Brief Definitive Report

IN VIVO TREATMENT OF (NZB × NZW)F_1 LUPUS-LIKE NEPHRITIS WITH MONOCLONAL ANTIBODY TO γ INTERFERON

BY CHAIM O. JACOB,* P. H. VAN DER MEIDE,‡ AND HUGH O. MCDEVITT*

*From the *Departments of Medical Microbiology and Medicine, Stanford University, Stanford, California 94305; and the ‡Primate Center, TNO, 2288 GJ Rijswijk, The Netherlands*

The F_1 hybrids of the autoimmune New Zealand Black (NZB) mice and the phenotypically normal New Zealand White (NZW) mouse strain, develop severe systemic autoimmune disease, more fulminant than that found in the parental NZB strain. These mice manifest several immune abnormalities including antibodies to nuclear antigens and subsequent development of a fatal, immune complex–mediated glomerulonephritis with female predominance, remarkably similar to systemic lupus erythematosus (SLE) in humans.

As a reflection of their autoimmune nature, both the human and murine forms of the disease show a strong association with MHC gene products. HLA DR2 and HLA DR3 individuals are at a higher risk than the general population to develop SLE (1), while in NZB/W F_1 mice (H-$2^{d/u}$) a gene linked to the H-2^u haplotype derived from the NZW parent contributes to the development of the lupus-like nephritis (2). The role of MHC genes in SLE and murine lupus is unknown. Similarly, it has been difficult to clarify the regulatory and functional abnormalities in the immune system that allow the tissue damage to occur in autoimmune disease. No doubt, with the large range of cellular interactions required for normal immunological function and tolerance, defects in the control or modulation of these interactions could occur at several levels and at any or all of these might result in reactivity to self antigens.

In this study we pursue the hypothesis that IFN-γ plays a crucial role in the pathogenesis of autoimmune processes. Earlier reports from this laboratory (3) and from others (4–5) have indicated that treatment of NZB/W F_1 mice with partially purified type I or type II interferon, resulted in an increased incidence of glomerulonephritis and death. If this hypothesis is correct, administration of IFN-γ may upregulate the autoimmune process, while blocking the effect of IFN-γ might downregulate such a process. We have tested this hypothesis in vivo, in the NZB × NZW/F_1 lupus nephritis murine model.

Materials and Methods

IFN-γ and Treatment Regimen. Murine IFN-γ manufactured in *Escherichia coli* by recombinant DNA technology and of >95% purity (7.2×10^6 U/mg) was kindly provided by Dr. H. Michael Shepard, Genentech, Inc., South San Francisco, CA. NZB/W F_1 female mice were given intraperitoneal injections of 5×10^4 U of rIFN-γ or PBS three times weekly for a period of 3 mo.

This work was supported by National Institutes of Health grants AI-11313 and AI-07757.

 J. EXP. MED. © The Rockefeller University Press · 0022-1007/87/09/0798/06 $2.00
Volume 166 September 1987 798–803

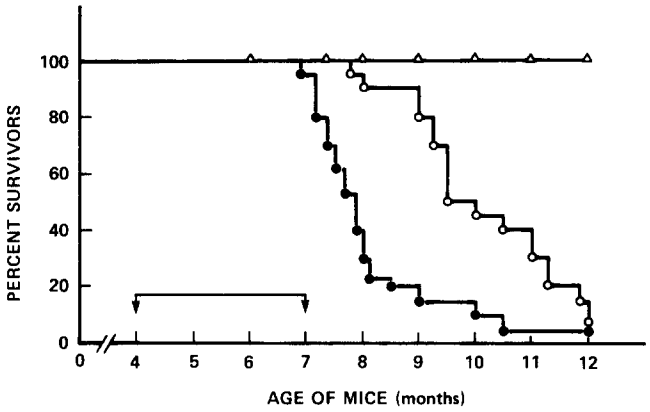

FIGURE 1. Survival of NZB/W F_1 female mice treated with IFN-γ. 25 NZB/W F_1 mice treated with IFN-γ (●) are compared with 25 age- and sex-matched NZB/W F_1 animals given PBS (○) and to 16 NZW parental strain treated with IFN-γ (△). Arrows indicate the time period of treatment.

Monoclonal Antibodies. DB-1 is an IgG1 mouse anti-rat IFN-γ mAb that exhibits high-affinity binding for rat and mouse IFN-γ and efficiently neutralizes the antiviral activity of both animal interferon species (6). TE33 is an IgG1 mouse mAb specific to residues 50–64 of cholera toxin B subunit, and was used as a control antibody.

DB-1 monoclonal anti IFN-γ antibodies were purified from ascites fluid of BALB/c mice injected with 5×10^6 DB-1 hybridoma cells. Antibodies were purified by 45% ammonium sulfate precipitation (twice) followed by chromatography on DEAE-Sephacel column. The ability of DB-1 to inhibit IFN-γ–induced Ia expression was assayed in vitro on the murine myelomonocytic cell line WEHI-3. Treatment of these cells with murine IFN-γ for 24–48 h induces the expression of Ia antigens on their surface (7). Treatment of WEHI-3 cells with 10 U/ml of murine IFN-γ in the presence of DB-1 at 0.5 mg/ml caused inhibition of ~70% of Ia expression as detected with fluorescein-conjugated MK-D6 (anti-Ia^d) mAb on a FACS IV (Becton Dickinson & Co., Mountain View, CA).

Measurement of Anti-DNA Antibodies. Anti-DNA–specific antibodies in sera were assayed by an ELISA as described (8).

Proteinuria. Proteinuria was measured colorimetrically by the use of Uristix (Miles Laboratories Inc., Elkhart, IN).

Results

Two groups of 25 female NZB/W F_1 mice, 4 mo old, received rIFN-γ or equivalent volumes of PBS over a period of 3 mo. As an additional control group, 16 age- and sex-matched mice of the parental strain NZW, were similarly treated with IFN-γ. Death occurred at an earlier age in the NZB/W F_1 group that received IFN-γ compared with the PBS control mice (Fig. 1). The difference in survival between treated and control mice was statistically significant ($p \leq 0.001$).

While PBS-treated NZB/W F_1 control mice began to die at ~8 mo of age (50% survival, 9.5 mo), in the IFN-γ-treated group 75–80% of NZB/W F_1 were dead by 8 mo. The life span of the NZW control group was not affected by the IFN-γ treatment. High grade proteinuria was detected significantly earlier in the IFN-γ-treated NZB/W F_1 group compared with the control group (Fig. 2*A*). The onset of death correlated closely with the onset and amount of proteinuria. Similarly, anti-DNA antibodies reached peak levels earlier in the IFN-γ treated-group than in the control group (Fig. 2*B*), although no significant differences in the peak levels of anti-DNA antibodies were observed between the two groups as a whole.

In a separate experiment, treatment with IFN-γ was initiated at different ages

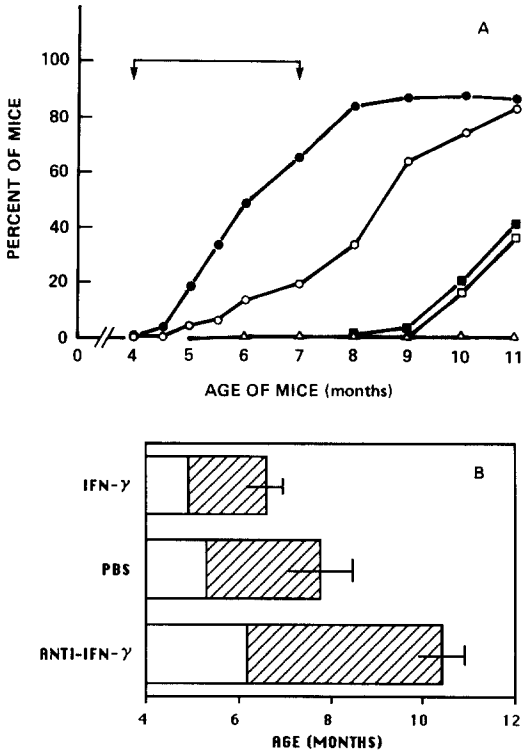

FIGURE 2. (*A*) Cumulative frequency of significant proteinuria (≥3⁺) in NZB/W F_1 mice treated with IFN-γ (●), PBS or nonrelevant mAb (○), or monoclonal anti-IFN-γ, either 3 times per week (■) or once per week (□). Since no significant differences were found between PBS- versus nonrelevant antibody–treated animals, they are presented together as a single control group. To reflect more accurately the development of renal disease in all mice (alive and dead), a correction factor was induced. Thus, each point reflects the current level of proteinuria as well as the last measurement of proteinuria in deceased mice. (*B*) Effect of the various treatments on the appearance of anti-DNA antibodies. The open bars represent the mean age at which anti-DNA antibodies were detected in the various groups, while dashed bars represent the mean age ± SD at which peak levels of anti-DNA antibody occurred. Note that at the age when anti-DNA antibody was detected, the difference between the PBS- and the IFN-γ-treated groups is not significant, while the difference between PBS- and DB-1-treated groups is significant ($p \leq 0.05$). The differences in age at peak levels between PBS-treated versus IFN-γ- or DB-1-treated groups are significant ($p < 0.005$ and $p \leq 0.0005$, respectively).

between 2.5 and 6.5 mo, using the same treatment protocol. Treatment starting at various ages between 2.5 and 6 mo resulted in significantly earlier appearance of high grade proteinuria and accelerated mortality when compared with age-matched control groups. When IFN-γ treatment started at 6.5 mo, no significant difference in lifespan was observed between treated and controls (not shown).

Given these results we asked whether we could block disease development by using mAb to IFN-γ. Two groups of 20 female NZB/W F_1 mice received purified anti-IFN-γ mAb (DB-1), and as controls, 10 mice received an irrelevant monoclonal IgG1 antibody (TE33) and 20 animals received PBS. All mice were 4 mo old at the beginning of the experiment. One group was treated with 2 mg DB-1 intraperitoneally three times per week and the other received 2 mg DB-1 once a week. Treatment was given for a period of 3 mo.

Fig. 3 shows the improved survival rate of mice treated with DB-1. At the age of 11 mo, 80–85% of both control groups were dead, while 95% of mice were alive in both DB-1-treated groups. No difference was found between mice given weekly 2-mg injections of anti-IFN-γ compared with those receiving 2 mg antibody three times per week. In parallel with the dramatic prolonged survival of DB-1-treated mice, the development of severe proteinuria was significantly delayed (Fig. 2*A*). By 9 mo >60% in each control group had high grade proteinuria, but none of the mice treated with DB-1 had proteinuria of this degree. At the age of 11 mo ~30–40% of treated animals showed severe proteinuria. The titer of anti-DNA antibody reached maximal levels at 7–8 mo in the control groups, while DB-1-treated animals showed similar levels at ~10 mo of age (Fig. 2*B*). It is noteworthy that the PBS control group and the

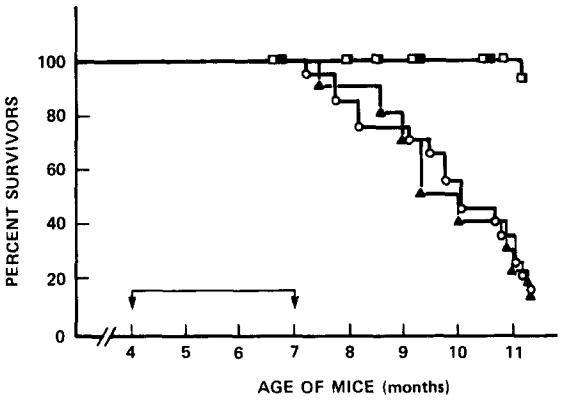

FIGURE 3. Prolonged survival of NZB/W F_1 mice treated with anti-IFN-γ mAbs. Groups of 20 age-matched female mice were treated intraperitoneally with PBS (▲), nonrelevant mAb (○), or with monoclonal anti IFN-γ either at 2 mg three times per week (■) or 2 mg once per week (□).

irrelevant antibody control groups are very similar in terms of survival, proteinuria, and anti-DNA antibody profiles.

Discussion

During the past decade many diverse activities have been attributed to IFN-γ. Despite the ability of IFN-γ to mediate such a variety of functions, and to exert remarkably pleomorphic effects on the immune system (9), surprisingly little is known about the biological relevance of IFN-γ to the homeostasis of the immune system in vivo.

Two lines of experimental observations led us to undertake these experiments. First, IFN-γ has been established as the prototype lymphokine to induce enhancement of synthesis and surface expression of MHC class II antigens in a wide variety of cell types both in vitro and in vivo (10, 11). Inappropriate expression of MHC class II molecules has been shown in several autoimmune processes both in animal models (12) and human diseases (13). Moreover, it seems possible that the induction of MHC molecules is due to release of IFN-γ by activated T cells. Thus, rat astrocytes induced in vitro to express Ia molecules by IFN-γ were able to present myelin basic protein to encephalitogenic T cell lines in a MHC-restricted manner (14). Second, studies in this laboratory have demonstrated that in vivo administration of mAbs specific for an Ia region gene product ($I\text{-}A^u$) induced remission in NZB/W F_1 mice with moderate renal disease (15). Anti-Ia mAb therapy was effective in several other autoimmune disease models such as experimental allergic encephalitis, experimentally induced myasthenia gravis, and in spontaneous autoimmune diabetes and thyroiditis in BB/W rats.

Our results clearly suggest that IFN-γ might have a major biological role in aberrant immune regulation causing the development of murine lupus nephritis.

The fact that administration of IFN-γ accelerated the development of the nephritis does not by itself prove that this lymphokine has a major role in the development of the disease, since other lymphokines and cellular factors share some activities with IFN-γ: thus, for example, in addition to IFN-γ, class II MHC expression has been enhanced by IFN-α or -β (16) and IL-4 (BSF-1) (17). Therefore, the blocking of IFN-γ might have no effect on other possible regulatory factors and thus have no effect on the progression of the disease. On the other hand, if antibody to IFN-γ is able to block or delay progression of disease, this would argue in favor of IFN-γ playing a principal role in the

pathogenesis of the disease. Thus, the experiments with monoclonal anti-IFN-γ were essential to confirm this point.

Whether the results of this study are applicable in a more general sense to the initiation and propagation of other autoimmune processes, or even during normal ongoing immune responses, remains an open question at the present time. Similarly the mechanism of IFN-γ activity in this in vivo system remains to be determined. While we favor the hypothesis that IFN-γ activates the immune system by upregulating class II MHC antigen expression as initially proposed by Bottazzo et al. (18), it must be noted that IFN-γ has both activating and inhibiting effects on B cell differentiation and B cell responses, and at least some of the autoimmune abnormalities in NZB/W F_1 mice may be attributed to B cell effects (19). Alternatively, it is possible that IFN-γ has an indispensable physiological function that is entirely distinct from any yet defined.

In considering potential therapeutic applications of IFN-γ (20), our observations indicate that this lymphokine should not be considered exempt from possible untoward consequences. The present study, coupled with other observations (21), suggests that IFN-γ may be contraindicated in patients with certain autoimmune diseases.

Conversely, we have shown that in vivo therapy with monoclonal anti-IFN-γ can significantly alter the course of murine lupus-like nephritis. This may have implications for the treatment of SLE in man.

Summary

The (NZB × NZW)F_1 mouse is recognized as an important animal model of the human disease systemic lupus erythematosus (SLE). Groups of NZB/W F_1 mice were treated either with IFN-γ or with PBS. The results demonstrate that IFN-treated animals have accelerated development of fatal immune complex glomerulonephritis relative to age-matched controls. On the other hand, administration of mAbs specific for IFN-γ to such mice from 4 to 7 mo of age resulted in significant remission. Development of both proteinuria and anti-DNA antibodies were delayed and survival at 11 mo was increased from <20% to 95% in treated mice relative to controls ($p \leq 0.001$). These findings may have therapeutic implications for the treatment of SLE.

We thank Dr. H. Michael Shepard for the generous gift of recombinant IFN-γ. We also thank Peggy Sullivan for her expert technical contribution and Drs. Arthur van Es and Michael McDermott for their help and advice.

Received for publication 4 May 1987 and in revised form 11 June 1987.

References

1. Reinertsen, J. L., H. J. Kleppel, A. H. Johnson, A. D. Steinberg, J. C. Decker, and D. C. Mann. 1978. B-lymphocyte alloantigens associated with systemic lupus erythematosus. *N. Engl. J. Med.* 299:515.
2. Knight, J. G., and D. D. Adams. 1978. Three genes for lupus nephritis in NZB × NZW mice. *J. Exp. Med.* 147:1653.
3. Engleman, E. G., G. Sonnenfeld, M. Dauphinee, J. S. Greenspan, N. Talal, H. O. McDevitt, and T. C. Merigan. 1981. Treatment of NZB/NZW F_1 hybrid mice with

Mycobacterium Bovis strain BCG or type II interferon preparations accelerates autoimmune disease. *Arthritis Rheum.* 24:1396.

4. Heremans, H., A. Billan, A. Colombatti, J. Hilders, and P. DeSomer. 1978. Interferon treatment of NZB mice: accelerated progression of autoimmune disease. *Infect. Immun.* 21:925.
5. Sergiescu, D., I. Cerutti, E. Efthymiou, A. Kahan, and C. Chany. 1979. Adverse effects of interferon treatment on the life span of NZB mice. *Biomed. Exp.* 31:48.
6. Van Der Meide, P. H., M. Dubbeld, K. Vijerberg, T. Kos, and H. Schellekens. 1986. The purification and characterization of rat gamma interferon by use of two monoclonal antibodies. *J. Gen. Virol.* 67:1059.
7. King, D. P., and P. P. Jones. 1983. Induction of Ia and H-2 antigens on a macrophage cell line by immune interferon. *J. Immunol.* 131:315.
8. Zouali, M., and B. D. Stollar. 1986. A rapid ELISA for measurement of antibodies to nucleic acid antigens using UV-treated polystyrene microplates. *J. Immunol. Methods.* 90:105.
9. Trinchieri, G., and B. Perussia. 1985. Immune interferon: a pleiotropic lymphokine with multiple effects. *Immunol. Today.* 6:131.
10. Wong, G. H. W., I. Clark-Lewis, J. L. McKinn-Breschkin, A. W. Harris, and J. W. Schrader. 1983. Interferon-γ induces enhanced expression of Ia and H-2 antigens on B lymphoid, macrophage and myeloid cell lines. *J. Immunol.* 131:788.
11. Ameglio, F., R. Tosi, N. Tanagaki, and A. Doley. 1986. Regulation of HLA class II antigen expression. *In* HLA Class II Antigens. A Comprehensive Review of Structure and Function. B. G. Solheim, E. Moller, and S. Ferron, editors. Springer-Verlag New York Inc., New York. p 299.
12. Sakai, K., T. Tabira, M. Endoh, and L. Steinman. 1986. Ia expression in chronic relapsing experimental allergic encephalomyelitis induced by long-term cultured T cell lines in mice. *Lab. Invest.* 54:345.
13. Bottazzo, G. F., I. Todd, R. Mirakian, A. Belfiore, and R. Pujol-Borrel. 1986. Organ specific autoimmunity: a 1986 overview. *Immunol. Rev.* 94:137.
14. Fontana, A., W. Fierz, and H. Wekerle. 1984. Astrocytes present myelin basic protein to encephalitogenic T-cell lines. *Nature (Lond.).* 307:273.
15. Edelman, N. E., D. L. Watling, and H. O. McDevitt. 1983. Treatment of (NZB × NZW) F_1 disease with anti-I-A monoclonal antibodies. *J. Exp. Med.* 158:1350.
16. Basham, T., W. Smith, L. Lanier, V. Morhenn, and T. Merigan. 1984. Regulation of expression of class II MHC antigens on human peripheral blood monocytes and Langerhans cells by interferons. *Hum. Immunol.* 10:83.
17. Noma, Y., P. Sideras, T. Naito, S. Bergstedt-Lindquist, C. Azuma, E. Severinson, T. Tanabe, T. Kinashi, F. Matsuda, Y. Yaoita, and T. Honjo. 1986. Cloning of cDNA encoding the murine IgG_1 induction factor by a novel strategy using SP6 promoter. *Nature (Lond.).* 319:640.
18. Bottazzo, G. F., R. Pujol-Borrell, T. Hanafusa, and M. Feldmann. 1983. Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. *Lancet.* ii:1115.
19. Izui, S., P. J. McConahey, and F. J. Dixon. 1978. Increased spontaneous polyclonal activation of B lymphocytes in mice with spontaneous autoimmune disease. *J. Immunol.* 121:2213.
20. Nathan, C. F., G. Kaplan, W. R. Levis, A. Nusrat, M. D. Witmer, S. A. Sherwin, C. K. Job, C. R. Horowitz, R. M. Steinman, and Z. A. Cohn. 1986. Local and systemic effects of intradermal recombinant interferon-γ in patients with lepromatous leprosy. *N. Engl. J. Med.* 315:6.
21. Panitch, H. S., A. S. Haley, R. L. Hirsch, and K. P. Johnson. 1986. A trial of gamma interferon in Multiple Sclerosis: clinical results. *Neurology.* 36(Suppl. 1):285.