TREATMENT OF (NZB × NZW)F₁ DISEASE WITH ANTI-I-A MONOCLONAL ANTIBODIES*

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(NZB × NZW)F₁ (NZB/W F₁) mice spontaneously develop a disease remarkably similar to systemic lupus erythematosus (SLE) in humans (1). The disease is characterized by the appearance of antibodies to nuclear antigens and subsequent development of a fatal, immune complex-mediated glomerulonephritis. In both the human and murine forms of the disease, a strong association between major histocompatibility complex (MHC) gene product genotype and manifestation of the disease state have been reported. HLA-DRw2 and HLA-DRw3 individuals are at higher risk than the general population for the development of SLE, while in NZB/W F₁ ($H\text{-}2^{d/z}$), a gene tightly linked to the $H\text{-}2^z$ haplotype contributes to the renal disease (2–5). The role of MHC genes in SLE and murine lupus is unknown.

Several studies have demonstrated that in vivo administration of anti-Ia antibodies to mice can greatly alter immune responsiveness (6–13, 17, 21). We have recently shown that in vivo treatment of $H\text{-}2^{b/k}$ mice with anti-I-A^k monoclonal antibodies can suppress humoral responses to (H,G)-A--L (controlled by I-A^k) without markedly suppressing the response to (T,G)-A--L (controlled by I-A^b) (13). We have now extended these experiments to the treatment of the NZB/W F₁, a hybrid in which a gene derived from by the $H\text{-}2^z$ parent appears to contribute to the development of a lupus-like nephritis. The results provide evidence that in vivo administration of monoclonal antibodies specific for an *I* region gene product (*I-A^z*) of the $H\text{-}2^z$ haplotype induces a long-lasting remission in female mice with moderate renal disease.

Materials and Methods

Mice. NZB/W F₁ mice were bred in our mouse colony at Stanford University. Only female mice between the ages of 4 and 5 mo with at least 1+ proteinuria were used.

Monoclonal Antibodies and Treatment Regimen. Two monoclonal antibodies were used in these experiments. H10-3.6 is an IgG₂ₐ that fixes complement and reacts with public specificity Ia.17 found on *I-A$^{k,r,s,f,\ and\ z}$* products (20). BP107 (donated by Dr. F. Symington and Dr. J. Sprent of the Wistar Institute, Philadelphia, PA) recognizes a public specificity associated with I-A^d and is an IgG₁ (19). Hybridomas were maintained in ascites form and the ascites fluids had titers >10⁴ in cell-binding assays (13).

NZB/W F₁ mice were treated for 4 mo with weekly intraperitoneal injections of 0.5 ml of anti-I-A^z- or anti-I-A^d-containing ascites, control ascites (Ig-containing ascites devoid of

anti-I-A activity) or phosphate-buffered saline (PBS). The mice were evaluated weekly for changes in weight and proteinuria, and for mortality.

Proteinuria.   Proteinuria was assayed with tetrabromphenol paper (Albustix; Ames Co., Inc., Elkhart, IN) on fresh urine samples. The test is relatively specific for albumin and is graded 1-4$^+$ (1$^+$, <30 mg/100 ml; 2$^+$, <100 mg/100 ml; 3$^+$, <300 mg/100 ml; and 4$^+$, >2,000 mg/100 ml). In these experiments, low grade proteinuria was designated as 1+ to 2+, and high grade proteinuria was designated as >2+.

Results and Discussion

In two experiments, a total of 35 female NZB/W F_1 received H10-3.6 (anti-I-A^z), 25 received BP107 (anti-I-A^d), and, as controls, 10 received ascites fluid containing no demonstrable anti-I-A Ig and 30 received PBS. All mice were 4–5 mo old and had documented proteinuria at the beginning of the experiment. Fig. 1 shows the improved survival rate of NZB/W F_1 females treated with anti-I-A^z antibody. At 1 yr, almost 90% of control animals were dead. Treatment with anti-I-A^z resulted in a highly significant improvement ($P < 0.0001$) in the survival rate (90%) and anti-I-A^d therapy enhanced survival by 60%. High grade proteinuria was observed in <10% of anti-I-A^z-treated NZB/W F_1 mice compared with 44% of surviving anti-I-A^d-treated animals and 70% of surviving control animals (Fig. 2).

A marked difference in weight gain of control and anti-I-A–treated mice was also observed. The anti-I-A^z-treated mice consistently gained or maintained their weight throughout the experiments, while control animals showed fluctuations in weight with a gradual decline as the disease progressed (data not shown).

Significantly, this study showed that in vivo therapy with anti-I-A antibodies can alter the course of NZB/W F_1 disease after onset of renal pathology. The majority of experimental lupus studies are prophylactic in nature and therefore have little applicability to the treatment of SLE (22–29). However, studies using high dose cyclophosphamide, total lymphoid irradiation, and ribavirin have demonstrated suppression of the disease after the development of proteinuria (30–32).

The mechanism by which anti-I-A therapy reverses NZB/W F_1 renal disease is unknown. However, it probably involves altering the immune status of NZB/W F_1 mice. Considerable evidence indicates that in vivo administration of anti-
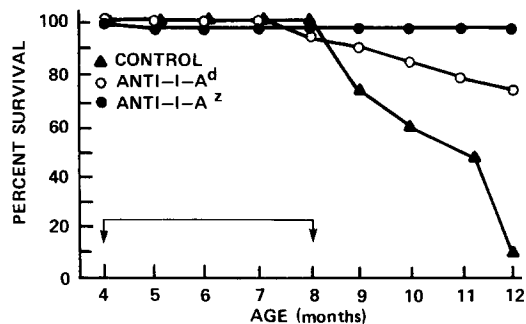

FIGURE 1.   Effect of anti-I-A monoclonal treatment on the survival of NZB/NZW F_1 mice up to 1 yr of age. (●) 10-3.6 (anti-I-A^z) group, (○) BP107 (anti-I-A^d) group, (▲) control group. Arrows indicate the time period of monoclonal antibody treatment.

FIGURE 2.   Effect of treatment on the cumulative progression to high grade proteinuria (2^+, ≤ 100 mg/100 ml) up to 1 yr of age. (●) 10-3.6 (anti-I-A^z) group, (○) BP107 (anti-I-A^d), (▲) control group. Arrows indicate the time period of monoclonal antibody treatment.
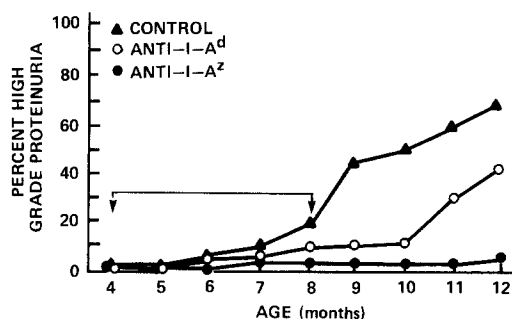
Ia antibodies can markedly alter immune responsiveness. Studies demonstrate that treatment with anti-Ia Ig can enhance allograft survival (6), modulate immune responses to tumors (7, 8) and to schistosomes (9), block delayed hypersensitivity reactions to azobenzenearsonate and a methylcholanthrene-induced fibrosarcoma (10, 11), and prevent the induction of helper T cells required for Ig secretion (12). Further, results from our laboratory show that it is possible to alter an Ir gene-controlled humoral response in a haplotype-specific manner in F_1 mice by in vivo treatment with anti-I-A monoclonal antibodies directed against the product of one parental haplotype (13). This in vivo regimen results in an interesting form of suppression: it is long term, lasting at least 3 mo after cessation of treatment; it can alter ongoing I-A-controlled humoral responses; and it has no significant effect on the response to an antigen that is not controlled by the I-A allele under study (13, 14). All of these factors are advantages for any potential in vivo therapy. Results from adoptive transfer studies indicate that this suppression is maintained by a suppressor T cell population(s) (14). The work of other investigators provides evidence that suppressor cell induction may be a common occurrence after anti-Ia or anti-DR (the human analog of Ia) administration. Broder et al. (15) identified an anti-DR–induced T cell that can suppress mitogen-triggered Ig secretion in vitro, while Muchmore et al. (16) were able to block antigen-induced proliferation with anti-DR–treated monocytes. Using an in vitro murine system, Berzofsky and Richman (17) described transferable suppression of Ir gene-controlled responses to myoglobin with anti-Ia–treated macrophages. Perry and Greene (18) showed that a T cell population is responsible for depression of delayed hypersensitivity and for tumor rejection seen in mice after in vivo anti-I-A treatment.

Experiments are in progress to determine if the induction of suppressor cells is the mechanism responsible for the alteration in NZB/W F_1 renal disease after anti-I-A treatment. It is possible that the anti-I-A may be affecting other Ia$^+$ cells in NZB/W F_1 mice and that those actions may be responsible for altering the disease course. However, preliminary evidence indicates that anti-Ia monoclonals are not functioning simply at the level of B cells, since anti-ds DNA and antinuclear antibody titers are not reduced in the anti-I-A^z–treated group relative to the control group.

The observed difference in the effectiveness of the anti-I-A^z and the anti-I-A^d monoclonals to alter morbidity and mortality in the present experiments suggests that the choice of monoclonal antibody may be critical. It is unclear whether this variation resulted from differences in affinity, quantity, specificity, or isotype of the antibodies used. It is unlikely that the experimental variation was due to differences in affinity and quantity since both monoclonals had similar binding potentials in solid-phase antigen-binding assays. Possibly, the reduced ability of BP107 to avert renal disease stems from its specificity; i.e., I-A^d gene product(s) is not the principle product involved in the course of lupus nephritis. The work of Knight and Adams (4) provides some support for this hypothesis. Their study showed that three dominant or codominant genes are associated with glomerulonephritis in NZB/W F_1 mice. Significantly, one of the two genes derived from the NZW (H-2^z) parent is very tightly linked to the H-2 complex while the gene derived from the NZB (H-2^d) parent is weakly associated with H-2 and the nephritis. Additionally, the isotype and the biological activity associated with particular Ig subclasses may be very important in altering immune responsiveness; i.e., IgG$_1$ monoclonals may be less efficient than IgG$_{2a}$ antibodies in triggering suppression.

The results of this study coupled with other reports (10–13, 21) indicate that administration of anti-Ia antibodies is a safe method for manipulating immune responses. In particular, NZB/W F_1 mice that are genetically prone to renal difficulties tolerated weekly infusions of milligram quantities of Ig without obvious problems. The ability of anti-I-A monoclonals to manipulate H-2-linked autoimmune syndromes (21, 33) without visible damage to the host suggests that the present experiments may have potential application to the treatment of autoimmune diseases associated with certain HLA-D or DR types.

Summary

(NZB × NZW)F$_1$ mice spontaneously develop an autoimmune syndrome characterized by a fatal immune complex glomerulonephritis. Administration of monoclonal antibodies specific for an I region gene product (I-A^z) of the H-2 haplotype associated with susceptibility to glomerulonephritis in these animals produced a remission in female mice with established renal disease. The results demonstrated that anti-I-A therapy stabilized the level of proteinuria and increased the 1-yr survival rate from 10% to >90% in treated animals relative to control mice. These findings may ultimately have therapeutic potential for the treatment of systemic lupus erythematosus.

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