Interleukin-4 Protects against a Genetically Linked Lupus-like Autoimmune Syndrome

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Summary

Interleukin-4 (IL-4) provides support for humoral immune responses through upregulation of T helper (Th) type 2 cell differentiation, but it is not known whether IL-4 promotes antibody-mediated autoimmune diseases such as systemic lupus erythematosus (SLE). Here, we show that the constitutive expression of an IL-4 transgene by B cells completely prevents the development of lethal lupus-like glomerulonephritis in the (NZW × C57BL/6. Yaa)F1 murine model of SLE. This was associated with marked changes in the serum levels of IgG subclasses, rather than in the total levels of anti-DNA antibodies, with a lack of IgG3, a decrease of IgG2a, and an increase in IgG1 subclasses, and by a strong reduction in the serum levels of gp70-anti-gp70 immune complexes. This effect of the transgene appears to result from a modulation of the Th1 versus Th2 autoimmune response, since the protected mice displayed comparably modified IgG2a and IgG3 antibody response against exogenous T cell–dependent antigen, but not against T cell–independent antigens. Thus, IL-4 prevents the development of this lupus-like autoimmune disease, most likely by downregulating the appearance of Th1-mediated IgG subclasses of autoantibodies such as the IgG3 autoantibodies which have been shown to be especially nephritogenic.

Mice of the (NZB × NZW)F1 hybrid, mice of the MRL strain homozygous for the lpr gene (lymphoproliferation), and of the BXSB/MpJ strain carrying an autoimmune acceleration gene present on the Y chromosome, Yaa (Y-linked autoimmune acceleration), spontaneously develop a severe immune complex–mediated glomerulonephritis resembling human SLE (1). The spontaneous production of pathogenic IgG autoantibodies in these lupus-prone mice reflects the intrinsic abnormal hyperactivity of B cells and their interaction with CD4+ Th cells in which the pattern of cytokine expression may have important immunopathological consequences (2). Among cytokines produced by Th cells, IL-4 has been shown to have important regulatory properties on humoral immune responses. First described as a costimulator for the proliferation of resting B cells, IL-4 has been also shown to enhance the expression of Ia molecules on resting B cells, to induce IgG1 and IgE antibody production, and to play a predominant role in vitro in the differentiation of CD4+ T cells into the Th type 2 subset (3). Notably, Th2 cells play an essential role in some humoral responses through the action of their cytokines such as IL-5, -6, and -10 on B cells (4–6).

In view of the B cell–stimulatory properties and Th2 promoting activity ascribed to IL-4, it can be speculated that IL-4 may be involved in the development of autoimmune diseases such as SLE. In fact, IL-4 has been shown to be an important mediator in the pathogenesis of murine lupus-like diseases induced by graft-versus-host and host-versus-graft reactions, or by chemicals (7). This notion is also supported by the findings that treatment of lupus-prone (NZB × NZW)F1 mice with mAbs specific for the Th2-related cytokines IL-6 or -10 markedly delays onset of autoimmune disease (8, 9), and that administration of IL-5 or -10 is able to induce autoimmune anemia in anti-RBC autoantibody transgenic mice (10). It is, however, not yet known whether IL-4 promotes disease development in spontaneous murine models of SLE.

To address this question, we determined the effect of constitutive expression of an IL-4 transgene by B cells on the development and progression of lupus-like syndrome. For this purpose, we have used the (NZW × C57BL/6. Yaa)F1 murine model of SLE (11), in which the development of an autoimmune syndrome in male mice is dependent on the abnormal autosomal NZW genome, and the presence of an unidentified mutant gene Yaa located on the Y chromosome derived from the lupus-prone BXSB strain (12). The IL-4 transgene was introduced into this lupus-prone background by crossing C57BL/6. Yaa (B6.Yaa) males with females of the 129/Sv–IL-4 transgenic strain, which contains a single copy of murine IL-4–encoding cDNA

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under the control of IgH enhancer/promoter (13), and then crossing the F1 male progeny with NZW females. Our analysis of the development of SLE in these lupus-prone mice indicates that the constitutive expression of the IL-4 transgene in B cells does not promote, but prevents, the development of lupus-like autoimmune syndrome, and provides a possible explanation for the capacity of IL-4 to control SLE by downregulating Th1-mediated IgG subclasses of autoantibodies.

Materials and Methods

Mice. The 129/Sv IL-4 transgenic mutant strain (pEP-IL-4), which contains one copy of murine IL-4–encoding cDNA under the control of IgH enhancer/promoter, was generated by transfecting a murine-embryonic stem cell line with an IL-4 expression vector, described previously (13). B6.Yaa mice were established by backcross procedures, as described previously (11). NZW mice were purchased from Harlan Olac Ltd. (Oxon, UK). NZW × (pEP-IL-4 × B6.Yaa) mice were obtained by local breeding in our own animal facility. The inheritance of IL-4 transgene was ascertained by assessing an increased 1a expression by peripheral blood B lymphocytes. Blood samples were collected by orbital sinus puncture, and the sera were stored at −20°C until use.

mAb and Cytofluorometric Analysis. The following mAbs were used: LO-MM-9 (anti–mouse μ chain), Y-3P (anti-I-A^b), and H81.98.21.1 (anti-I-E). For immunofluorescence analysis, mAbs were purified on protein G–sepharose and coupled to FITC or biotin; staining the cells was done as described by Rolink et al. (14). PE-labeled avidin was purchased from CALTAG Labs. (South San Francisco, CA). Spleen cells from 2-mo-old mice were first stained with FITC-labeled anti–mouse μ chain mAb, and then incubated in absence or in presence of biotinylated anti-I-A or I-E mAb’s, before incubation with PE-labeled avidin. 10^4 events were analyzed with a lymphocyte gate as defined by light scatter with a FACScan® (Becton Dickinson, Mountain View, CA).

Antigens and Immunization Protocols. Mice were immunized intravenously with 400 μg of heat-aggregated human IgG (HGG)1 in PBS or 10 μg (4-hydroxy-3-iido-5-nitrophenyl) acetyl (NIP)–Ficoll in PBS. NIP–Ficoll was a gift from Dr. A. Rolink (Basel Institute for Immunology, Basel, Switzerland). Sera were collected 10 d later. LPS-induced polyclonal Ig responses were determined by injecting mice intraperitoneally with 25 μg LPS from Salmonella minnesota R595, and collecting sera 8 d later for IgG and 15 d later for IgG subclass analysis.

Serological Assays. Total levels of serum IgM and IgG subclasses were determined by ELISA as previously described (15), using alkaline phosphatase–labeled goat antibodies specific for mouse Ig classes and subclasses (purchased from Southern Biotechnology Assoc., Birmingham, AL). The Ig concentrations were determined by referring to standard curves obtained with known concentrations of mouse Ig (Southern Biotechnology Assoc., and ICN Biomedicals, Inc., Costa Mesa, CA).

The presence of IgG anti-DNA antibodies was assessed by ELISA as previously described (15). Results are expressed in titration units (U/ml) in reference to a standard curve obtained from 3–4-mo-old MRL-lpr/lpr mice. Titters of IgG subclass anti–DNA were determined by ELISA using IgG subclass specific antibodies (Southern Biotechnology Assoc.). The titers are the highest dilutions still giving a positive signal (log2 titers) in the ELISA, in which twofold serum dilutions were tested starting from 1:100 dilution.

Serum levels of gp70-anti–gp70 immune complexes (gp70 IC) were quantified by ELISA combined with the precipitation of the serum with polyethylene glycol (average molecular weight 6,000), which precipitates only the antibody-bound gp70, and not free gp70, as described (16). Results are expressed as μg/ml of gp70 complexed with anti-gp70 autoantibodies by referring to a standard curve obtained from NZB sera with known amounts of gp70.

Serum levels of IgM and IgG subclasses of anti–HGG and anti–NIP antibodies were measured by ELISA in which HGG or NIP10-BSA were used as coating antigens. Titters of anti–HGG and anti–NIP of individual Ig isotypes are expressed as the highest serum dilution giving a positive signal (log2 titers) in the ELISA, in which twofold serum dilutions were tested starting from 1:100 dilution.

Histopathology. Samples of all major organs were obtained at autopsy, and histological sections were stained with periodic acid–Schiff reagent. Glomerulonephritis was evaluated based on the intensity and extent of pathological changes as described (11).

Statistical Analysis. Statistical analysis was performed with the Wilcoxon two-sample tests. P >5% were considered insignificant.

Results

Prevention of Lupus-like Nephritis By IL-4 Transgene Expression in the (NZW × B6.Yaa)F1 Murine Model of SLE. To determine whether constitutive IL-4 transgene expression may influence the development of the autoimmune SLE syndrome in (NZW × B6.Yaa)F1 mice, pEP-IL-4 female transgenic mice were crossed with B6.Yaa males, and the resulting (pEP-IL-4 × B6.Yaa)F1 male mice bearing the IL-4 transgene and the Yaa gene were then crossed with NZW females, thus generating lupus-prone NZW × (pEP-IL-4 × B6.Yaa) male mice. Consistent with previous analysis of pEP-IL-4 transgenic mice (13), most splenic B cells of IL-4 transgenic NZW × (pEP-IL-4 × B6.Yaa) mice exhibited a markedly increased (5–10-fold) expression of I-A and I-E molecules, as compared with nontransgenic littermates (Fig. 1 A).

The male nontransgenic littermates spontaneously developed a lethal form of lupus-like glomerulonephritis, as observed in (NZW × B6.Yaa)F1 male mice (11). 46% (16/35) of the nontransgenic males died of glomerulonephritis by 9 mo after birth and 86% (30/35) at 12 mo. In contrast, the development of lupus-like glomerulonephritis was dramatically prevented in the male transgenic mice; only 1/38 transgenic mice died of glomerulonephritis within the first year (Fig. 1 B) and beyond (18 mo). At 8 mo, transgenic males showed only minimal glomerular alterations, which markedly contrasted with the severe glomerular lesions observed in the surviving nontransgenic male littermates (Fig. 1 C).

Serological Characteristics in the IL-4 Transgenic Mice. The protective effect of the IL-4 transgene did not appear to be
associated with a reduction of total serum IgG anti-DNA autoantibodies (although titers were 1.5-fold lower in IL-4 transgenic mice; 4 mo: P <0.05, 8 mo: P >0.1, Fig. 2 A), but rather with marked changes in IgG subclasses (Fig. 2 B). In fact, IgG3 anti-DNA antibodies were barely detectable in transgenic mice (P <0.0001) even at one year of age (data not shown), and IgG2a anti-DNA antibodies were reduced (P <0.001), while IgG1 anti-DNA levels were increased in the transgenic mice (P <0.01). Total IgG subclass levels also differed in transgenic mice in a comparable way; there was a two- to fourfold increase of IgG1 (P <0.005), but two-fold and 10-fold decreases of IgG2a (P <0.001) and IgG3 (P <0.0001), respectively (Fig. 2 C).

To further characterize the basis for the IL-4 protection, transgenic mice were analyzed for the serum levels of gp70 IC, which have been demonstrated to best correlate with the development of murine lupus nephritis (17–19). Results of this analysis, presented in Fig. 2 D, indicate that amelioration of clinical manifestations was accompanied by a strong reduction of circulating gp70 IC levels at 4 and 8 mo in nontransgenic mice (4 mo: 9.2 ± 7.3 μg/ml, 8 mo: 9.2 ± 8.6 μg/ml), as compared to transgenic males (4 mo: 0.4 ± 0.5 μg/ml, P <0.0001, 8 mo: 2.2 ± 2.3 μg/ml, P <0.0001).

Reduced Production of IgG2a and IgG3 versus IgG1 Antibodies Against T Cell–dependent, But Not T Cell–independent, Antigens in IL-4 Transgenic Mice. The changes in IgG subclasses may result from a direct effect of the transgenic IL-4 expressed by the B cells on these cells, or from an IL-4 action modulating the Th1 and Th2 responses; IFN-γ produced by Th1 cells and IL-4 secreted by Th2 cells are known to regulate reciprocally IgG class switching, the former promoting the Ig switch to IgG2a and IgG3 subclasses, and the latter to the IgG1 subclass (20, 21). To ad-

Figure 1. Prevention by the IL-4 transgene of the spontaneous development of lupus-like glomerulonephritis in lupus-prone NZW × (pEP-IL-4 × B6.Yaa) mice. (A) Increased expression of MHC class II molecules on surface IgM+ B cells in 2-mo-old NZW × (pEP-IL-4 × B6.Yaa) male mice bearing the IL-4 transgene, as compared with nontransgenic littermates. Spleen cells were stained with FITC-labeled anti–MHC class II mAb, and then incubated in absence (normal line) or in presence (bold line) of biotinylated anti-I-A (upper panel) or anti-I-E (lower panel) mAb’s, followed by PE-conjugated avidin. (B) Cumulative mortality with glomerulonephritis in IL-4 transgenic (Tg) and nontransgenic (N-Tg) male mice. Note increased glomerular cellularity and obliteration of the glomerular architecture in nontransgenic males, which contrast with the minimal alterations in glomeruli from transgenic mice. The tissues were stained with periodic acid–Schiff reagent (×200).

Figure 2. Effect of the IL-4 transgene expression on the spontaneous autoantibody and IgG production in lupus-prone NZW × (pEP-IL-4 × B6.Yaa) mice. (A) Levels of serum IgG anti-DNA antibodies in IL-4 transgenic (Tg) and nontransgenic (N-Tg) male mice 4 and 8 mo-old. Results are expressed in U/ml by reference to a standard curve obtained with a serum pool of 3–4-mo-old MRL-lpr/lpr mice. (B) IgG1, IgG2a, and IgG3 subclass distribution of anti-DNA antibodies in the sera of 6-mo-old IL-4 transgenic and nontransgenic male mice. Twofold serum dilutions were tested starting with 1:100 dilution. The titers are the highest dilutions still giving a positive signal in the ELISA. (C) Serum levels of IgG1, IgG2a, and IgG3 subclasses in 4-mo-old IL-4 transgenic and nontransgenic male mice. Ig concentrations are expressed in mg/ml. The means of 16 mice (± 1 SD) are represented. (D) Serum levels of gp70 IC in IL-4 transgenic and nontransgenic male mice 4 and 8 mo old. Results are expressed in μg/ml. Each symbol represents the value from individual animals. The median values are indicated as horizontal lines.
The role of IL-4 in systemic autoimmunity was investigated here by studying the effects of an IL-4 transgene on the development of autoimmune disease in the (NZW × B6.Yaa)F1 murine model of SLE. Our data demonstrate that the constitutive expression of an IL-4 transgene by B cells did not promote, but protected mice against the spontaneous development of lupus-like glomerulonephritis. We also showed that this protection occurred in association with marked changes in the IgG subclasses rather than in the total levels of anti–DNA autoantibodies, with an absence of IgG3 and a decrease of IgG2a subclasses. This was accompanied by a similar modulation of the IgG subclass of T cell–dependent antibody responses, but not T cell–independent antibody responses, suggesting a causal link between IL-4, downregulation of Th1 autoimmune responses, and protection against SLE disease.

The protection from SLE in association with the changes in IgG subclass responses as a result of the IL-4 transgene expression by B cells can be mediated either by a direct effect of the transgenic IL-4 on B cells, or by an IL-4 action on the modulation of the Th1 and Th2 responses. However, the lack of effect of the IL-4 transgene on the induction of IgG1 antibody production during T cell–dependent (antigen-specific and nonspecific) activation of B cells strongly argues against a direct effect of the IL-4 transgene on the IgG class switching in B cells. This may be due in part to a low level of expression of the IL-4 transgene (13), as the pEP-IL-4 transgenic mice do not spontaneously exhibit any significant isotype alterations, although the transgene expression is sufficient to maintain Ia hyperepression on B cells. Instead, the results obtained with the stimulation with T cell–dependent antigen suggest that the decrease of IgG3 and IgG2a autoantibody production in the transgenic mice is most likely due to the inhibitory effect of IL-4 on the development of the Th1 subset. This is consistent with the previous demonstration that pEP-IL-4 transgenic mice are more susceptible to Leishmania major infection than nontransgenic mice (22) in which Th1 cells play a crucial role in protection against L. major infection (23). The lack of effect on IgM and IgG3 antibody responses to T cell–independent antigens, together with the absence of significant effect on the serum levels of IgM antibodies reactive with bromelain-treated mouse red blood cells and the total number of IgMbright IgD−, Mac-1+ B-1 peritoneal cells (our unpublished results), further rule out an IL-4 effect not mediated by T cells.

The beneficial effect of the IL-4 transgene is thus likely to be a result of a downregulation of Th1 responses involved in autoantibody production in our murine SLE model. This notion is supported by our recent observation that the progression of lupus nephritis in MRL mice bearing the Yaa or lpr (Fas) mutation correlates with an increased production of IgG2a and IgG3 versus IgG1 antibodies in parallel to an enhanced expression of IFN-γ versus IL-4 mRNA by CD4+ T cells (24). In addition, it has been shown that repeated injections of recombinant IFN-γ can accelerate the development of SLE, and treatment with anti–IFN-γ mAb or soluble IFN-γ receptors can inhibit the progression of SLE in (NZB × NZW)F1 mice (25, 26). Although one cannot rule out the possibility that proinflammatory Th1 cytokines may be involved in the immunopathogenetic process of glomerular lesions thus
participating in the progression of the disease, an attractive possibility is that IFN-γ producing Th1 cells may promote the production of autoantibodies with more immunopathological consequences. It has been shown that murine IgG3 mAb are extremely nephritogenic, and generate lupus-like glomerular lesions through their cryoglobulin activity related to the unique structure of the γ3 constant region (27, 28). Several studies have also shown that IgG3 levels correlate well with the development of murine lupus nephritis (24, 29–31). In addition, the complement activating IgG2a antibodies may be more nephritogenic than IgG1 antibodies. Although the IgG subclasses present in the IgG2a antibodies may be more nephritogenic than those of IgG3, the IgG3 mAb are extremely nephritogenic, and generate lupus-like glomerular lesions through their cryoglobulin activity related to the unique structure of the γ3 constant region (24, 29–31). Clearly, it will be important to determine whether IL-4 transgene expression also leads to protection against SLE in other murine models such as (NZB × NZW)F1 mice, and to address the issue of whether IL-4-induced immune deviation can provide protection from ongoing disease (35), since this would help establish new strategies for the development of therapeutic approaches in SLE and related human autoimmune diseases.

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