Interleukin 6 Promotes Murine Lupus in NZB/NZW F_1 Mice

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Abstract

To investigate the role of IL-6 in systemic lupus erythematosus (SLE), we selectively inhibited IL-6 in lupus-prone NZB/NZW F_1(B/W) mice by chronic administration of a rat mAb to mouse IL-6. Anti-IL-6 alone elicited an anti-rat response that blocked its biologic effects. To circumvent this problem, we rendered B/W mice tolerant to the rat mAb by administration of anti-CD4 concurrent with the first dose of anti-IL-6. Thereafter, the mice received weekly injections of anti-IL-6 alone. There were two control groups: one group received the tolerizing regimen of anti-CD4 along with a control rat IgG1 mAb (GL113) instead of anti-IL-6; the other control group received PBS. Mice that received anti-CD4 were tolerant to the rat mAb for 6 mo. Throughout this period, treatment with anti-IL-6 prevented production of anti-dsDNA, significantly reduced proteinuria, and prolonged life. Mice that received anti-IL-6 without anti-CD4 developed an immune response to the rat mAb and then developed anti-dsDNA antibodies, proteinuria, and mortality comparable with control mice. These findings establish that IL-6 promotes autoimmunity in B/W mice. They further indicate that, although mAb to IL-6 can suppress murine lupus, the development of host immunity to the mAb abrogates its beneficial effects. Finally, this is the first study to demonstrate that a brief course of anti-CD4 can induce tolerance to another therapeutic mAb, in this case an anti-cytokine mAb. (*J. Clin. Invest.* 1994. 94:585–591.) Key words: systemic lupus erythematosus • interleukin 6 • anti-CD4 • monoclonal antibody • cytokines

Introduction

Interleukin 6 (IL-6) is a multifunctional cytokine that was first described as a B-cell differentiation factor (1–6). In addition to its capacity to stimulate B-cell differentiation and Ig secretion, IL-6 promotes differentiation of cytotoxic T cells (7, 8), enhances production of hepatic acute phase reactants (9, 10), stimulates multipotential progenitors in the bone marrow (11, 12), and promotes growth of renal mesangial cells (13–15). IL-6 is produced primarily by macrophages and monocytes (10, 16, 17), but it can also be produced by a wide variety of cells including T cells and B cells (3, 9, 10, 18–22). The production of IL-6 by diverse cell types and its multiple activities suggest that IL-6 contributes to a broad range of biological functions.

Recent indirect evidence has implicated IL-6 in the pathogenesis of several human autoimmune diseases (15, 23–28), including systemic lupus erythematosus (SLE) (29–36). For example, IL-6 levels are elevated in the sera of patients with active, but not inactive, SLE (29–32). Similarly, IL-6 is elevated in the cerebrospinal fluid of patients with central nervous system (CNS)[1] lupus, but not in patients without CNS involvement (35, 36). Increased IL-6 has also been detected in the urine and renal glomeruli of patients with lupus nephritis (15, 26). Analyses of peripheral blood mononuclear cells from patients with SLE are consistent with these clinical observations. Specifically, several studies have shown increased production of IL-6 by both T cells and B cells from patients with SLE (37–41). Both mRNA for IL-6 and cytoplasmic IL-6 have been detected in freshly isolated monocytes as well as lymphocytes from SLE patients, but not from normal controls (29). B cells from patients with SLE have been reported to be hypersensitive to IL-6 (29, 38–40). Moreover, in vitro production of IgG anti-dsDNA antibodies by SLE B cells is promoted by IL-6 and can be blocked by monoclonal antibodies (mAb) to IL-6 (29, 38). Finally, peripheral blood monocytes/macrophages from SLE patients, but not from patients with rheumatoid arthritis or from atopic control subjects, produce IL-6 in response to UVA and UVB wavelengths (41). These findings suggest that IL-6 may contribute to disease activity in SLE, including flares provoked by UV light.

IL-6 has also been implicated in a number of autoimmune diseases in mice (42–45). In particular, murine lupus is characterized by IL-6 abnormalities that closely parallel the IL-6 abnormalities in humans with SLE. In MRL/*lpr* mice, for example, serum IL-6 levels rise steadily as autoimmune disease progresses (46). In lupus-prone NZB/NZW F_1 (B/W) mice, serum IL-6 levels are elevated, and B cells are hyperresponsive to IL-6 (17, 47–50).

The observations summarized above establish an association between enhanced IL-6 activity and autoimmune phenomena, including those seen in human and murine lupus, but they do not establish a causal relationship. Therefore, in this study, we treated B/W mice with mAb to IL-6 in order to test directly the hypothesis that IL-6 promotes lupus-like autoimmune disease.

Methods

Mice. B/W mice were purchased from The Jackson Laboratories (Bar Harbor, ME). Athymic BALB/c (*nu/nu*) mice were purchased from Simonsen Laboratories (Gilroy, CA). The mice were housed in the

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1. *Abbreviations used in this paper:* BGG, bovine gamma globulin; B/W, NZB × NZW F_1 mice; CNS, central nervous system; HRP, horseradish peroxidase.

AAALAC-accredited animal care facility at the San Francisco Veterans Administration Medical Center.

Production and purification of mAb. Rat IgG1 mAb to mouse IL-6 (hybridoma MP5-20F3.11) and control rat IgG1 mAb to *E. coli* B-galactosidase (hybridoma GL113) were derived from hybridomas that were generously provided by Dr. John Abrams (DNAX, Palo Alto, CA). Some mice also received rat IgG2b mAb to CD4 (hybridoma GK1.5). The mAb were harvested as ascites from athymic BALB/c mice, partially purified by ammonium sulfate precipitation, dialyzed in PBS, pH 7, and quantitated by cellulose acetate electrophoresis and measurement of optical density, as described previously (51).

Experimental design. Four groups of 3-mo-old female B/W mice were studied. Groups of 10 mice received weekly injections of either anti-IL-6 (500 μg ip) or PBS (0.2 ml). Based on preliminary studies indicating that weekly administration of rat mAb to IL-6 would elicit a host immune response that could inhibit the biologic effects of the mAb, we included two additional groups that were tolerized to either the rat anti-IL-6 mAb or to a control rat mAb (GL113). This was accomplished by administering the first dose of rat anti-IL-6 or control mAb concurrent with a brief course of rat mAb to CD4. This strategy was based on previous studies indicating that anti-CD4 can induce tolerance to some, but not all, antigens when they are administered concurrently (52). Specifically, two groups of 10 mice received anti-CD4 (1 mg/d on days −1, 0, and +1) surrounding the first dose of either anti-IL-6 or GL113 (500 μg on day 0). Thereafter, the mice received weekly injections of either anti-IL-6 or GL113 (500 μg) alone.

Fluorescence analysis of lymphocyte subpopulations. Circulating mononuclear cells from individual mice were analyzed by flow cytometry as described previously (53). Cells were stained with the following mAbs: anti-Thy-1.2 (hybridoma 30-H12) to identify all T cells, anti-CD4 (hybridoma GK1.5), and anti-CD8 (hybridoma 53-6) to identify the major T cell subsets, and anti-B220 (hybridoma RA3.6B2) to identify B cells.

Measurement of the immune response to rat IgG. Mouse antibodies to rat IgG were measured by ELISA. Briefly, microtiter plates were coated with chromatographically purified rat IgG (Cappel Laboratories, Durham, NC) in 0.1 M Tris HCl (0.5 μg/well) at room temperature for 1 h. The plates were then washed with tap water and blocked with 2% BSA for 30 min and then washed again. Serum samples were diluted 1:50 in 2% bovine gamma globulin (BGG) and 1% BSA, titered by double dilution, and allowed to sit at room temperature for 1 h. The plates were washed, blocked, and washed as above. Horseradish peroxidase (HRP)-conjugated goat anti-mouse Ig (heavy and light chain) absorbed with rat Ig (Jackson ImmunoResearch Laboratories, West Grove, PA) was used as the second antibody at a 1:3,000 dilution in 2% BGG and 1% BSA (75 μl/well) and allowed to sit at room temperature for 1 h. The plates were washed and developed with *o*-phenylenediamine in phosphate citrate buffer for 10 min. The reaction was stopped with 2N H_2SO_4 and plates were read on an ELISA reader (Bio-Tek, Winooski, VT) at 490 nm. Monoclonal mouse anti-rat kappa chain (MAR 18.5) and normal mouse serum served as positive and negative controls, respectively.

To measure anti-idiotype as well as anti-isotype antibodies, the results obtained with the assay described above were confirmed using plates coated with $F(ab')_2$ fragments of the rat anti-IL-6 mAb or the control GL113 mAb. $F(ab')_2$ fragments of the rat IgG1 mAb were prepared by pepsin digestion as described (54). First, the mAb were purified over a recombinant protein A-column (Clinetics, Tustin, CA) and concentrated to 3 mg/ml using a Centriprep 30 spin column (Amicon Corp., Beverly, MA). The purified mAbs were then dialyzed in sodium acetate buffer, pH 4.0, for 4 h at 4°C, and concentration was determined by optical density at 280 nm. Pepsin (0.1 mg/ml) in sodium acetate buffer, pH 4.0, was added at a 1:20 ratio of mAb to pepsin, and the mixture was heated to 37°C for 2 h. The reaction was stopped with 2N Tris-base. Pepsin digestion of rat IgG1 frequently produces F(ab)-Fc fragments as well as $F(ab')_2$ fragments, both having a MW of approximately 110–120 kD on nonreducing SDS-PAGE. Therefore, to remove F(ab)-Fc and Fc fragments, the digest was purified over a recombinant protein A column. The $F(ab')_2$ fragments thus obtained were then used to coat the plates in the ELISA to assess the mouse immune response to the anti-IL-6 and GL113 mAb. HRP-conjugated goat anti-mouse IgG (Fc) (Cappel Laboratories) diluted 1:1,000 in 2% BGG and 1% BSA was used as the second antibody.

Measurement of anti-dsDNA antibodies. Antibodies to dsDNA in sera from individual mice were measured by ELISA as previously described (51, 55). Briefly, microtiter plates were coated with 75 μl/well poly-L-lysine (50 μg/ml) (Sigma Chemical Co., St. Louis, MO) and allowed to sit overnight. The plates were washed and then coated with poly (dA-dT)·poly (dA-dT) (Sigma Chemical Co.) 1.5 μg/well and allowed to sit for 1 h. The plates were washed, blocked, and washed as described above. Serum samples were diluted 1:50 and titered by double dilution as described above. HRP-conjugated goat anti-mouse IgG (Fc) (Cappel Laboratories) was used as the second antibody at a concentration of 1:1,000 and allowed to sit for 1 h at room temperature. The plates were washed and developed with *o*-phenylenediamine and read as above. Serum from MRL/*lpr* mice previously titered for anti-dsDNA antibodies and normal mouse serum were used as positive and negative controls, respectively.

Measurement of Ig concentration. Mouse IgM, IgG1, IgG2a, IgG2b, and IgG3 levels were measured by ELISA. Microtiter plates were coated with either goat mAb to mouse IgM, rat mAb to mouse IgG1, IgG2a, IgG3 (Tagoimmunologics, Camarillo, CA), or purified rat polyclonal antibody to mouse IgG2b (Sigma Chemical Co.) at 10 μg/ml in 0.1 M Tris-HCl (75 μl/well) and incubated overnight at 4°C. The plates were washed and blocked with 2% BSA between each step as above. Serum samples were diluted 1:500 in 2% BGG and 1% BSA and then titered by serial double dilution. Chromatographically purified mouse IgM, IgG1, IgG2a, IgG2b (Caltag), and IgG3 (Pharmingen, San Diego, CA) were used as standards. Human serum and rat IgG were used as negative controls. The serum samples were allowed to sit for 1 h at room temperature. HRP-conjugated goat anti-mouse IgM (1:1,000 dilution) or HRP-conjugated goat anti-mouse IgG (heavy and light chain specific) (1:3,000 dilution) in 2% BGG and 1% BSA (75 μg/well) was added for the IgM and IgG assays, respectively, and allowed to sit for 1 h at room temperature. The plates were then washed, developed with *o*-phenylenediamine, and read at 490 nm. A standard curve was generated for each Ig isotype using Delta Soft II 3.3.67B (BioMetallics, Princeton, NJ). The concentration of IgM, IgG1, IgG2a, IgG2b, and IgG3 in each serum sample was determined by interpolation on the standard curve.

Assessment of renal function. Proteinuria was measured monthly using Uristix test strips (Miles Inc., Elkhart, IN). Significant proteinuria was prospectively defined as proteinuria > 100 mg/dl.

Statistical analysis. Mean antibody titers and mean antibody concentrations were compared using the Student's *t* test. Proteinuria and survival were analyzed by chi-squared analysis.

Results

Anti-IL-6 alone elicited a brisk immune response, but anti-CD4 given concurrently with the first dose of anti-IL-6 induced sustained tolerance to the rat anti-IL-6 mAb. Weekly injections of rat mAb to IL-6 without anti-CD4 elicited a brisk immune response to the IL-6 mAb in B/W mice (Fig. 1). In these mice, the mean titer of anti-rat antibodies rose to 1:800 within one month after the first injection and remained elevated thereafter. In contrast, mice that received anti-CD4 concurrent with the first dose of anti-IL-6 or GL113 were tolerant to weekly injections of the rat mAb for 6 mo. Specifically, titers of anti-rat antibodies in mice treated with anti-CD4 were identical to titers in PBS-treated controls (mean titer < 1:50) whether the sera were tested on ELISA plates coated with purified rat IgG (Fig. 1) or on ELISA plates coated with $F(ab')_2$ fragments of either anti-IL-6 or GL113 (data not shown).

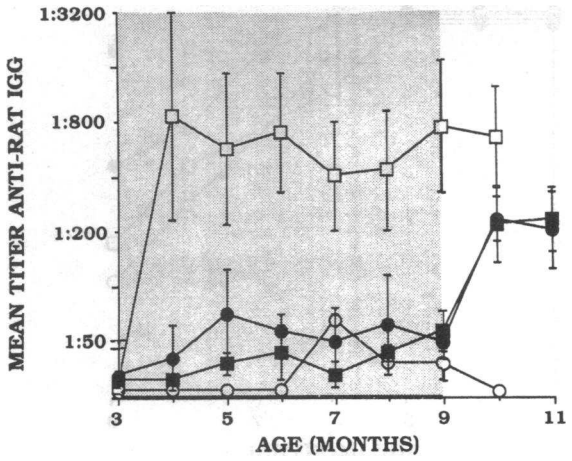

Figure 1. Geometric mean titer of mouse antibodies to rat Ig (±SEM). (*Shaded portion*) Mice that received weekly injections of anti-IL-6 alone (□) developed an immune response to the rat mAb. In contrast, mice that received anti-CD4 concurrent with the first dose of either anti-IL-6 (■) or an isotype-matched control mAb, GL113, (●) were rendered tolerant to the rat mAb despite weekly injections of either anti-IL-6 or GL113. Through the ensuing 6 months of therapy, the titers of anti-rat Ig in these mice were indistinguishable from the titers in control mice that were treated with PBS (○). (*Unshaded portion*) At 9 mo of age, tolerance was broken simultaneously in the two groups of mice that had received anti-CD4 with the initial dose of anti-IL-6 (■) or GL113 (●). Thereafter, continued weekly injections of rat mAb elicited an immune response to rat Ig. Although this response was readily detectable, the titer of anti-rat antibodies in these groups did not reach the levels that were documented in mice that had received weekly injections of anti-IL-6 without prior tolerization (□). Control mice that received weekly injections of PBS (○) did not make antibodies to rat Ig.

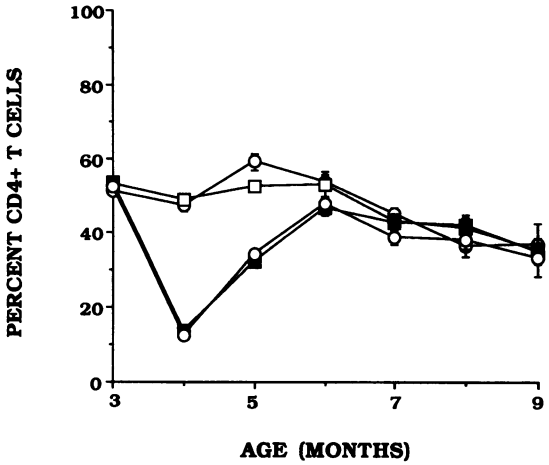

Figure 2. Percentage of CD4+ T cells (±SEM). Mice that received anti-CD4 concurrent with the first dose of anti-IL-6 (■) or GL113 (●) had a significant reduction in the percentage of CD4+ T cells immediately after receiving anti-CD4. CD4+ T cells then gradually returned to normal levels within 3 mo, so that by age 6 mo there was no difference in the frequency of CD4+ T cells between these mice and mice that had received either anti-IL-6 alone (□) or PBS (○).

We have shown previously that tolerance induction by anti-CD4 does not depend on sustained depletion of CD4+ T cells, nor does it cause long-term generalized immune suppression (51, 52). The results of the current study are consistent with these observations. In this study, the percent of CD4+ T cells was significantly reduced for 2 mo after the tolerizing dose of anti-CD4 ($P < 0.01$ for groups that received anti-CD4 compared to groups that did not receive anti-CD4). During this period, there was a reciprocal increase in the percent of CD8+ T cells and B cells (data not shown). However, by age 6 mo, the CD4 subset was fully reconstituted (Fig. 2), and the other lymphocyte subsets had returned to baseline. Despite recovery of CD4+ T cells by age 6 mo, unresponsiveness to the rat mAb was sustained until age 9 mo (Fig. 1). This unresponsiveness cannot be explained on the basis of generalized immune suppression because, as demonstrated below (Fig. 3), autoantibody production in the mice that received anti-CD4 plus GL113 was comparable with autoantibody production in the untreated control B/W mice.

Chronic administration of anti-IL-6 suppresses murine lupus in B/W mice if, and only if, the mouse immune response to therapy is blocked. Mice that received anti-IL-6 alone developed anti-dsDNA antibodies in titers that were comparable to PBS-treated controls (Fig. 3). In contrast, mice that were tolerant to the anti-IL-6 mAb did not produce anti-dsDNA antibodies. Suppression of autoantibody production was due to the effects of anti-IL-6 and not anti-CD4, because the mice that received the control GL113 mAb after tolerance induction by anti-CD4 produced high titers of anti-dsDNA antibodies (Fig. 3). At age 9 mo, for example, the mean titer of anti-dsDNA Ab was < 1:50 in the mice that were tolerant to the anti-IL-6 mAb, compared to 1:280 in tolerant mice that received GL113 ($P < 0.01$), 1:245 in mice that received anti-IL-6 alone ($P < 0.05$), and 1:980 in mice that received PBS ($P < 0.01$). In mice that were tolerant to chronic administration of anti-IL-6, the reduction in autoantibody levels relative to the control groups was statistically significant throughout the period from

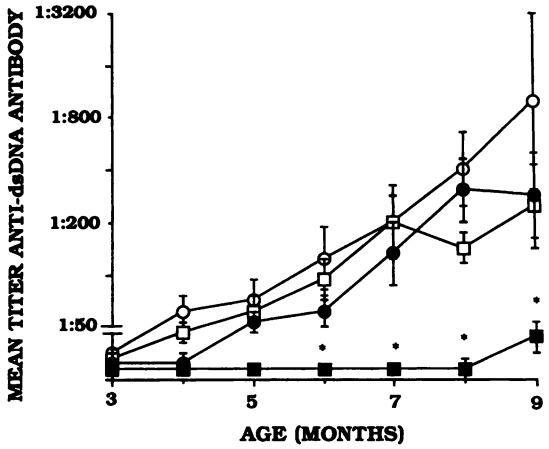

Figure 3. Effect of treatment with anti-IL-6 on the geometric mean titer (±SEM) of anti-dsDNA antibodies. In mice that were tolerant to the anti-IL-6 mAb (■), weekly administration of anti-IL-6 suppressed production of anti-dsDNA antibodies. In contrast, tolerant mice that received GL113 (●), mice that received anti-IL-6 without prior tolerization (□), and mice that received PBS (○) all produced anti-dsDNA antibodies. The asterisks denote points at which the titer of anti-dsDNA antibodies in tolerized mice treated with anti-IL-6 differed significantly ($P < 0.05$) from each of the other groups.

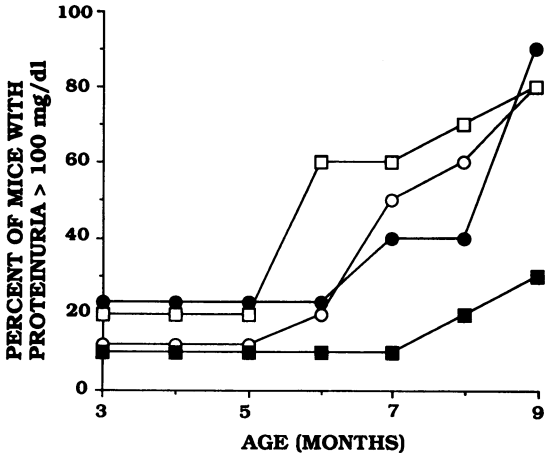

Figure 4. Effect of treatment with anti–IL-6 on renal disease. This graph shows the frequency of significant proteinuria (> 100 mg/dl) in mice that were tolerant to weekly administration of anti–IL-6 (■), control mice that received GL113 instead of anti–IL-6 (●), mice that received anti–IL-6 without prior tolerization (□), and mice that received PBS (○). To reflect accurately the development of renal disease in all mice, each point reflects the current level of proteinuria in surviving mice, as well as the last measurement of proteinuria in deceased mice. By 9 mo of age, only 30% of mice that were tolerized and treated with anti–IL-6 (■) had developed proteinuria, compared to 80% of mice in each of the other groups.

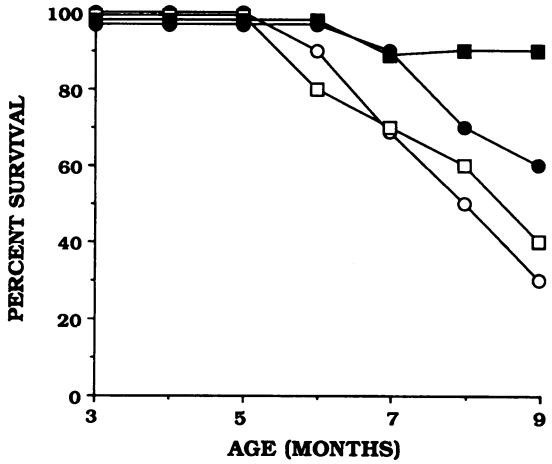

Figure 5. Effect of treatment on survival. Groups of 10 female B/W mice were rendered tolerant to rat mAb therapy by administration of anti-CD4 concurrent with the first dose of anti–IL-6 or GL113 at age 3 mo. These mice then received weekly injections of either anti–IL-6 (■) or GL113 (●). Control mice received weekly injections of anti–IL-6 without prior tolerization (□) or PBS (○).

age 6 to 9 mo, whereas none of the differences among the other groups was statistically significant at any time during the experiment.

Inhibition of autoantibody production in mice that were tolerant to chronic administration of anti–IL-6 was accompanied by significant inhibition of renal disease (Fig. 4). By age 9 mo, proteinuria (> 100 mg/dl) had developed in only 30% of tolerized mice treated with anti–IL-6, compared with 80–90% in each of the other groups ($P < 0.05$ for each comparison). At this time, 90% of the tolerant mice that had been treated with anti–IL-6 were still alive, compared with 60% survival among control mice treated with GL113 ($P > 0.10$, NS), 40% survival among mice treated with anti–IL-6 alone ($P < 0.02$), and 30% among PBS controls ($P < 0.01$) (Fig. 5).

Administration of anti–IL-6 did not significantly reduce serum Ig concentrations. To examine whether the reduction in anti-dsDNA antibodies and the amelioration of SLE reflected a generalized reduction in Ig production, we measured serum Ig levels (Table I). We found that treatment with anti–IL-6 did not significantly reduce total IgM or IgG concentration, nor did it significantly reduce the concentration of any of the IgG isotypes.

Loss of tolerance to the anti–IL-6 mAb coincided with the development of murine lupus. At age 9 mo, after 6 mo of weekly injections of rat mAb, tolerance was broken simultaneously in both the tolerized anti–IL-6 and GL113 treated mice (Fig. 1, unshaded portion). Although the anti–rat IgG titers in these groups did not reach the levels seen in mice that received anti–IL-6 alone, the mean titer of anti–rat IgG antibodies rose from < 1:50 before age 9 mo to 1:200 at age 10 mo. Coincident with this loss of tolerance, anti-dsDNA antibodies appeared for the first time in mice that had previously been tolerant to administration of anti–IL-6, although the mean titer remained considerably lower than in any of the control groups (1:76 at 11 mo of age, compared with ≥ 1:250 in each of the other groups by 9 mo of age). The percentage of mice in this group with proteinuria increased from 30% at 9 mo of age to 70% at 11 mo of age, and their survival rate declined from 90% at 9 mo to 50% by 11 mo. Thus, like the control mice that received anti–IL-6 alone, the mice that had been tolerized to the anti–IL-6 mAb developed signs of lupus once tolerance was broken and they developed an immune response to the rat mAb.

Discussion

Previous studies have provided circumstantial evidence that IL-6 promotes SLE both in mice and in humans (17, 29–41, 46–50). Our findings support these studies by establishing three important points. First, by demonstrating that chronic administration of anti–IL-6 suppresses autoantibody production and retards autoimmune disease in lupus-prone B/W mice, our studies provide the first direct evidence that IL-6 promotes lupus in a murine model for SLE. Second, our studies indicate that the development of a host immune response to the anti–IL-6 mAb abrogates its beneficial effects. Third, our studies demonstrate that a short course of mAb to CD4 can be used to induce tolerance to another mAb with potential therapeutic value. This is the first demonstration that anti-CD4 can be used successfully to accomplish this objective.

There are several mechanisms by which IL-6 might promote autoimmunity. IL-6 was first described as a B-cell stimulatory factor (1–6), and it has been shown to augment the secretion of IgG by activated B cells (1, 29, 38, 44). Therefore, IL-6 may contribute to the polyclonal hypergammaglobulinemia that occurs in SLE (56, 57). To evaluate this possibility, we measured Ig levels in sera from individual mice. These studies showed that anti–IL-6 did not significantly reduce IgM or IgG concentrations, suggesting that anti–IL-6 did not suppress auto-

*Table I. Analysis of Immunoglobulin Isotypes in Serum**

Treatment group‡	Immunoglobulin isotype§				
	IgM	IgG1	IgG2a	IgG2b	IgG3
			μg/ml		
Tolerized anti-IL-6	2050±450	560±90	1690±380	560±130	460±120
Tolerized GL113	1780±660	570±160	1910±600	500±90	240±70
Anti-IL-6	2660±1010	580±220	1280±400	510±170	340±100
Saline	860±200	730±500	2620±1450	740±420	680±130

* Immunoglobulin isotype levels were determined in serum at age 8 mo at which time there was a significant difference in the mean-dsDNA titer between tolerized anti-IL-6-treated mice and controls. ‡ Mice were tolerized to the anti-IL-6 mAb or the control mAb, GL113, by administration of mAb to CD4 concurrent with the first dose of anti-IL-6 or GL113. § Immunoglobulin isotype levels were determined by ELISA. A standard curve was generated for each isotype and concentrations were determined for each sample by comparison of the optical density in the linear range.

immunity simply by inhibiting polyclonal B-cell activation. Despite this finding, anti-IL-6 might selectively suppress certain isotypes that are strongly represented among autoantibodies. In female B/W mice, for example, anti-dsDNA antibodies are predominantly IgG2a or IgG2b antibodies (58). However, our studies showed that there was no selective reduction in any isotype. Alternatively, IL-6 may preferentially stimulate autoantibody-producing cells. This possibility is consistent with previous studies indicating that anti-DNA producing B cells may have increased IL-6R (38). Specifically, Kitani et al. (38) found that IL-6R were preferentially expressed on low density B cells from patients with active SLE. These cells produced high levels of IgG anti-DNA antibodies, and their production of autoantibodies could be inhibited by either anti-IL-6 or anti-IL-6R mAb (38). Thus, anti-IL-6 may preferentially effect autoantibody-producing B cells, without significantly altering the total Ig or isotype levels. This may simply reflect the activated state of autoreactive B cells in B/W mice.

Finally, IL-6 affects many cell types, including T cells and multipotential progenitor cells as well as B cells (11, 12). Thus, anti-IL-6 could conceivably have had an immunosuppressive effect by changing the relative proportions of distinct lymphocyte subsets. However, in this study we found that administration of anti-IL-6 did not alter lymphocyte subsets. Specifically, there was no alteration of B cells or CD8+ T cells at any point during the study. Mice that received anti-CD4 had significant depletion of CD4+ T cells for several months, but the percentage of CD4+ T cells returned to normal levels by 6 mo of age even though the mice continued to receive anti-IL-6.

We have shown previously that chronic therapy with anti-CD4 retards murine lupus in B/W mice (51, 55). However, brief therapy caused only a brief delay in the development of lupus nephritis (59). The results of the current study are consistent with this observation, in that the control mice that received a tolerizing dose of anti-CD4 along with GL113 at age 3 mo had a slight, albeit statistically insignificant, delay in the onset of proteinuria and mortality relative to the other two control groups (see Figs. 4 and 5).

Our studies do not establish whether chronic administration of anti-IL-6 suppresses normal immune function as profoundly as it suppresses autoimmunity. However, the development of a brisk host immune response to the anti-IL-6 mAb suggests that anti-IL-6 alone does not completely abrogate normal immune function. It should be emphasized, however, that the development of an immune response to anti-IL-6 may inhibit its biologic effects. Thus, no conclusions can be drawn regarding the long-term effects of IL-6 suppression on normal immune function. Studies are currently underway in our laboratory to delineate the extent of immune suppression caused by anti-IL-6.

In our experiments, the development of an immune response to the anti-IL-6 mAb abrogated its effectiveness. Although our study did not address the mechanism by which the immune response to the mAb abrogated its effect, previous studies suggest that this may be due either to the formation of blocking antibodies or to the development of associated immune-complex disease (60). In order to circumvent these problems, we tolerized the mice to the rat mAb by giving anti-CD4 concurrent with the first dose of anti-IL-6 or the control mAb, GL113. In previous studies, anti-CD4 has been used to induce tolerance to classic laboratory antigens, such as HGG, but attempts to induce tolerance to mAb directed against mouse cell surface molecules have been unsuccessful (61, 62). Thus, the induction of tolerance to an anti-cytokine mAb represents the first successful use of anti-CD4 to facilitate therapy with another mAb.

Loss of tolerance to the rat mAb at age 9 mo occurred simultaneously in mice that were receiving anti-IL-6 and in mice that were receiving GL113. Previous studies in non-autoimmune mice indicate that the duration of tolerance induced by anti-CD4 is limited, although it can be prolonged by repeated antigen challenge as was done in our experiment (63). Therefore, the eventual loss of tolerance may simply reflect the finite duration of the effects of anti-CD4. However, it is also possible that the loss of tolerance was due in part to underlying defects in the B/W strain. This possibility is consistent with previous studies indicating that B/W mice are relatively resistant to tolerance induction and that this resistance increases with age (64–66). It is unlikely that the loss of tolerance was due to progressive disease, since tolerance was broken simultaneously in both the anti-IL-6 and GL113 groups even though the GL113 group had evidence of SLE several months before the loss of tolerance to the rat mAb.

Previous studies in mice have shown that selective inhibition of IL-6 can prevent autoimmune diabetes (43) and protect against lethal bacterial sepsis (67). Based in part on these observations, preliminary trials of anti-IL-6 have recently been initi-

ated in humans (68). Our demonstration that IL-6 is involved in the pathogenesis of murine lupus suggests an even broader range of potential clinical applications of such therapy, although it remains to be determined whether practical therapeutic strategies can be developed.

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