen and urinary protein

Blood urea nitrogen values for all groups are presented in Figure 7. Although each strain started at a somewhat different blood urea nitrogen, only B10.D2 and NZB showed a rise in the level of the blood urea nitrogen during apoferritin injection and neither rise was statistically significant.

Twenty-four-hour urinary protein excretions are illustrated in Figure 8. Once again, although each strain started at a somewhat different level, only B10.D2 and NZB mice had substantial rises in their twenty four hour protein excretion. Of these, only the rise in the B10.D2 mice were of significance ($P < 0.05$ for B10.D2 vs. DBA/2J at week 4).

Discussion

Some forms of human glomerulonephritis occur more commonly in patients with certain HLA antigens [1–3]. However, not all patients with a specific HLA allele will develop the disease. For example, although patients with HLA-DR3 are at higher risk of developing idiopathic membranous glomerulonephritis, most persons with HLA-DR3 will not develop membranous glomerulonephritis [2]. This may be because HLA-DR3 as

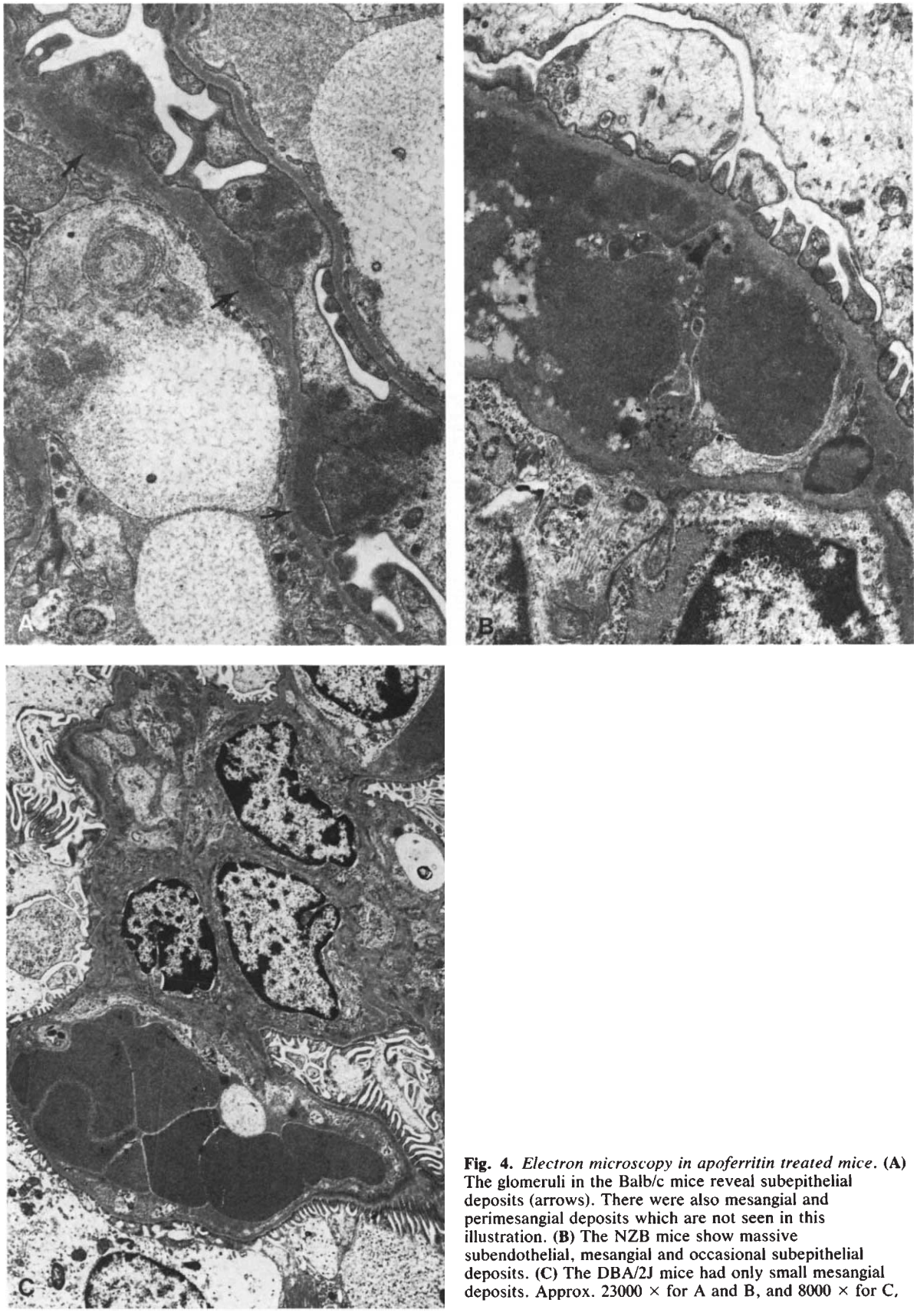

Fig. 4. *Electron microscopy in apoferritin treated mice.* **(A)** The glomeruli in the Balb/c mice reveal subepithelial deposits (arrows). There were also mesangial and perimesangial deposits which are not seen in this illustration. **(B)** The NZB mice show massive subendothelial, mesangial and occasional subepithelial deposits. **(C)** The DBA/2J mice had only small mesangial deposits. Approx. 23000 × for A and B, and 8000 × for C.

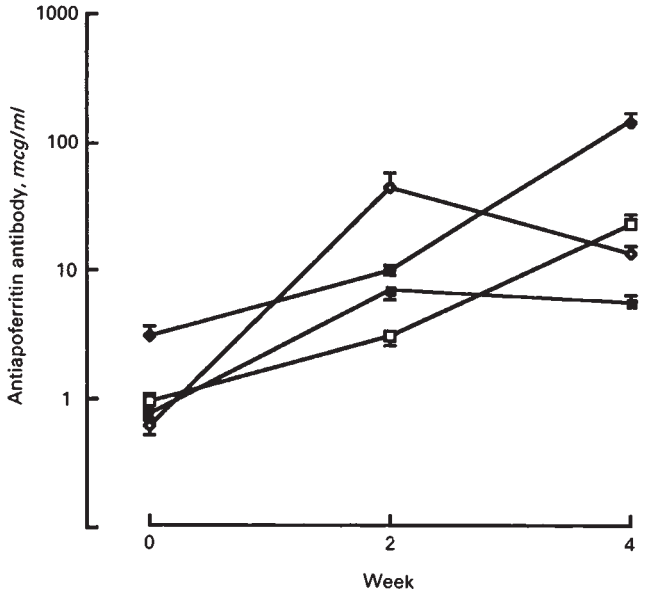

Fig. 5. *Anti-apoferritin antibody levels.* The antibody levels were determined semiquantitatively by ELISA. The data for the B10.D2 mice [6] are included for comparison. At day 0, the levels were slightly elevated in the NZB mice vs. the other strains ($P < 0.05$). The levels had risen by day 14 in all strains but the elevation in the B10.D2 was the most marked ($P < 0.05$ vs. the other strains). By Day 32 the levels in the B10.D2 and the DBA/2J mice had decreased but the NZB and Balb/c mice continued to rise and this was significant for the NZB mice ($P < 0.05$ vs. all other strains). Saline injected controls average less than 2 μg/ml with the exception of the NZB mice in which the saline injected controls had 8.5 ± 1.1 μg/ml. The anti-apoferritin antibody levels are significantly elevated in each strain in the apoferritin injected vs. the normal saline injected controls ($P < 0.05$). The NZB saline injected controls are elevated vs. the DBA/2J apoferritin-injected mice at day 32 ($P < 0.025$). Symbols are: Balb/c (□), NZB (◆), DBA/2J (■), and B10.D2 (◇).

defined serologically may be quite diverse at the DNA level and disease risk may be associated with only some subtypes of HLA-DR3 as demonstrated in diabetes [14]. It is also possible that other factors such as genes outside of the major histocompatibility complex may be important in the pathogenesis of human glomerulonephritis.

The purpose of the current experiments was to study a series of inbred mice with the genes in the H-2 region known to be at high risk for developing Apo-ICGN, that is, H-2^d [6], but with entirely different non-H-2 genes. In the present studies, Balb/c mice also developed severe ICGN with crescents. The glomeruli had deposition of IgG, C3 and apoferritin. Compared to B10.D2 mice, Balb/c mice had somewhat lower pathologic scores although the differences were not significant. In addition, Balb/c mice did not develop proteinuria or azotemia, suggesting that pathologic abnormalities are a more sensitive indicator of glomerular pathology in this disease model. Previous work with Balb/c has shown similar findings [5, 15, 16] although the degree of pathologic abnormalities was somewhat different.

DBA/2J mice behaved in a quite different manner despite having the susceptible H-2 genotype, H-2^d. These mice developed only minimal pathologic lesions or were within normal limits and the pathology scores did not differ from saline injected controls. In addition, DBA/2J mice had normal physi-

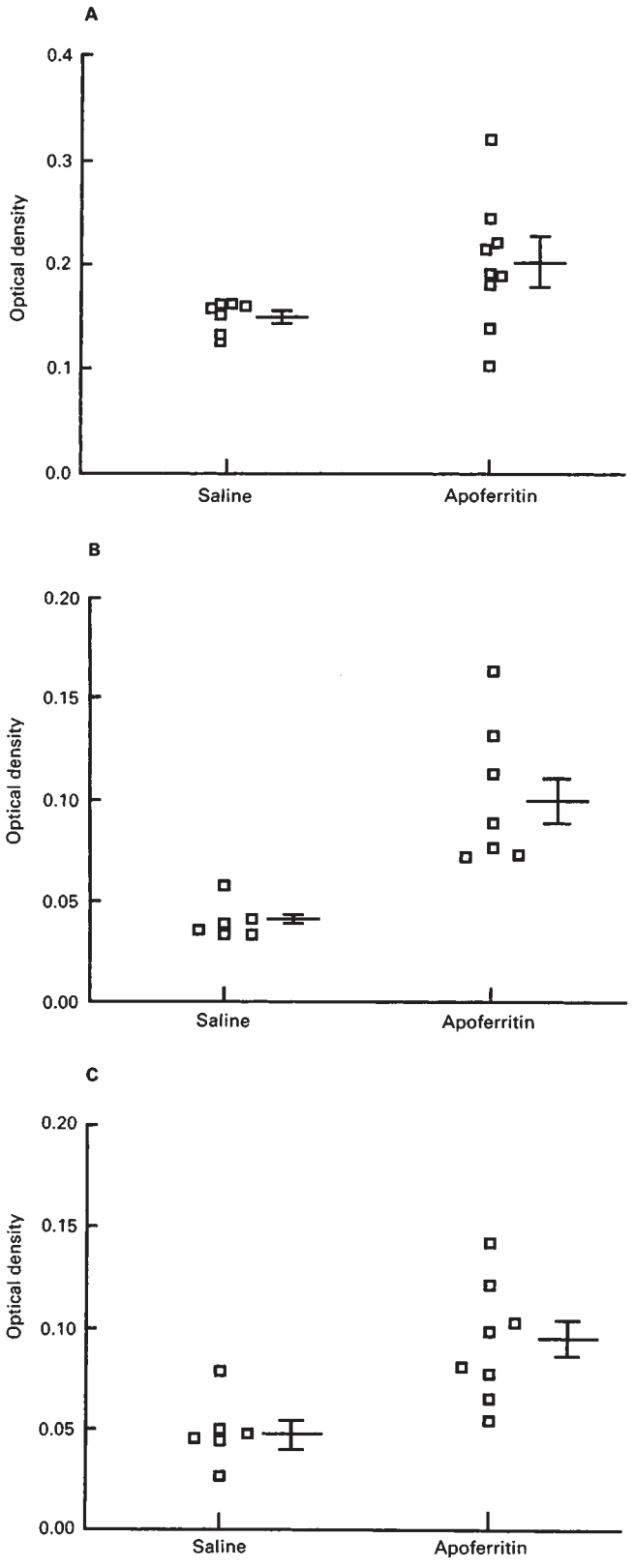

Fig. 6. *Anti-nuclear antibodies in NZB mice.* (**A**) The serum anti-dsDNA antibodies were elevated in seven of nine apoferritin injected mice. The data do not reach statistical significance. The renal eluate anti-dsDNA (**B**) and anti-ssDNA (**C**) antibodies, however, were markedly elevated and reached statistical significance ($P < 0.01$).

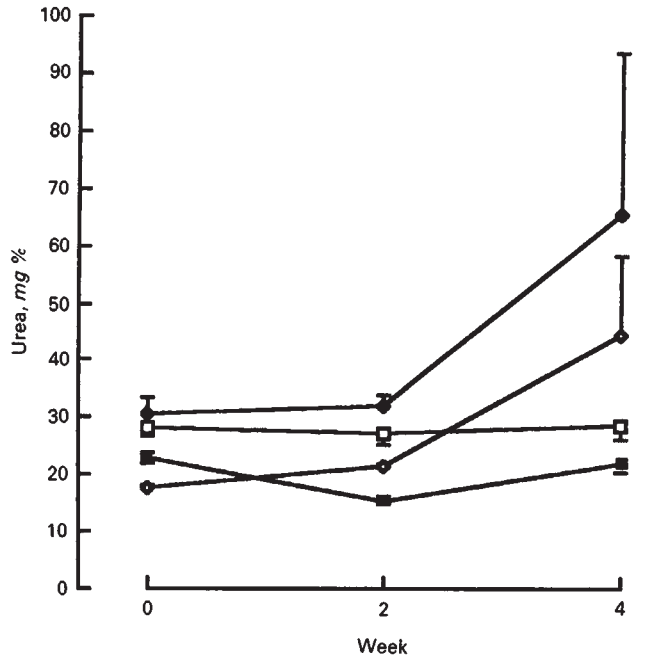

Fig. 7. *Blood urea nitrogen.* The blood urea nitrogen was elevated at week four in the B10.D2 and NZB mice but the elevations were not statistically significant. Symbols are: Balb/c (□), NZB (◆), DBA/2J (■), and B10.D2 (◇).

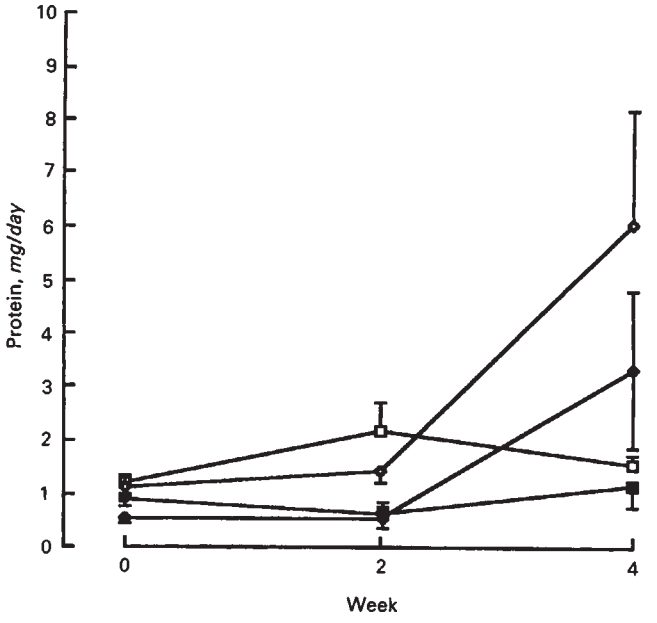

Fig. 8. *Urinary protein excretion.* Only the B10.D2 and NZB mice had elevated levels of 24-hour urinary protein excretion, and of these only the B10.D2 were significant ($P < 0.05$ for the B10.D2 vs. DBA/2J at week 4). Symbols are: Balb/c (□), NZB (◆), DBA/2J (■), and B10.D2 (◇).

ological parameters and developed only a modest antibody response compared to the other three strains studied. Interestingly, B10.BR (H-2^k) mice, which develop mesangial proliferation in response to apoferritin [6], actually have antibody levels somewhat lower than the levels in the DBA/2J mice, suggesting that other factors such as antibody avidity [16] or isotype may be playing a role. It is clear from this data that the H-2^d genotype is not sufficient for the development of severe Apo-ICGN. Genes outside of H-2 must also play a role.

This phenomenon occurs in other animal disease models. In murine interstitial nephritis, Balb/c and B10.D2 mice are susceptible, but DBA/2J mice are not [17]. Neilson and coworkers suggested that interactions between the H-2K locus and the Igh-1 locus (which codes for IgG2a) were important in the pathogenesis of this disease. A similar phenomenon was described in experimental allergic orchitis [18]. In murine collagen II autoimmune arthritis, disease susceptibility has been associated with genes within H-2 [19], and with non-H-2 genes such as Igh-1 and C5. Indeed, the essential role that C5 plays in Apo-ICGN was also shown [4]. In humans the influence of genes outside of the major histocompatibility complex has been demonstrated for the Gm loci on chromosome 14, which code for human immunoglobulin heavy chains, in multiple sclerosis [20] and lupus nephritis [21].

The glomerular lesions in NZB mice were in many ways the most interesting. These mice developed severe proliferative ICGN with massive deposits similar to the "wire loop" lesions seen in human lupus glomerulonephritis. These deposits were large and located in the subepithelial and subendothelial positions. In other strains studied by this laboratory, such extensive subendothelial deposits have not been seen. These deposits contained large amounts of apoferritin by immunofluorescence microscopy. In addition to the pathological abnormalities suggesting active lupus nephritis, renal eluates from NZB mice contained anti-ssDNA and dsDNA antibodies.

NZB mice spontaneously develop autoimmune hemolytic anemia and anti-nuclear antibodies after three months of age [22]. In addition, glomerulonephritis develops spontaneously in NZB mice. However, severe glomerulonephritis is not seen until late in life [23] and significant azotemia is seen only in elderly mice [22]. In our experiments, no significant glomerulonephritis or proteinuria was seen in the normal saline controls at the time of sacrifice at the age of three months. The saline injected controls did not develop autoantibodies in serum or kidneys.

Several phenomena appear to accelerate the development of glomerulonephritis in NZB mice. For example, the NZB X NZW F_1 hybrid develops fulminant lupus nephritis [24] as do the NZB X SWR F_1 hybrids [25]. Infection with lymphocytic choriomeningitis virus [26] and polyoma virus [27] was associated with more aggressive glomerulonephritis and elevated anti-nuclear antibodies. Injections of the synthetic double stranded ribonucleic acid, polyinosinic-polycytidylic acid, also accelerates the disease [28]. In non-autoimmune mice, autoantibodies and glomerulonephritis can be produced by injections with lipopolysaccharide [29, 30].

In the current studies, the acceleration of the development of a glomerular lesion suggestive of lupus nephritis and the development of autoantibodies came about as a result of the injection of an irrelevant antigen, apoferritin. The mechanisms of this phenomenon are not clear. Some authors have suggested that the B cells of the NZB mouse are hyperactive [31] and the data

in these studies would support that notion. NZB mice had the highest antiapoferritin antibody levels at the time of sacrifice of any strain we have studied. In addition, NZB mice had the highest levels of antiapoferritin antibody prior to immunization and saline-injected NZB mice had higher levels of antiapoferritin antibody on day 32 than apoferritin injected DBA/2J. Whether development of large deposits in the subepithelial and subendothelial spaces in these mice is a function of the extremely high antibody levels or some other factor such as antibody avidity [16], charge [32], or isotype [33] is not clear. Likewise, the role of the anti-dsDNA and anti-ssDNA antibodies in the pathogenesis of Apo-ICGN in NZB mice remains unclear. The presence of large amounts of apoferritin in the NZB kidneys suggests that apoferritin- anti-apoferritin immune complexes were involved in the pathogenesis of the glomerulonephritis. In addition, the presence of serum anti-nuclear antibodies in B10.D2 and DBA/2J suggests that these antibodies may form as a result of the immunization even in strains not predisposed to lupus. This has been seen in hyperimmunized mice [34] and mice immunized against tubercle bacillus [35]. Humans with chronic infections also develop autoantibodies [36]. However, in these studies only the NZB mice deposit these antibodies in their kidneys. We are currently examining the isotype classes and spectrotypes of these antibodies to identify any differences in NZB, B10.D2, DBA/2J and Balb/c which may have contributed to the development of lupus-like lesions in NZB mice.

A number of non-H-2 genes might be important in determining disease susceptibility. Genes coding for the T cell receptor, immunoglobulin allotypes, such as Igh-1, minor histocompatibility loci coded for on other chromosomes than chromosome 6, and complement loci [4] may all play a role.

Despite the dramatic changes that occur pathologically in these kidneys, the blood urea nitrogens were not significantly elevated. Perhaps this occurred because blood urea nitrogen is a poor marker of glomerular filtration rate. Another possibility is that the most azotemic mice died prematurely. Likewise, with the exception of the B10.D2 mice, the animals did not have proteinuria.

Genes in the H-2 region are crucially important in determining the susceptibility to Apo-ICGN and the pathology that results [6]. The present study demonstrates that genes outside of the major histocompatibility complex also can modify the expression of this disease. Non-H-2 genes in the DBA/2J strain can virtually eliminate the disease. Which non-H-2 genes are important in this effect remains to be determined. NZB background genes can alter the expression of Apo-ICGN so that it appears pathologically similar to lupus nephritis and is associated with the development of autoantibodies and severe glomerulonephritis. Genes in the Balb/c background do not alter the expression of Apo-ICGN as described in the B10.D2 mouse [6]. Susceptibility to Apo-ICGN is therefore dependent upon H-2 and non-H-2 genes.

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