IL-1β-deficient mice are resistant to induction of experimental SLE

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ABSTRACT. IL-1 is one of the most pleiotropic pro-inflammatory and immunostimulatory cytokines. Overproduction of IL-1 has been shown to be involved in the pathogenicity of various autoimmune inflammatory diseases, including systemic lupus erythematosus (SLE). However, the different contributions that the IL-1 agonistic molecules make in their *in vivo* native milieu, IL-1β which is mainly secreted against IL-1α which is mainly cell-associated, have not been established. Experimental SLE can be induced in mice by injection with monoclonal anti-DNA antibodies bearing a major idiotype designated, 16/6Id. In the present study, experimental SLE was induced in mice deficient in specific IL-1 molecules, *i.e.* IL-1α$^{-/-}$, IL-1β$^{-/-}$, IL-1α/β$^{-/-}$ (double KO) and in control BALB/c mice. Mice deficient in IL-1β , *i.e.* IL-1β$^{-/-}$ and IL-1α/β$^{-/-}$ mice, developed lower levels of anti-dsDNA antibodies after immunization with 16/6Id, as compared to IL-1α$^{-/-}$ or control BALB/c mice. Disease manifestations were milder in mice deficient in IL-1β expression. The representative cytokine cascade that is characteristic of overt experimental SLE was also shown to be reduced in groups of mice that lacked IL-1β as compared to mice deficient in IL-1α, which is mainly cell-associated. Altogether, our results point to the importance of secretable IL-1β, rather than cell-associated IL-1α, in the immunostimulatory and inflammatory phenomena that mediate the pathogenesis of experimental SLE.

Keywords: experimental SLE, IL-1α, IL-1β

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the increased production of autoantibodies and defective T cell-mediated responses [1]. These are associated with clinical manifestations of disease, such as immune complex deposition in the kidneys and other organs [1]. Anti-double-stranded DNA (ds-DNA) antibodies are characteristic of SLE and are central to the diagnosis of the disease [2-4].

Experimental SLE can be induced in mice by immunization with a monoclonal anti-DNA antibody bearing the common idiotype 16/6, designated 16/6Id [5]. After immunization, the mice produce high titers of antibodies to the 16/6 Id, and to DNA, and they develop SLE-related clinical manifestations that include leukopenia, proteinuria, and glomerulonephritis [5]. Cytokines are essential for the control of the specific immune responses that characterize SLE, as well as for the inflammatory response that induces the pathogenic effects of the disease [6-8]. In young-adult mice, experimental SLE is associated with a characteristic cytokine response pattern, manifested by an initial burst of pro-inflammatory cytokines, *i.e.*, IL-1 and TNF-α, followed by a wave of T_h1 cytokines (IL-2 and IFN-γ), while the T_h2-type cytokines (IL-4 and IL-10) peak later, at the time when the clinical manifestations are observed. At this stage, there is a reduction in the levels of T_h1-type cytokines. However, pro-inflammatory cytokine levels (IL-1 and TNF-α) remain high throughout the disease [6-8].

IL-1 is a master pro-inflammatory and immunostimulatory cytokine that acts mainly through the induction of a network of cytokines, including itself, chemokines and adhesion molecules in the microenvironment, and thus it activates an array of stromal and immune/inflammatory cells; this initiates and propagates immune and inflammatory responses (reviewed in [9, 10]). The IL-1 family consists of two major agonistic proteins, namely IL-1α and IL-1β, and one physiological antagonistic protein, the IL-1 receptor antagonist (IL-1Ra) that binds to the IL-1 receptor type I without transmitting an activation signal (reviewed in [9, 10]). IL-1α and IL-1β bind to the same receptors and there are no significant differences in the spectrum of activities induced by recombinant IL-1α and IL-1β. However, in the *in vivo* milieu, within the producing cells or their microen-

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vironment, IL-1α and IL-1β differ dramatically in the sub-cellular compartments in which they are active. Thus, IL-1β is active only as a secreted, mature product (17.5 kD) that is secreted by macrophages, as well as by many other cells, whereas its cytosolic precursor (31 kD) is inactive. On the other hand, IL-1α is mainly active in cell-associated forms, *i.e.*, the unprocessed, cytosolic precursor form (31 kD) and the membrane-associated form (23 kD). The mature, secreted form of IL-1α (17.5 kD) is less abundant in the body, as it is secreted only by activated macrophages, while other cells do not secrete IL-1α, but rather express cell-associated IL-1α.

In our previous studies, we have demonstrated different tissue distribution of IL-1α *versus* IL-1β, pointing to differential, *in vivo* functions of the two IL-1 molecules. Indeed, we have demonstrated that IL-1α and IL-1β exert differential effects on tumor invasiveness and tumor-host interactions [9, 11, 12]. Thus, cell-associated IL-1α expressed by tumor cells increases their immunogenicity and induces efficient anti-tumor cell immune responses that lead to tumor eradication, while secretable IL-1β increases tumor invasiveness and metastasis and also induces tumor-mediated suppression [9, 11].

The involvement of IL-1 in the pathogenesis of SLE has been documented [7, 8, 13-15]. However, most studies have considered both IL-1 molecules as synonymous, and have usually assessed only one of the IL-1 molecules. Studies on the different roles of IL-1α as compared to IL-1β in health and disease have only recently started to appear, mainly following the generation of specific IL-1 KO mice in which one can assess the role of IL-1α and IL-1β *in vivo*; IL-1β is mainly secreted and IL-1α is mainly cell-associated. In the present study, we have assessed the different *in vivo* involvement of the IL-1 molecules in experimental SLE, by using knockout (KO) mice that selectively lack IL-1 molecules as follows: IL-1$\alpha^{-/-}$, IL-1$\beta^{-/-}$ and IL-1α/$\beta^{-/-}$ (double KO) mice. The results point to a key role of IL-1β in the induction and propagation of experimental SLE.

MATERIALS AND METHODS

Mice

Female BALB/c mice were purchased from Harlan (Jerusalem, Israel). The generation of IL-1 KO mice, *i.e.*, IL-1$\alpha^{-/-}$, IL-1$\beta^{-/-}$ and IL-1α/$\beta^{-/-}$ (double KO mice) were previously described by us [16]. These mice were extensively backcrossed to BALB/c mice for more than eight generations. These strains of mice are homozygous for the relevant mutation. The IL-1$^{-/-}$ mice were bred and kept at the Animal Facilities of the Faculty of Health Sciences, Ben-Gurion University, under aseptic conditions. Mice were treated according to the Animal Care NIH guidelines adapted by our Animal Committee.

Monoclonal antibody

The human monoclonal anti-DNA antibody that bears the common idiotype 16/6Id (IgG1/k) has been characterized previously [17]. This antibody will be designated here as 16/6Id. The mAb were secreted by hybridoma cells in culture, and were purified using a protein G-Sepharose column (Pharmacia, Fine Chemicals, Uppsala, Sweden).

Induction of experimental SLE

In order to induce experimental SLE, mice were immunized with 1 μg of the human mAb 16/6Id. The primary injection was administered in Freund's complete adjuvant (CFA, Difco, MI, USA), intradermally into the hind footpads. Three weeks later, the mice received a booster injection of the same amount of the 16/6Id in PBS.

ELISA for determination of anti-mouse dsDNA and anti-16/6Id antibodies

For measuring anti-mouse dsDNA antibodies, 96 well Maxisorb microtiter plates (Nunc, Denmark) were coated with poly-L-lysine (Sigma). The plates were then washed and coated with lambda phage dsDNA (Worthington Biochemical Corporation, New Jersey, USA). After incubation with different dilutions of sera, goat antimouse IgG (γ-chain-specific) conjugated to horseradish peroxidase (Jackson Immuno Research, West Grove, PA, USA) was added to the plates. Plates were then incubated with the substrate, ABTS [2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); Sigma, Israel] and read at 405 nm using an ELISA reader [18]. For the determination of 16/6Id-specific Abs, plates were coated with 2 μg/mL of the monoclonal human 16/6Id. The assay was carried out as above [5, 19].

Detection of proteinuria

Proteinuria was measured by a standard, semi-quantitative test, using a Combur 10 Test kit (Roche Diagnostics GmbH, Mannheim, Germany). Results were graded according to the manufacturer as: negative, + = 0.3g/L, ++ = 1g/L, +++ = 3g/L, ++++ = ≥ 20g/L.

Immunohistology for detection of immune complex deposits in kidneys

Mice were sacrificed six to seven months following disease induction and kidneys were removed and immediately frozen in liquid nitrogen. Frozen cryostat sections of 5 μm were air-dried and fixed in acetone. For the detection of Ig deposits, sections were incubated with FITC-conjugated goat antimouse IgG (γ-chain-specific) (Jackson Immuno Research, West Grove, PA, USA). Staining was visualized using a fluorescence microscope. The immune complex deposits were scored using semi-quantitative grading as follows: 0, no complexes; 1, minimal deposition of complexes; 2, moderate complexes; 3, intense complexes.

Cytokine production and secretion

Assays of cytokine production by spleen cells were performed in mice with overt SLE, at sacrifice. Pooled cells of five mice from each experimental group were examined. Splenocytes (5 x 10^6/mL) from mice in each experimental group were incubated with 16/6Id 25 μg/mL in enriched culture medium consisting of RPMI-1640 supplemented with 10 % heat-inactivated FCS, 2 mM L-glutamine, penicillin G (100 u/mL), streptomycin (100 u/mL)

2-mercaptoethanol (5 x 10^{-6}M) for 48 h. Supernatants were tested for the presence of IFN-γ, IL-10, and TNF-α. Cytokine levels were determined by commercial ELISA kits, using the relevant standards, capture and detecting antibodies (Pharmingen, San Diego, CA, USA), according to the manufacturer's instructions. The results were measured using an ELISA reader at 405nm wavelength.

Detection of intracellular cytokines

Single spleen cell suspensions were permeabilized using the Cytoperm kit (Pharmingen, San Diego, CA, USA) according to the manufacturer's instructions. Size-gated splenic leukocytes were analyzed. Thereafter, cells were incubated with appropriate anti-cytokine-FITC conjugated antibodies and analyzed by a FACScan flow cytometer. The data were analyzed using Lysis software. The percentage staining shown is after substructing levels obtained with isotype controls.

Statistical analysis

Two experiments to induce experimental SLE in control and IL-$1^{-/-}$ mice were performed. Each group consisted of 8-10 mice. Similar patterns of antibody production and disease manifestations were observed in the two experiments. Results shown are from one representative experiment. To evaluate the significance of the difference between control and IL-1 KO groups, the Student's t-test and the non-parametric Mann-Whitney test were used. Values of $p \leq 0.05$ were considered significant.

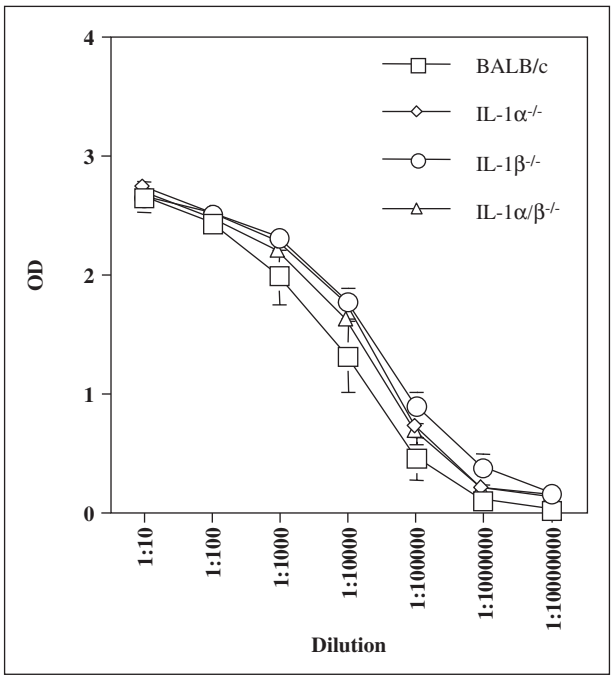

Figure 1
Serum levels of 16/6Id specific antibodies in IL-1-deficient mice. Control BALB/c mice and IL-1-deficient mice were immunized with the human monoclonal anti-DNA that bears the idiotype 16/6 Id (designated as 16/6Id). Anti-16/6Id antibodies were assessed by ELISA. Anti-16/6 antibody levels, 2 months after primary immunization with the 16/6Id are shown. The average values of O.D. of sera from 8-10 mice in each experimental point ± SD are shown.

RESULTS

Effects of endogenous IL-1 on antibody levels in experimental SLE

In this study, we attempted to assess the role of endogenous IL-1 on the induction and development of experimental SLE. We used different strains of IL-1 KO mice as follows: IL-$1\beta^{-/-}$, IL-$1\alpha^{-/-}$, IL-$1\alpha/\beta^{-/-}$ (double knockout) mice and BALB/c mice as a control. To induce experimental SLE, two-month-old mice were immunized and boosted (3 weeks later) with the16/6 Id, as described [5]. Levels of anti-16/6Id antibodies were assessed, two months after the primary immunization. As can be seen in *figure 1*, all types of mice, regardless of the patterns of IL-1 expression, generated similar high levels of anti-16/6 Id antibodies. The mice were bled periodically and levels of anti-dsDNA antibodies, which are characteristic of experimental SLE, were assessed. As can be seen in *figure 2*, similar levels of anti-dsDNA antibodies were detected in control BALB/c and IL-$1\alpha^{-/-}$, while lower levels were observed in mice deficient in IL-1β, *i.e.*, IL-$1\beta^{-/-}$ and IL-$1\alpha/\beta^{-/-}$ (double knockout) mice. These differences were observed at all time intervals after immunization until the sacrifice of mice with overt disease.

Clinical manifestations of experimental SLE in IL-$1^{-/-}$ and control mice

Clinical manifestations of experimental SLE were evaluated by assessing proteinuria and immune complex deposits in

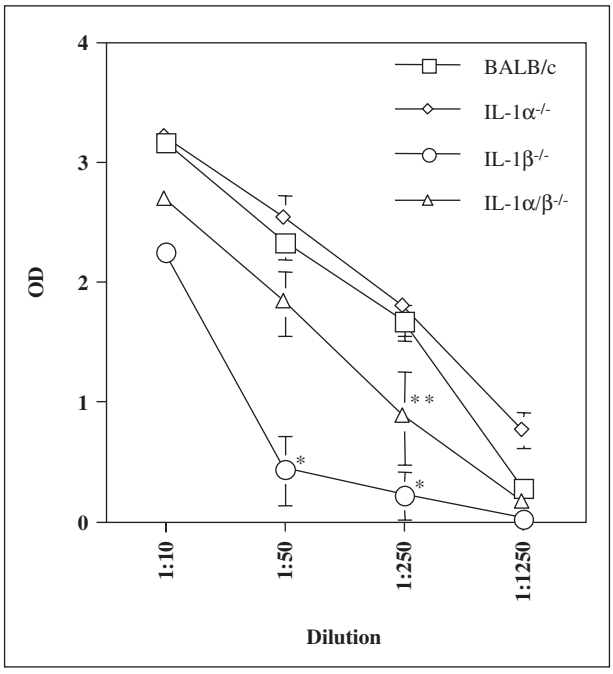

Figure 2
Serum levels of anti-dsDNA antibodies in IL-1-deficient mice. Control BALB/c mice and IL-1-deficient mice were immunized with the 16/6Id antibody, which leads to the development of experimental SLE. Serum anti-dsDNA levels, 3 months after the primary immunization with the 16/6 Id antibody, are shown. Results are expressed as mean values of O.D. of sera from 8-10 mice at each experimental point ± SD, *p = 0.0043, **p = 0.007 as compared to the control group of BALB/c mice.

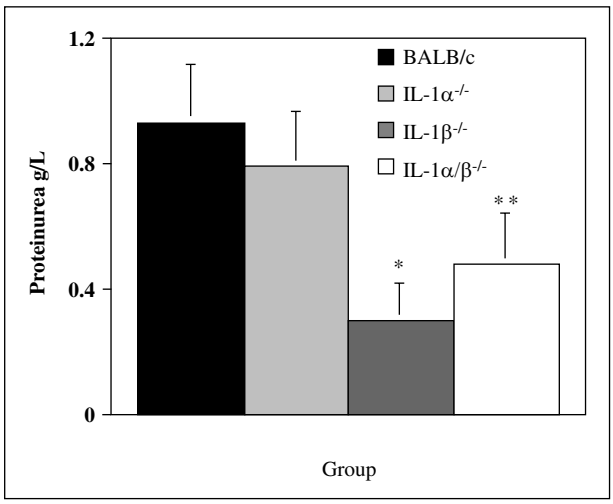

Figure 3

Proteinuria levels in IL-1-deficient mice following immunization with the 16/6/Id. Proteinuria was assessed, using a semiquantitative kit, as specified in Materials and Methods, in BALB/c control and IL-1-deficient mice, 7 months after the primary immunization with the 16/6 Id. Proteinuria levels (expressed as g/L) of 8-10 mice in each experimental group ± SD. *p = 0.004, **p = 0.025 as compared to the control group of BALB/c immunized mice are shown.

the kidneys, seven months following immunization with the 16/Id antibody. *Figure 3* shows similar levels of protein in the urine of control and IL-1$\alpha^{-/-}$ mice, while in IL-1$\beta^{-/-}$ and IL-1α/ $\beta^{-/-}$ mice, significantly lower levels of proteinuria were detected (p = 0.004 and 0.025, respectively). In IL-1$\beta^{-/-}$ and IL-1$\alpha/\beta^{-/-}$ mice, no immune complex deposits or minimal deposits, were observed in kidney sections. Although the intensity of immune complex deposits in kidneys of 16/6Id immunized-IL-1$\alpha^{-/-}$ mice was significantly lower than in kidneys of control BALB/c mice *(figure 4)*, the frequency and intensity of the immune deposits in the kidneys of the IL-1$\alpha^{-/-}$ mice were much more prominent than in IL-1$\beta^{-/-}$ and IL-1$\alpha/\beta^{-/-}$ mice. Similar patterns were observed when leucopenia was assessed (data not shown). These results indicate the dominant involvement of IL-1β in determining the susceptibility of mice to SLE induction and severity of disease.

Cytokine generation in 16/6 Id-immunized control and IL-1$^{-/-}$ mice

As cytokines were shown to play a major role in the pathogenesis of experimental SLE [6-8, 18, 20-23], it was of interest to find out whether endogenous IL-1 affects the cytokine profile of overt SLE, seven months post-immunization with the 16/6Id antibody. We assessed the expression of IL-2, IFN-γ and TNF-α, representatives of T_h1-type cells and IL-4 and IL-10, representatives of T_h2-type cells. IL-10, TNF-α and IFN-γ are also generated and secreted by macrophages and other innate cells. Intracellular cytokines were detected by immunofluorescence and FACS analyses, in freshly isolated spleen cells from immunized mice, as described in Materials and Methods. Secreted cytokines were assessed in supernatants of spleen cells from immunized mice that were challenged in culture with the 16/6Id. As can be seen, in *figures 5 and 6*, representing the expression of intracellular and secreted cytokines, respectively, cytokine levels were generally lower in IL-1$\beta^{-/-}$ and IL-1$\alpha/\beta^{-/-}$ mice, as compared to

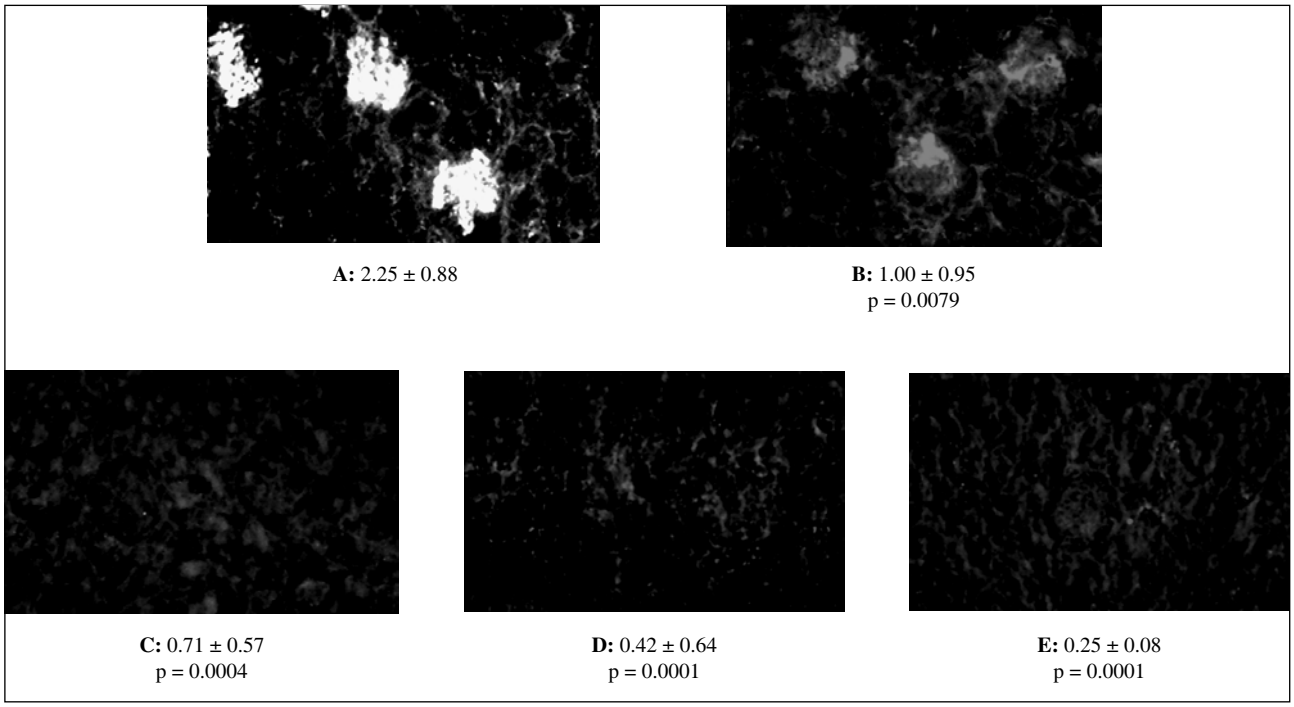

Figure 4

Immunohistology of kidney sections from IL-1-deficient mice following immunization with the 16/6Id. Frozen cryostat sections (5 μm) of kidneys of mice, 7 months following immunization with the 16/6Id were air-dried, fixed in acetone and stained with FITC-conjugated to goat antimouse IgG (γ chain specific) A- Control BALB/c mice; B- IL-1$\alpha^{-/-}$ mice; C- IL-1$\beta^{-/-}$ mice; D- IL-1$\alpha/\beta^{-/-}$ mice; E- Normal (non-immunized) BALB/c mice. This figure demonstrates representative kidney sections from individual mice (x 400). Means of scores (see *Materials and Methods*) of immune complex deposits in kidney sections from 8-10 mice per group ± SD are indicated.
P values for the significance between the 16/6Id immunized BALB/c mice and the IL-1$^{-/-}$ relevant groups of mice are also shown.

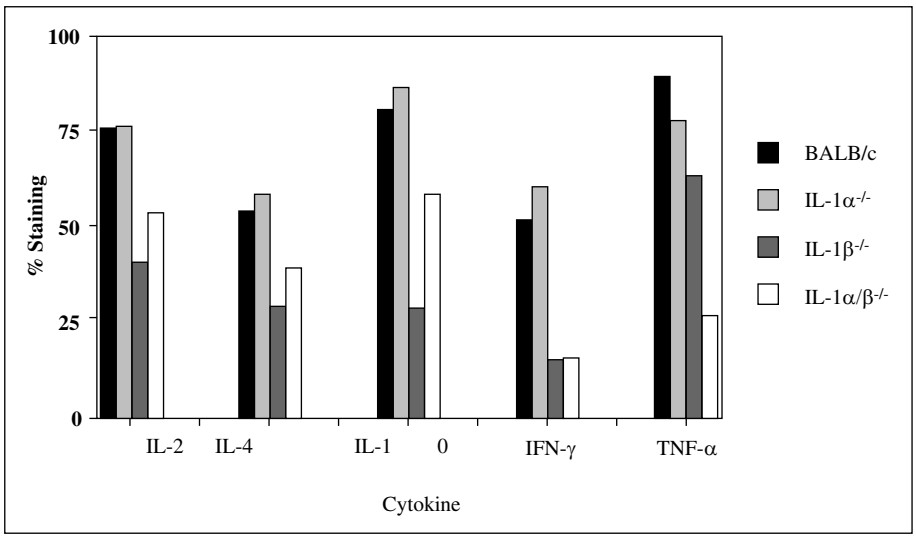

Figure 5

Intracellular levels of cytokines in spleens from IL-1 deficient and BALB/c mice following immunization with the 16/6Id. Mice were sacrificed at the end of the experiment, 7 months after immunization with the 16/6 Id. Intracellular levels of cytokines in spleen cells were assessed by FACS analyses, using specific kits for assaying intracellular cytokines, as specified in the *Materials and Methods*. Pools of spleens from 8-10 mice per group were assessed for intracellular levels of cytokines. Each cytokine was measured in 3 samples. A representative staining from one experiment is shown.

control and IL-1$\alpha^{-/-}$ mice. Thus, cytokine levels in the spleen correspond to the clinical manifestations of overt experimental SLE.

DISCUSSION

The present study emphasizes the role of secretable IL-1β in the initiation and propagation of experimental SLE. In mice deficient in IL-1β, *i.e.*, IL-1$\beta^{-/-}$and IL-1$\alpha/\beta^{-/-}$ (double KO) mice, the immunological and clinical manifestations of disease, *i.e.*, production of dsDNA specific antibodies, immune complex (IC) deposition in the kidneys and proteinuria, are diminished compared to control BALB/c or IL-1$\alpha^{-/-}$ mice. The effects of the IL-1 molecules on SLE may encompass regulatory effects on the immune response, leading to the development of pathogenic antibodies and T cells, as well as subsequent effects on the inflammatory response that mediate tissue-damage in the kidneys, small blood vessels and other target organs that are afflicted in experimental disease [5, 6, 8, 22].

The involvement of IL-1 in *in vivo* inflammatory responses in SLE and other autoimmune inflammatory diseases has been described. Its involvement is possibly due to its adjuvant-like effects on immune phenomena and its pro-inflammatory characteristics [10]. In mice with active SLE, manifesting kidney involvement, local and systemic low levels of the IL-1Ra were demonstrated, indicating the contribution of unattenuated levels of IL-1 to the pathogenesis of disease [13, 14, 24], which paved the way to some trials using the IL-1Ra in SLE patients [25]. In addition, intrarenal production of IL-1 was shown in MRL/lpr and in (NZBxNZW) F1 mice [26, 27]. Finally, an increase in IL-1β expression has been reported in different tissues of SLE-prone mice and in PBL of patients with active lupus [7, 8, 15]. Inhibition of IL-1 production or neutralization of preformed IL-1 were shown to alleviate disease symptoms in various experimental models of SLE, such as the NZB/NZW model or 16/6Id-induced SLE [21, 28].

We have previously shown that treatment of 16/6Id-induced SLE with Methotrexate [21], tamoxifen (an estrogen antagonist) [20], anti-TNF-α antibodies or pentoxiphylline (that downregulates TNF-α) [29] and a peptide based on the complementarity-determining region (CDR) 1 of the 16/6Id [23], all resulted in beneficial effects on the progression and severity of the experimental disease, highlighted by a significant decrease in expression and secretion of IL-1β.

Clinical symptoms of SLE are observed starting four months after disease induction, and full-blown disease appears in all immunized control mice after seven months; at this time mice were sacrificed. Disease symptoms are persistent for the whole life span of the mice, as they only seldom die from the disease. No signs of either antibodies or other clinical manifestations were seen in the IL-1β–deficient mice, excluding the possibility that disease is delayed rather than prevented.

The crucial role of IL-1 as a master cytokine in autoimmune diseases has been demonstrated in mice that are devoid of both IL-1 molecules (IL-1$\alpha/\beta^{-/-}$) and that are resistant to the development of collagen type II-induced arthritis [30-32]. In IL-1Ra$^{-/-}$mice, all females of BALB/c origin spontaneously develop RA, due to unattenuated levels of IL-1 [32-34]. Also, in IL-1 receptor type-1 (IL-R1$^{-/-}$) and interleukin-1-associated kinase 1 (IRAK1$^{-/-}$) mice, in which IL-1 signaling is impaired, the development of experimental autoimmune encephalomyelitis (EAE) [35] and autoimmune myocarditis [36], respectively, is decreased. It was also shown, that signaling through the IL-1R plays a necessary and non-redundant role in experimental autoimmune uveitis (EAU) and can, by itself, account for the lack of EAU development in MyD88 mice [37]. In all these autoimmune diseases, IL-1 contributes to the inflammatory response that is involved in tissue damage. We have shown here that in mice deficient in IL-1β, the disease is less severe than in mice deficient in IL-1α. Secretable IL-1β diffuses into the local microenvironment and activates diverse stromal and inflammatory cells to

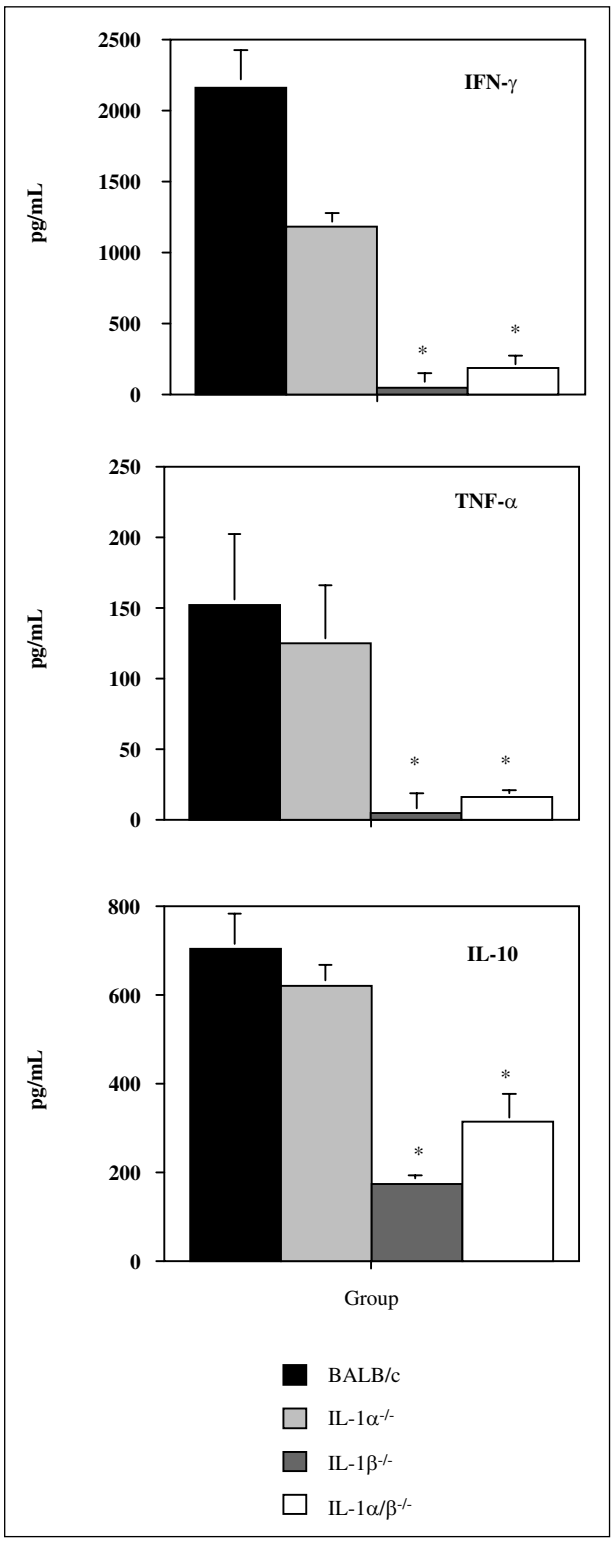

Figure 6

Cytokine levels in supernatants of 16/6Id-stimulated spleen cells. Mice were sacrificed at the end of the experiment, 7 months after immunization with the 16/6 Id. Spleen cells were stimulated for 48 h in culture with the 16/6Id, and cytokine levels in supernatants were determined using ELISA kits, as specified in Materials and Methods. Pools of spleens from 8-10 mice per group were cultured in triplicate. Shown are mean values ± SD.
*$p < 0.03$ as compared to the control group of BALB/c mice.

produce a broad pro-inflammatory cascade, which amplifies the inflammatory response, while the effects of IL-1α, which is mainly cell-associated are more restrained. This is in agreement with our previous results on the different contributions of IL-1α as compared to IL-1β, to tumor invasiveness [9, 11, 12].

In our experiments, all types of mice (control and the various IL-$1^{-/-}$ mice) produced equal levels of specific antibodies following immunization with the 16/6Id. This probably results from the supra-optimal immunization conditions that were used, applying CFA as an adjuvant that activates macrophages and other APCs to produce multiple pro-inflammatory/co-stimulatory cytokines and to express cell surface co-stimulatory molecules, such as B7. These conditions possibly override the homeostatic need for endogenous IL-1β in the production of anti-16/6 antibodies. However, significantly reduced anti-DNA antibodies were observed in IL-$1\beta^{-/-}$ mice and also in IL-$1\alpha/\beta^{-/-}$ mice, as compared to the high levels of anti-dsDNA antibodies that were generated in control BALB/c and in IL-$1\alpha^{-/-}$ mice. Using the same series of IL-$1^{-/-}$ mice, it was shown that homeostatic IL-1β, rather than IL-1α, is required for T cell-dependent antibody production [38]. It was demonstrated that endogenous IL-1β promotes antigen-specific T_H cell function through efficient interactions between APCs and the T cells that result from IL-1β-mediated enhanced expression of CD40L and OX40 on T cells [39]. On the other hand, IL-1α is more essential for the development of contact sensitivity, a form of cell-mediated immunity [40]. Effects of IL-1β on the function of various cellular components that are involved in antibody production, such as B cells, T_h cells and antigen-presenting cells have been described [9, 10]. Further studies will be aimed at assessing the mechanisms of involvement of endogenous IL-1β in the control of pathogenic antibodies in experimental SLE.

Many types of cells have the potential to produce IL-1, especially professional APCs. In the context of experimental SLE, in B cells from old MRL/lpr mice, elevated levels of IL-1β transcripts were observed, possibly reflecting post-transcriptional stabilization of IL-1β mRNA controlled by the lpr mutation [41]. IL-1β production by B cells may be one of the mechanisms that lead to the overproduction of autoantibodies in MRL/lpr mice.

SLE is characterized by high levels of T_h1 and T_h2, and pro-inflammatory cytokines [6, 8]. This manifests as high levels of intracellular *(figure 5)* as well as secreted *(figure 6)* IFN-γ, IL-10 and TNF-α in 16/6Id-stimulated spleen cell cultures of SLE-afflicted control BALB/c mice. In IL-$1\alpha^{-/-}$ mice, levels of secreted cytokines were lower when compared to control mice, and significantly lower levels of cytokines were observed in spleen cell cultures from IL-$1\beta^{-/-}$ and IL-$1\alpha/\beta^{-/-}$ mice that manifested a very mild disease or were disease-free. Assessment of secreted cytokines represents *in vitro* activation of memory T cells from immunized mice, while intracellular cytokine staining enumerates the pool of cells that express the cytokine under chronic stimulation *in vivo.* Significantly lower levels of all cytokines that are considered pathogenic in SLE (*i.e.* INF-γ, TNF-α and IL-10) could be observed in IL-$1\beta^{-/-}$ and IL-$1\alpha/\beta^{-/-}$ mice.

Our results demonstrate a dominant involvement of IL-1β in the initiation and propagation of experimental SLE, indicating a role of microenvironment-secreted IL-1 in this process. However, the results do not exclude some contribution of IL-1α to the disease, as observed by the lower rate of IC in the kidney in IL-$1\alpha^{-/-}$ compared to control

mice. The mutual *in vivo* interactions between IL-1α and IL-1β are complex, and synergism between them has been shown in different experimental systems [9, 10].

In conclusion, our study has emphasized the role of IL-1β as an important factor in the induction and pathogenesis of experimental SLE. Its major roles include the induction of the pathogenic autoantibodies and T cells, as well as the control of inflammatory responses that lead mainly to kidney disease in the experimental models. Thus, IL-1β seems to be an essential factor in the "mosaic" of genetic, environmental, microbial and immune/inflammatory factors that are involved in the pathogenesis of SLE. Therefore, approaches that efficiently neutralize IL-1β should be considered for the treatment of lupus.

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