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Efficacy of IVIG affinity-purified anti-double-stranded DNA anti-idiotypic antibodies in the treatment of an experimental murine model of systemic lupus erythematosus

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Abstract

Since the idiotypic network is an important mechanism for controlling the immune repertoire, we tested anti-idiotypic modulation employing concentrated specific natural polyclonal anti-double-stranded (ds) DNA anti-idiotypic antibodies obtained from a commercial IVIG in the treatment of experimental systemic lupus erythematosus (SLE). Specific natural polyclonal anti-dsDNA anti-idiotypic antibodies (IVIG-ID) were affinity purified from IVIG on an anti-dsDNA–Sepharose column constructed from anti-dsDNA idiotypes (ID) affinity purified from 55 patients with active SLE. NZB/W F_1 mice were treated i.v. with 3 weekly injections of IVIG-ID (2 mg/kg/injection) or regular IVIG (400 mg/kg/injection) both before (age 8 weeks) and after developing anti-dsDNA antibodies at the age of 21–22 weeks. The IVIG-ID-treated mice showed a decline in the titer of anti-dsDNA antibodies during the treatment, reaching maximum suppression 1 week after the last injection. A significant difference in the proteinuria level in the IVIG-ID-treated group compared to the control group was observed. Immunohistology showed different patterns of IgG deposition, with mesangial and capillary wall deposits in controls and in the IVIG-treated group, but only mesangial deposits in the IVIG-ID-treated group. The survival time of the IVIG-ID-treated group was longer than the IVIG-treated group. Treatment with concentrated specific anti-dsDNA anti-ID prepared from commercial IVIG is more effective in suppressing the humoral reaction and clinical signs of SLE than native IVIG. These results point to the considerable regulatory role of anti-ID in the mechanism of action of IVIG in SLE.

Introduction

Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disease with a great diversity of clinical manifestations, ranging from mild clinical findings with typical abnormal laboratory tests to a life-threatening condition. Laboratory abnormalities include high titers of autoantibodies to a vast array of tissue antigens. The most characteristic are those directed against components of the cell nucleus such as DNA, RNA, histones, nuclear proteins and protein–nucleic complexes. The clinical course of SLE is highly variable and unpredictable, frequently involving periods of remissions and relapses. The survival of patients with SLE has improved remarkably over the past decades, mainly due to the use of corticosteroids and cytotoxic drugs. While such medications have powerful anti-inflammatory and immunomodulatory effects, their use is severely limited by immunosuppression, myelosuppression and/or numerous other frequent side effects. A safe and efficient mode of immunomodulatory therapy for this disorder is still lacking. Administration of high-dose IVIG is immunoregulatory, but not immunosuppressive or myelotoxic. IVIG is capable not only of modulating SLE in animal models and in humans, but it also may provide a defense against infection rather than encouraging it. However,

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using IVIG in the treatment of SLE is at present limited by cost, a poor understanding of the mechanism of action and conflicting results presented in the literature regarding clinical efficacy (1,2)

IVIG appears to provide large amounts of immunoregulatory substances that have the capacity to regulate the immune system in various ways. The notion that IVIG contains anti-idiotypic antibodies comes from Sultan's observation that 95% of anti-factor VIII antibodies were cleared from patient's serum within 36 h of IVIG treatment (3). Consistent with this theory, SLE patients demonstrate a significant decline of anti-double stranded (ds) DNA autoantibodies after the treatment with IVIG in the majority of the cases reports (1). Evidently, commercial IVIG preparations contain anti-idiotypic antibodies against anti-factor VIII antibodies (3) and anti-DNA autoantibodies (4,5), as well as other autoantibodies such as anti-intrinsic factor antibodies (5), anti-thyroglobulin (Tg) autoantibodies (5), anti-neutrophil cytoplasmic antibodies (6), anti-microsomal antibodies (7), anti-neuroblastoma antibodies (8), anti-phospholipid antibodies (9), anti-platelet antibodies (10), anti-Sm idiotype (ID-434) (11) and anti-GM_1 antibody (12). These anti-idiotypic antibodies can inhibit the binding of the pathogenic autoantibodies to their corresponding antigen *in vitro* (4,5) and *in vivo*, thereby treating or preventing disease manifestations. (9,11,13). Another possible explanation for the beneficial effect of anti-idiotypic antibodies is their inhibitory effect on the spontaneous secretion of anti-dsDNA by peripheral B lymphocytes, as was demonstrated *in vitro* (14). Soluble circulating immune complexes may also become aggregated and insoluble following IVIG treatment via the idiotypic network mechanism, and thereby increasing their removal by the reticuloendothelial system.

We believe that the idiotypic network is an important mechanism for controlling the immune repertoire. Mice models of SLE treated with monoclonal anti-idiotypic antibodies have pointed to this potential effect; however, they have yielded conflicting results (15–17). Since human SLE is characterized by the presence of numerous autoantibodies and it is not yet known which are pathogenic (18–22), treatment with monoclonal anti-idiotypic antibody may produce an inhibitory effect in some patients while being useless in others. On the other hand, the amount of specific anti-ID in commercial IVIG preparations is extremely low; thereby use of isolated anti-ID against pathogenic autoantibodies may result in a more effective treatment with a fraction of the amount of IgG. Consequently, we decided to employ anti-idiotypic modulation by concentrated specific natural polyclonal anti-dsDNA anti-idiotypic antibodies from a commercial IVIG in the treatment of SLE. We used the most commonly employed animal model of SLE, NZB/W F_1 hybrid mice, which spontaneously develop autoimmune disease characterized by the production of high levels of IgG autoantibodies to nuclear antigens and the development of severe immune complex-mediated glomerulonephritis (23).

The aim of the present study was to evaluate the effect of IVIG affinity-purified anti-dsDNA anti-idiotypic antibodies (IVIG-ID) on the immunological and clinical findings in the genetic model of the SLE in mice.

Methods

Affinity purification of anti-dsDNA antibodies

Anti-dsDNA antibodies were purified from sera of 55 active patients satisfying the ACR diagnostic criteria for SLE. Native DNA–cellulose (Pharmacia Biotech, Uppsala, Sweden) was equilibrated with buffer, containing 0.025 M Tris/0.145 M NaCl, pH 7.4 (TBS). Patient serum (2 ml) diluted 5 times with TBS was allowed to pass through the column for 4 h at room temperature. The column was then washed with TBS and adherent antibody was eluted with 2.5 M $MgCl_2$. Protein-containing fractions were extensively dialyzed against TBS. Sera, affinity column eluates and flow-through were tested for anti-dsDNA activity using an ELISA lit (ORGenTec Diagnostika, Mainz, Germany).

Construction of dsDNA idiotypic column

Affinity-purified anti-dsDNA antibodies (2 mg) were dialyzed against coupling buffer [0.1 M $NaHCO_3$ (pH 8.3) containing 0.5 M NaCl] overnight at 4°C and covalently bound on CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden; cat. no. 17-098-01) according to the manufacturer's instructions. The remaining active NH groups were blocked by incubation with 0.2 M glycine (pH 8.0) overnight at 4°C. Three cycles of washing with coupling buffer followed by 0.1 M acetate buffer (pH 4.0) were used to remove the excess of unbound anti-dsDNA antibodies. The washed column was equilibrated in sterile Tris–HCl buffer (pH 7.4).

Construction of a human IgG–Fcγ column

A Fcγ column was constructed in the same way using 5 mg of human IgG–Fcγ (Jackson ImmunoResearch, West Grove, PA) dissolved in the coupling buffer.

Purification of IVIG-ID

Five hundred milligrams (10 ml) of the commercial IVIG preparation OMRIGAM (Omrix, Biopharmaceuticals Ltd, Nes-Ziona, Israel) was dialyzed against loading buffer [0.05 M Tris containing 0.5M NaCl (pH 8.0)], diluted in 50 ml of loading buffer, filtered through a 0.45-ìm filter (Minisart; Sartorius, Millipore Corp., Bedford, MA) and passed through the anti-dsDNA idiotypic column for 16 h at 4°C. Unbound material was washed out and the bound anti-idiotypic antibody was eluted with 0.2 M glycine–HCl (pH 2.7) and immediately neutralized with 2 M Tris. The IgG-containing fractions were pooled, dialyzed against PBS, sterilized by membrane filtration on a low protein binding acetate filter (Minisart 0.2 μm) and frozen in aliquots at –70°C.

Purification of anti-Fcγ–IVIG and anti-Fcγ-depleted IVIG-ID (fIVIG-ID)

Commercial IVIG was first passed through the Fcγ column (16 h at 4°C) to deplete anti-Fcγ antibodies. The eluate (anti-Fcγ–IVIG) was dialyzed against 1% maltose and used for the treatment. The flow-through was immediately loaded to the anti-dsDNA idiotypic column (for 16 h at 4°C) and after washing out the unbound material the fIVIG-ID was eluted with 0.2 M glycine–HCl, immediately dialyzed against 1% maltose and stored at 4°C.

Assay for anti-idiotypic activity

Inhibition experiments were performed to confirm the presence of the anti-dsDNA–anti-ID in IVIG-ID preparations. The affinity purified human or mouse anti-dsDNA antibodies at 50% of maximal binding to dsDNA, were mixed with different concentrations (1000–0.01 μg/ml) of IVIG, IVIG-ID, flow-through or anti-Fc fraction of IVIG and were incubated at 4°C overnight. The mixtures were added to ELISA plates coated with dsDNA and the remaining activity of the human or mouse antibodies was detected using corresponding secondary antibodies. The specificity of the IVIG-ID was confirmed by inhibition assays with affinity-purified human and mouse unrelated anti-Tg antibodies.

Treatment of murine lupus in NZB/W F_1 mice with IVIG and IVIG-ID

Early treatment. Fifty female NZB/W F_1 mice (8–10 weeks old) were purchased from Jackson Laboratory (Bar Harbor, ME). To exclude the effect of the diluent of IVIG, i.e. maltose, or the non-specific effect of anti-Fcγ antibodies in the IVIG compound, two additional controls were added: mice were subjected to 1% maltose (the diluent of the IVIG) and mice were treated with anti-Fcγ antibodies. To confirm the 200 times greater efficiency of IVIG-ID (preliminary results), an additional group of mice was treated with a low dose of IVIG comparable in concentration (60 μg/injection) to IVIG-ID. The mice were randomly distributed to five groups (10 mice per group) and treated i.v. via the tail vein once a week for 3 weeks as follows: (i) control group, with 1% maltose; (ii) anti-Fcγ–IVIG group, with 2 mg/kg (60 μg/mouse) of anti-Fcγ–IVIG in 1% maltose; (iii) high-dose IVIG group, with 400 mg/kg (12 mg/mouse) of IVIG; (iv) low-dose IVIG group, with 2 mg/kg (60 μg/mouse) of IVIG; and (v) fIVIG-ID group, with 2 mg/kg (60 μg/mouse) of fIVIG-ID antibodies in 1% maltose. Following the treatments, the mice were bled by a retro-orbital puncture under anesthesia every 2 weeks and the anti-DNA autoantibody titers were determined in the sera by ELISA.

Late treatment. Thirty female NZB/W F_1 mice 9 –10 weeks old were bled by retro-orbital puncture under anesthesia every 2 weeks from the age of 17–18 weeks and the anti-DNA autoantibody level titers were determined in the sera by ELISA. After developing the IgG anti-dsDNA antibodies at the age of 21–22 weeks the mice were treated with either IVIG-ID at 2 mg/kg (60 μg/mouse) or IVIG at 400 mg/kg (12 mg/mouse) i.v. via the tail vein once a week for 3 weeks. Ten untreated mice served as controls.

Detection of anti-dsDNA antibodies in mice sera

Anti-dsDNA activity in the sera were detected by ELISA using commercially prepared DNA-coated plates (see above). Mice sera diluted 1:200 in sample buffer were incubated for 30 min at room temperature. Bound antibodies were detected using peroxidase-conjugated goat anti-mouse IgG (H + L) (Jackson ImmunoResearch) diluted 1:5000 in sample buffer followed by the addition of TMB (3,3′,5,5′-tetramethyl benzidine) substrate solution. Color development was stopped by adding 1 M hydrochloric acid and read in a Titertrek ELISA reader using a 450 nm filter/620 nm reference filter.

Evaluation of renal disease

Mice were evaluated for proteinuria using Multistix strips (Bayer Diagnostics, Puteau, France). Urine samples were graded 0–4+, corresponding to the following approximate protein concentrations: 0 = negative or trace, 1+ = 30 mg/dl, 2+ = 100 mg/dl, 3+ = 300 mg/dl and 4+ = ⩾2000 mg/dl. Mice with significant proteinuria (⩾3+) on two consecutive examinations were designated positive for renal disease. Severely ill and wasted mice with significant proteinuria were sacrificed when they appeared moribund. These mice were considered as dead at the time they were killed and were included in the survival evaluation.

Histologic analysis of the kidneys

Kidneys were obtained from mice killed by cervical dislocation and were frozen immediately in liquid nitrogen. Frozen cryostat sections (6–8 μm thick) were dried and subsequently fixed in acetone (Merck, Darmstadt, Germany) for 10 min. For detection of Ig deposits, cryostat sections were incubated with FITC-conjugated anti-mouse or anti-human $F(ab')_2$ IgG (Sigma, St Louis, MO). Evaluation was performed by a pathologist (J. K.) who was unaware of the group from which the samples were obtained. In both treatment protocols the survival time was monitored till 45 weeks of mouse age.

Statistical analysis

The χ^2-test for categorical variable was applied for comparison between groups. Continuous values among groups were tested using ANOVA.

Results

Anti-idiotypic activity of IVIG-ID

The recovery of specific bound IVIG-ID was ~0.05%. *In vitro* inhibition studies with IVIG and IVIG-ID showed a dose-dependent inhibition of SLE sera binding to the dsDNA by IVIG-ID as well as IVIG. At a concentration of IVIG or IVIG-ID of 10 μg/ml, inhibition was observed in seven out seven SLE sera with the IVIG-ID, while just one of seven sera showed inhibition with the regular IVIG ($P < 0.01$). Figure 1 depicts representative results of the inhibition study with three different human (Fig. 1A) or mouse (Fig. 1C) anti-dsDNA antibodies. In both experiments ~100 times higher concentration of whole IVIG was required to inhibit human and mouse affinity-purified anti-dsDNA antibody binding to dsDNA by 50%. No inhibition of anti-dsDNA activity was observed using the column non-extracted fluid or anti-Fc preparation. The affinity-purified IVIG-ID (Fig. 1) demonstrated specificity for dsDNA recognition since anti-dsDNA antibodies failed to inhibit specifically the binding of anti-Tg antibody from human (Fig. 1B) or mouse (Fig. 1D) origin to Tg.

In vivo inhibition of anti-dsDNA antibody activity

Early treatment of NZB/W F_1 mice at the age of 8 weeks resulted at the age of 27 weeks with a clear significant difference in anti-dsDNA antibodies level between the control

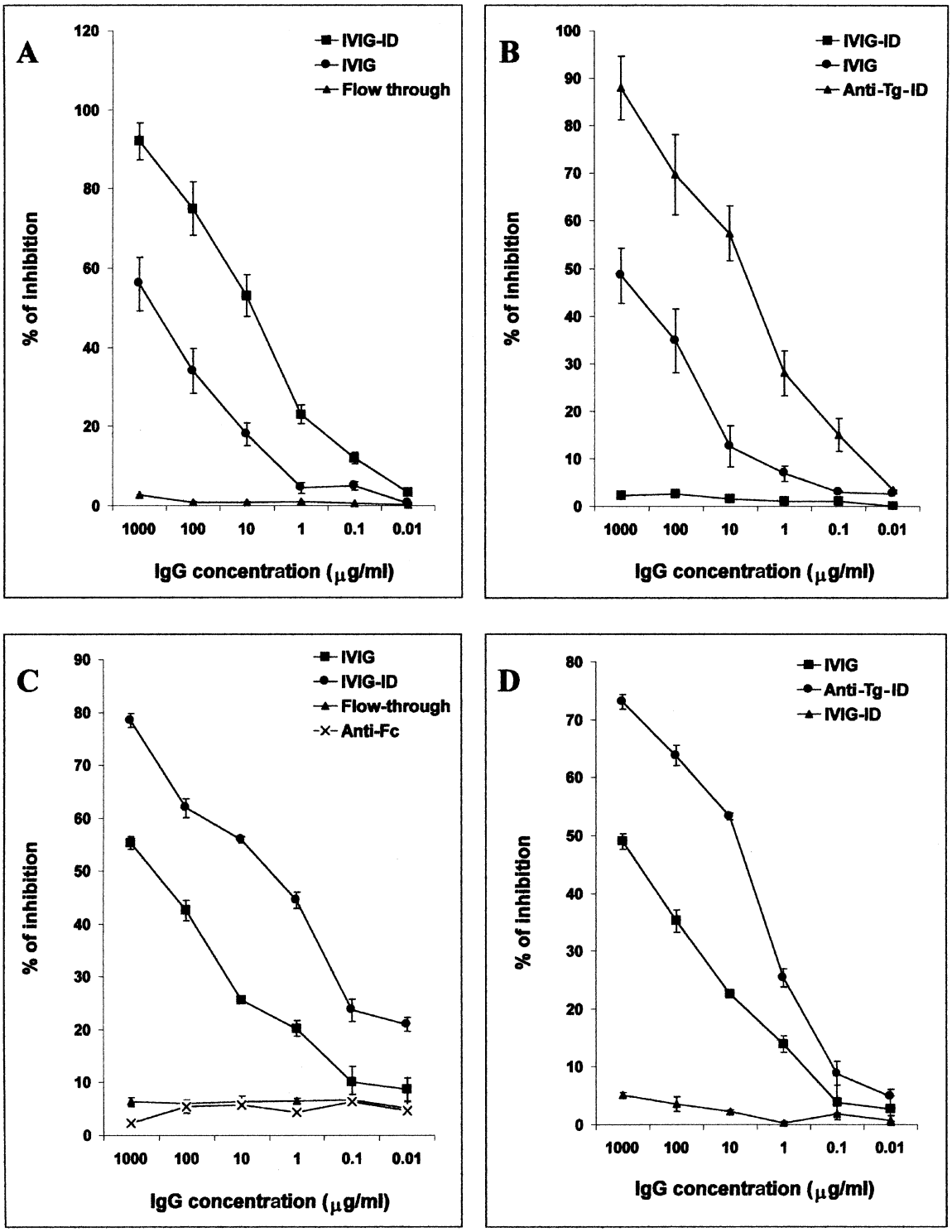

Fig. 1. The efficacy and the specificity of anti-idiotypic activity of IVIG-ID was confirmed by inhibition studies: (A) inhibition of affinity-purified human anti-dsDNA antibody to dsDNA by IVIG-ID, (B) inhibition of human anti-Tg antibodies binding to Tg by IVIG-ID, (C) inhibition of affinity-purified anti-dsDNA antibodies from sera of NZB/W F_1 to dsDNA by IVIG-ID and (D) inhibition of mouse anti-Tg antibodies to Tg by IIVIG-ID.

group treated with maltose and the group treated with fIVIG-ID. Mice treated with anti-Fcγ–IgG and low-dose IVIG respectively reached similar levels of anti-dsDNA antibodies as the control mice (Fig. 2A). NZB/W F_1 mice developed anti-dsDNA antibodies by the age of 20 weeks and the late treatment (after antibody development) with IVIG-ID (Fig. 2B) led to stabiliza-

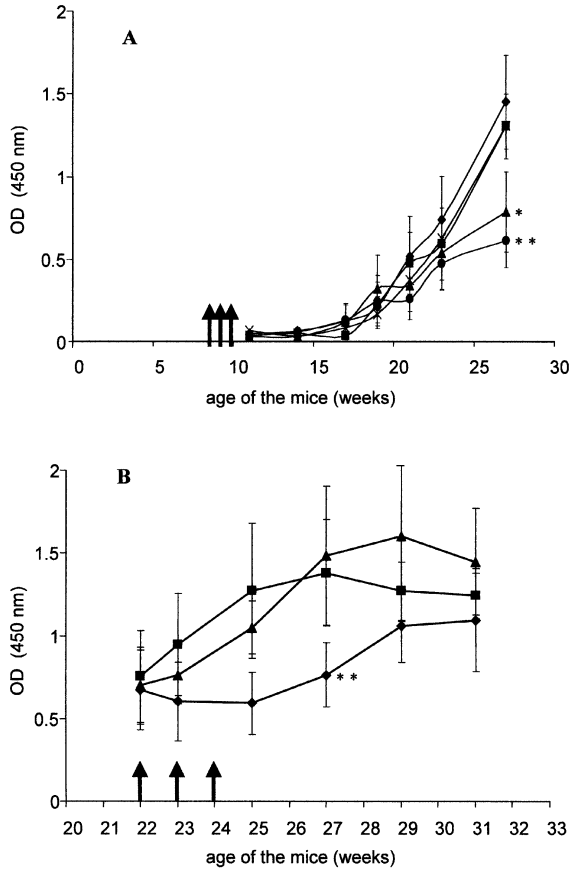

Fig. 2. Reduction in anti-dsDNA autoantibodies levels (mean ± SD) estimated by ELISA in NZB/W F_1 mice treated at the age of 8 weeks (A, early treatment) or 22 weeks (B, late treatment) with 1% maltose (control group; diamonds), anti-Fcγ–IgG (crosses), high-dose (400 mg/kg) IVIG (triangles), low-dose (2 mg/kg) IVIG (squares) and fIVIG-ID (2 mg/kg) (circles) respectively. Mice treated with IVIG-ID or high-dose IVIG had at the age of 27 weeks significantly lower levels of anti-dsDNA antibodies than mice treated with low-dose IVIG, anti-Fcγ–IgG or control mice. Arrows indicate the i.v. injections. $^*P < 0.05$, $^{**}P < 0.04$

tion of the constant levels of anti-dsDNA antibodies in IVIG-ID-treated mice during and immediately following the treatment, while increasing antibody levels were observed in IVIG-treated and in untreated control mice. Autoantibody levels started to rise in the IVIG-ID-treated group 3 weeks following completion of the treatment, but never reached the levels of the control group for as long as 2 months after the final injection. The control untreated group reached maximal levels of anti-dsDNA antibodies at the age of 27 weeks. At this time point the maximal difference in anti-dsDNA levels in the sera of IVIG-ID-treated mice compared to untreated control mice was observed (mean OD_{405} = 1.48 versus 0.76; $P < 0.04$). The anti-dsDNA antibody levels in IVIG-treated mice did not differ significantly from the control group. In contrast to the late treatment (after detecting the autoantibodies in periphery), the early therapy with high-dose IVIG also affected the anti-dsDNA antibodies, with titers of anti-dsDNA comparable to those achieved with fIVIG-ID group (Fig. 2A).

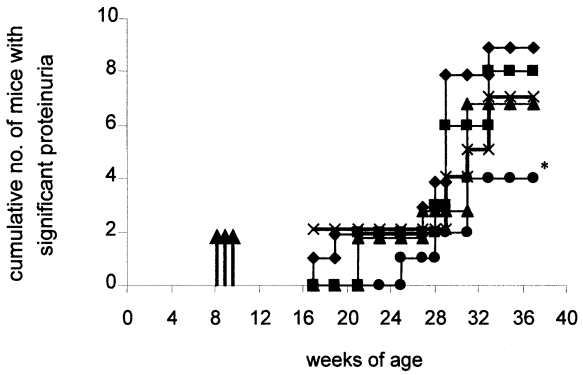

Fig. 3. Cumulative number of mice with significant proteinuria (stick reading ⩾3) during the early treatment with 1% maltose (control group; diamonds), anti-Fcγ–IgG (crosses), high-dose (400 mg/kg) IVIG (triangles), low-dose (2 mg/kg) IVIG (squares) and fIVIG-ID (2 mg/kg) (circles) respectively. Arrows indicate the i.v. injections. $^*P < 0.05$.

IVIG-ID and IVIG reduce the development of severe renal disease in NZB/W F_1 mice

Early treatment. Development of the renal disease was followed by weekly monitoring of proteinuria at the time and after the treatment. Proteinuria occurred as early as at the age of 17 weeks in two mice in the group treated with anti-Fcγ–IgG (Fig. 3). At the age of 35 weeks, nine out of 10 mice from the control group, while only four mice from the fIVIG-ID-treated group, had a significant proteinuria ($P < 0.05$). To examine the effect of IVIG-ID on the mesangial IgG deposits, three mice from each group were sacrificed and kidney specimens were examined by immunofluorescent microscopy for the presence of IgG deposits. There was clearly less intense immunofluorescent staining of mouse IgG in the mesangium of fIVIG-ID-treated mice than in the control group (Fig. 4A and D) and groups treated with anti-Fcγ–IgG or low-dose IVIG (Fig. 4C). Mice treated with high-dose IVIG had slightly more intense staining then fIVIG-ID-treated mice (Fig. 4B). No deposition of human IgG in the kidneys was observed in any IVIG treatment modality (data not shown). Consistent with this was the trend toward a better survival in the fIVIG-ID- and high-dose IVIG-treated groups compared to the control group and group treated with anti-Fcγ–IgG (Fig. 5). Four mice from the fIVIG-ID-treated group and three mice from the IVIG-treated group were still alive at the age of 45 weeks.

Late treatment. Eight weeks after the final injection, development of the renal disease was assessed. Mice were evaluated for the presence of proteinuria on three occasions within a 1-week period. Mice with a stick reading of 3+ or higher on at least two consecutive examinations were considered positive for renal disease. Significant proteinuria was seen least in the IVIG-ID mice (in three mice out of 10, $P < 0.05$), followed by the group treated with regular IVIG (in five of 10, $P > 0.05$), while in the untreated group severe proteinuria was observed most frequently (in nine of 10) (Fig. 6). Two out of the untreated mice and one out of the

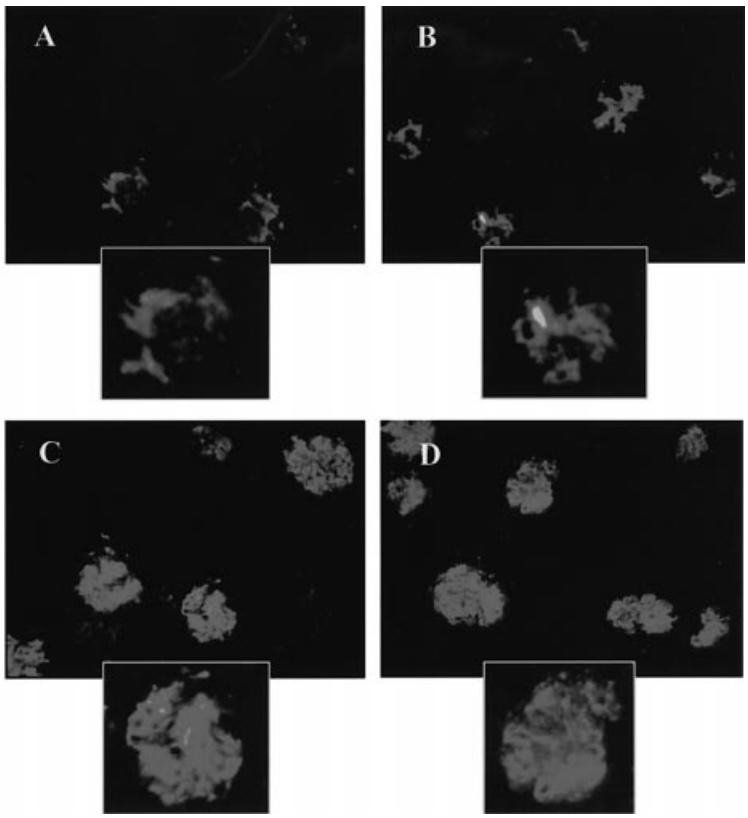

Fig. 4. Prevention of mesangial mouse IgG deposits in NZB/W F_1 mice treated with IVIG-ID. (A) Treatment with fIVIG-ID, (B) high-dose IVIG treatment, (C) low-dose IVIG treatment and (D) non-treated control group.

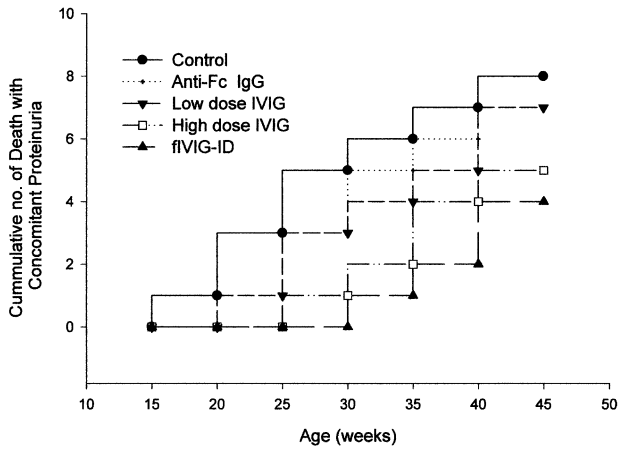

Fig. 5. Cumulative number of deaths with concomitant proteinuria. Eight mice per group were included in the experiment. Severely ill and wasted mice with significant proteinuria sacrificed when they appeared moribund were included in the counts.

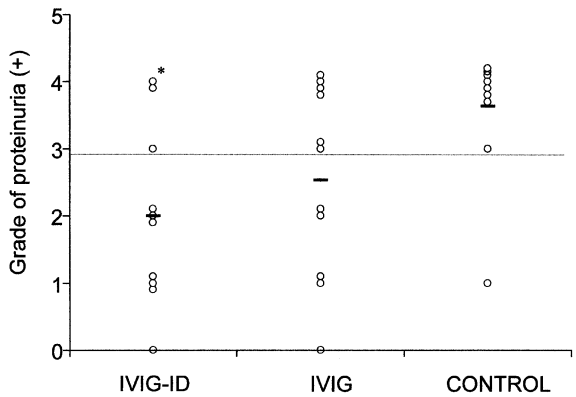

Fig. 6. Proteinuria in NZB/W F_1 mice treated at the age of 22–23 weeks with IVIG-ID 2 mg/kg or regular IVIG 400 mg/kg and in the untreated control group. At the age of 32–33 weeks (8 weeks following completing the treatment), nine out of 10 control mice developed heavy proteinuria, while only three out of 10 IVIG-ID-treated and five out of 10 IVIG-treated mice developed severe proteinuria with a Multistix reading ⩾3+ as indicated by the cut-off line. $^*P < 0.05$.

IVIG-treated mice appeared moribund, and were sacrificed during 4–8 weeks following the last injection. They all had proteinuria 4+ when sacrificed. To complete immunohistological evaluation of five kidneys from each group 8 weeks after the final injection, three mice from the control group, four mice from the IVIG-treated group and five mice from the IVIG-ID-treated group were sacrificed.

Kidney specimens were examined by immunofluorescent microscopy for the presence of IgG deposits. The immunofluorescence staining revealed extensive mouse IgG deposits in all mice. However, the patterns were clearly different. Intense mesangial and capillary wall deposits were observed in the control mice (Fig. 7C). In contrast, capillary wall staining

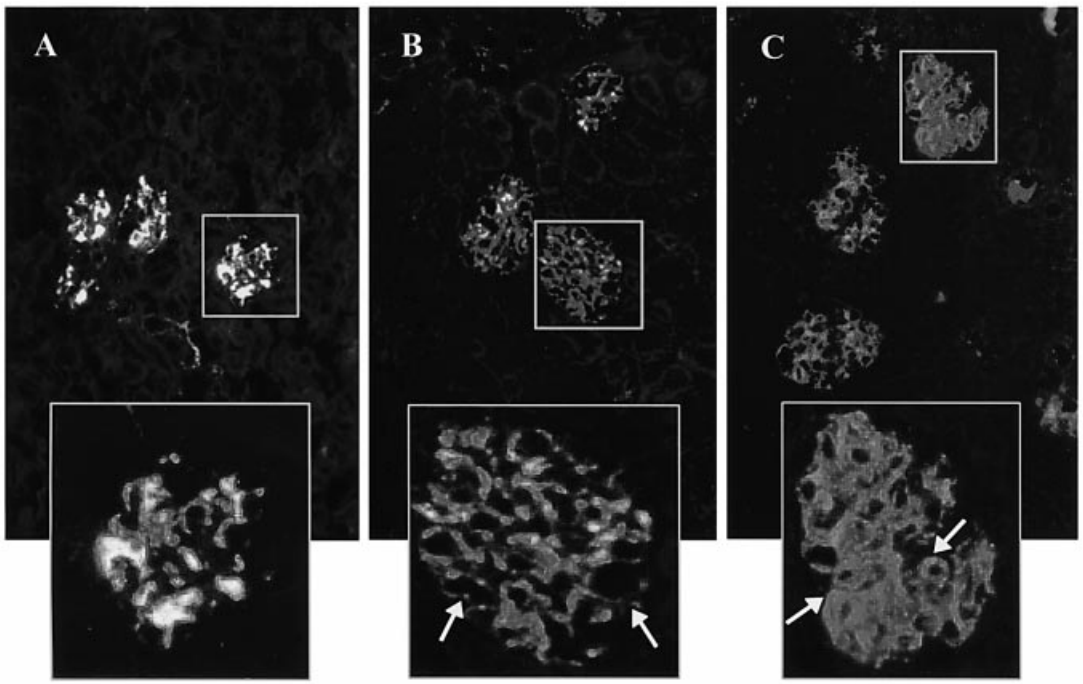

Fig. 7. Immunohistochemical staining of IgG deposits in kidneys of NZB/W F_1 mice. Arrows indicate the peripheral capillary staining of IgG deposits in the glomerular basement membrane. In IVIG-ID-treated group (A) there are mesangial deposits with no staining in capillary walls. Slight peripheral staining was observed in the IVIG-treated group (B). Control mice (C) showed extensive mesangial deposits with a strong peripheral pattern of IgG deposits in the glomerular basement membrane.

was only slightly seen in the IVIG-treated group (Fig. 7B) and was not detected in the IVIG-ID-treated groups (Fig. 7A). Consistent with the immunofluorescence results was the trend toward better survival in the IVIG-ID- and IVIG-treated groups compared to the control group in the remaining animals (Fig. 8). Four mice from the IVIG-ID-treated group and two mice from the IVIG-treated group were still alive when the last mice from the control group died at the age of 44 weeks.

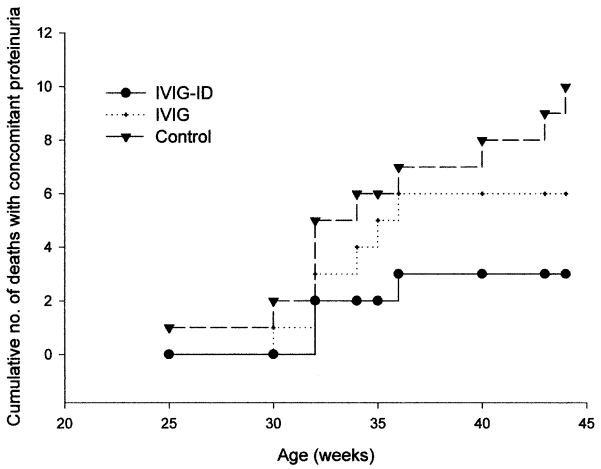

Fig. 8. Cumulative number of deaths with concomitant proteinuria. Ten mice per group were included in the experiment. Five mice from each group were sacrificed for immunohistology staining of IgG deposits in the kidneys at the age of 32 weeks. Severely ill and wasted mice with significant proteinuria sacrificed when they appeared moribund were included in the counts.

Discussion

Antibodies reactive with dsDNA are generally considered to be a highly specific serological marker for the diagnosis of SLE. The levels of anti-idiotypic antibodies correlate with the disease activity in SLE patients, with a remarkable drop during the exacerbation and an increase during the remission of the disease (24). IVIG contains a series of anti-idiotypic antibodies capable of inhibiting the *in vitro* activity of various autoantibodies (5,25). Manipulation of the idiotypic network by these anti-ID may be the main mechanism of action of IVIG in the treatment of SLE. Since the relative amount of specific anti-idiotypic antibodies in commercial IVIG preparations is extremely low, use of isolated anti-ID against pathogenic autoantibodies might result in a more effective treatment. We prepared polyclonal anti-dsDNA anti-idiotypic antibodies (IVIG-ID) by affinity purifying commercial IVIG on a column constructed with an array of different anti-dsDNA ID purified from sera of 55 active SLE patients. We have proven *in vitro* that this preparation in low concentration is more effective than regular IVIG in inhibiting the binding of SLE sera to dsDNA plates.

To test the efficacy of this preparation as a treatment for SLE, we treated the most frequently used murine model of human SLE—NZB/W F_1 mice. It has been previously shown that common anti-DNA antibody ID may be shared by both

lupus-prone mice and human SLE patients (26). The suppressive effect of regular IVIG on the immunological and clinical manifestations of experimental SLE has been demonstrated by Krause *et al*. (27). The authors assumed that this effect was mediated through manipulation of the idiotypic network and the neutralization of pathogenic autoantibodies since treatment with $F(ab)_2$ fragments was as efficient as the native antibodies, while Fc fragments were ineffective. The model of SLE we used has a distinct genetic background, a different pathogenesis and probably a different idiotypic repertoire of anti-dsDNA antibodies. In a preliminary study with experimental SLE induced by active immunization with human mAb MIV-7, IVIG as well as IVIG-ID, we were able to completely abrogate both the production of anti-dsDNA antibodies and proteinuria (data not shown). The effect of IVIG in the NZB/W F_1 model was not as profound as in the experimental SLE induced in BALB/c mice. In fact, the late treatment with IVIG had no significant effect on anti-dsDNA levels in NZB/W F_1 mice. Nevertheless, it improved the outcome of renal disease with fewer mice developing heavy proteinuria and prevented IgG deposits in the glomerular capillary walls. Our preparation containing concentrated polyclonal anti-dsDNA anti-ID was able to suppress autoantibody response and the development of the renal disease in a genetic murine SLE model in a dose 200 times less IgG than the commercial IVIG. The most remarkable suppressive effect on autoantibody levels was found during and immediately following the treatment. Three weeks after the last IVIG-ID infusion the levels of anti-dsDNA antibodies started to rise. However, they still remained lower than in the control or IVIG-treated group for as long as 10 weeks following treatment. A similar effect on suppression of anti-dsDNA antibody production by IVIG-ID was observed after the early treatment at the age of 8 weeks. Moreover, in this early treatment, high-dose IVIG treatment produced a similar effect.

Glomerulonephritis is considered to be one of the most severe manifestations in SLE. High-avidity anti-dsDNA antibodies play a pivotal role in the pathogenesis of SLE nephritis and their serum titer correlates with disease activity. Thus, it is conceivable that neutralizing these autoantibodies in the periphery could prevent renal involvement in SLE. The early treatment was designed to test the capability of IVIG-ID to prevent mesangial IgG deposits. To avoid the potential anti-Fc effect we used IVIG-ID which was depleted of anti- Fc antibodies and we treated an additional control group with these anti-Fc IgG. The fIVIG-ID was able to significantly reduce the IgG deposits in the kidney mesangium, while the anti-Fc–Ig had no effect. IVIG, at a dose 200 times higher than fIVIG-ID, was also able to decrease the deposits in the kidney mesangium, while the same low dose of IVIG had no effect.

NZB/W F_1 mice treated with IVIG-ID (late treatment) developed significantly less proteinuria compared to non-treated mice. This finding was consistent with IgG deposits in the kidneys of these mice restricted to the mesangium. At a first glance one might consider the inability of both IVIG-ID and IVIG to prevent mesangial IgG deposits as disappointing. However, mesangial deposits develop in NZB/W F_1 early in their life, before they produce high levels of anti-dsDNA antibodies in the periphery. In contrast to this, glomerular capillary wall deposits occur later in the disease concomitantly with severe proteinuria (23). Our immunohistology examination showed that IVIG-ID, as well as the 200 times higher IgG dose of IVIG, were able to prevent the progression of IgG deposition to the capillary walls. However, neither treatment was able to dissociate existing mesangial immune deposits as is presumed to occur in anti-dsDNA SLE patients treated with IVIG (28). Interestingly, *in vitro* studies have demonstrated the disappearance of a mouse IgG from the glomerular deposits following incubation of the kidney sections, only with an excess of homologic IgG. In contrast, no such effect was seen with heterologic Ig (28,29).

The suppression of the pathogenic antibodies to dsDNA in NZB/W F_1 mice was also observed after i.p. injection of a monoclonal anti-idiotypic antibody (16). The early treatment at the age of 6 weeks resulted in a depletion of the target ID only. Neither the total level of circulating anti-dsDNA antibodies, the onset of nephritis nor the survival was affected. There was, however, a transient drop of the total autoantibody levels, delayed onset of nephritis and a better survival following 10 weeks of therapy of 20-week-old mice who already had significant levels of circulating anti-DNA antibodies. However, despite the continued treatment, at 30 weeks of age the quantities of anti-dsDNA again rose rapidly and remained as high as in the control group. Similar results were observed after therapy of 6-week-old NZB/W F_1 mice with anti-Id33. The transient reduction in Ig-expressing Id33 was followed by a rise at 30 and 34 weeks that was significantly higher than in the untreated mice. The total anti-dsDNA antibody levels were significantly higher in the treated mice than in the untreated mice already at 10 and 18 weeks (17). These results indicate that suppression or even depletion of one pathogenic ID is not sufficient to suppress the *overall* disease and may lead to the expansion of remaining clones of autoantibody-producing cells or to an ID switch (30). In contrast to these studies, treatment with our polyclonal anti-ID preparation was not followed by the overproduction of other pathogenic anti-DNA ID. The induction of the experimental lupus in naive mice by anti-dsDNA ID is done upon active immunization and disregulation of the idiotypic cascade, thus generating anti-anti-ID mouse antibodies (e.g. anti-dsDNA). The mechanisms which lead to the development of experimental lupus were detailed by us previously (32–34). IVIG employed as a passive infusion may act as an ID neutralizing antibodies and down-regulate the B cells carrying the ID, as well as turning off the idiotypic cascade network. The anti-idiotypic activity of IVIG may involve cellular responses as well, via neutralization of the variable region of the TCR, regulation of production of T_h cytokines, induction of T suppressor cells and/or by inhibition of lymphocyte proliferation (35,36).

In summary, although IVIG has proven safe and efficacious in several autoimmune human diseases including SLE, its efficiency:cost ratio remains low. Concentrated polyclonal anti-ID antibodies to anti-dsDNA autoantibodies could increase both efficacy and efficiency. In fact, Silvestris *et al*. (31) recently reported the successful treatment of two SLE patients with nephritis using the eluate of a commercial IVIG preparation absorbed on a Sepharose column coupled with DC-305-39 myeloma protein. Moreover, results of our studies have shown that both early and late therapy with IVIV-ID in an experimental SLE model improve the survival of the NZB/W F_1 mice.

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Abbreviations

ds	double stranded
fIVIG-ID	anti-Fcγ-depleted IVIG anti-dsDNA anti-idiotypic antibodies
ID	idiotype
IVIG	i.v. Ig
IVIG-ID	IVIG affinity-purified anti-dsDNA anti-ID antibodies
NZB/W F_1	F_1 hybrids of New Zealand Black and New Zealand White mice
SLE	systemic lupus erythematosus
Tg	thyroglobulin

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