Clin Exp Immunol 1997; **108**:375–380

IL-12 inhibits *in vitro* immunoglobulin production by human lupus peripheral blood mononuclear cells (PBMC)

F. A. HOUSSIAU, F. MASCART-LEMONE*, M. STEVENS†, M. LIBIN*, J.-P. DEVOGELAER, M. GOLDMAN* & J.-C. RENAULD†‡ *Rheumatology Unit, Louvain Medical School, *Immunology Department, Université Libre de Bruxelles, †Ludwig Institute for Cancer Research, Brussels Branch and ‡Experimental Medicine Unit, Louvain Medical School, Brussels, Belgium*

(Accepted for publication 13 January 1997)

SUMMARY

Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease characterized by polyclonal B cell activation and by the production of anti-double-stranded (ds) DNA antibodies. Given the inhibitory effects of IL-12 on humoral immune responses, we investigated whether IL-12 displayed such an activity on *in vitro* immunoglobulin production by SLE PBMC. Spontaneous IgG, IgG1, IgG2, IgG3 and IgM antibody production was dramatically reduced by addition of IL-12. These results were confirmed by Elispot assays detecting IgG- and anti-dsDNA-secreting cells. While IL-6 and TNF titres measured in PBMC supernatants were not modified by addition of IL-12, interferon-gamma (IFN-γ) titres were up-regulated and IL-10 production down-regulated. Since addition of IFN-γ did not down-regulate immunoglobulin production and since the inhibitory activity of IL-12 on immunoglobulin synthesis was not suppressed by anti-IFN-γ antibody, we concluded that the effect of IL-12 on immunoglobulin production was not mediated through IFN-γ. Our data also argue against the possibility that down-regulation of endogenous IL-10 production was responsible for the effect of IL-12. Thus, inhibition of IL-10 production by IFN-γ was not accompanied by inhibition of immunoglobulin production, and conversely, restoration of IL-10 production by anti-IFN-γ antibody did not suppress the inhibitory activity exerted by IL-12 on immunoglobulin production. Taken together, our data indicate that reduction of excessive immunoglobulin and anti-dsDNA antibody production by lupus PBMC can be achieved *in vitro* by IL-12, independently of IFN-γ and IL-10 modulation.

Keywords IL-12 lupus immunoglobulins B lymphocytes autoimmunity

INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypical systemic autoimmune disease characterized by polyclonal B cell activation and by the production of anti-dsDNA antibodies. Recent advances in our understanding of the biology of cytokines have shed new light on the mechanisms underlying this inappropriate antibody production. Thus, it was demonstrated that IL-10 is overexpressed in lupus patients [1,2] and mediates, at least in part, the excessive immunoglobulin production and the autoantibody response associated with human and murine SLE [3,4]. Such results demonstrate that cytokines are closely involved in the pathogenesis of lupus and suggest that cytokine manipulation might be useful for the control of the disorder. In this respect, molecules down-regulating immunoglobulin production, e.g. by inhibiting endogenous production of B cell growth and differentiation factors, would be of potential therapeutic interest.

Correspondence: Frédéric A. Houssiau, Rheumatology Unit, UCL 5390, Louvain Medical School, Avenue Hippocrate 10, B-1200 Brussels, Belgium.

The recently reported *in vivo* inhibitory activity of IL-12 on B cell function in some murine experimental models [5–9] prompted us to investigate whether this cytokine, that promotes cell-mediated immunity but suppresses humoral immune responses (see [10] for review), displayed a similar activity in human lupus. Thus, in a murine intraperitoneal immunization model, *in vivo* treatment with IL-12 was found to cause the disappearance of peritoneal B1 lymphocytes, purportedly implicated in the development of autoimmunity [5]. It was also shown that *in vivo* treatment with IL-12 decreases serum immunoglobulin levels and autoantibody production in a murine model of chronic 'parent into F_1' graft-*versus*-host disease (GVHD), a syndrome sharing some features with human SLE [6]. These results, by suggesting that IL-12 might inhibit humoral autoimmunity, led us to test whether the excessive immunoglobulin production by lupus patients could be down-regulated by IL-12. Here, we report that IL-12 reduces *in vitro* immunoglobulin and anti-dsDNA antibody production by lupus PBMC, and that this inhibitory activity is not mediated by up-regulation of interferon-gamma (IFN-γ) nor down-regulation of IL-10.

PATIENTS AND METHODS

Patients

Fourteen female patients (age 36 ± 11 years (mean ± s.d.)) suffering from SLE, according to the ARA criteria [11], were included in the study. They suffered from active lupus disease characterized by active biopsy-proven lupus glomerulonephritis ($n = 8$; biopsy performed at the time of analysis), fever, arthritis, serositis or skin rashes. High titre anti-dsDNA IgG antibodies (1108 ± 800 U/ml (mean ± s.e.m.); range 26–11 400 U/ml; normal values <8 U/ml) were detected in the serum of all patients by radioimmunoassay (Farr assay). Patients were not treated by azathioprine, cyclosporin A or cyclophosphamide for the last 6 months before blood sampling. Eight patients were on low-dose glucocorticoid treatment (≤7·5 mg of prednisolone/day). Their data were pooled with those on untreated patients, as no differences were noted between the two groups regarding the effect of IL-12 on immunoglobulin and cytokine production. Ten female normal volunteers served as controls. Informed consent was obtained from patients and controls.

Cytokines and antibodies

Human rIL-12 produced in chinese hamster ovary (CHO) cells was kindly provided by Dr S. Wolf (Genetics Institute Inc., Cambridge, MA). It displayed a sp. act. of $5{\cdot}26 \times 10^6$ U/mg, one unit corresponding to half maximal stimulation of phytohaemagglutinin (PHA) blast proliferation. The IL-12 concentration used in our experiments (2 ng/ml) was chosen on the basis of a dose–response curve performed on PHA-stimulated human purified T cells and corresponded to a saturating dose, half-maximal proliferation of T cells being obtained with a concentration of 30 pg/ml. Human rIL-10 was produced in COS cells transfected with the human IL-10 cDNA, a gift from Dr K. W. Moore (Dnax Research Institute of Molecular and Cellular Biology, Palo Alto, CA). The biological activity of the COS cell supernatant was tested on a murine mast cell line (MC-9) and was inhibited by a specific anti-hIL-10 blocking MoAb purchased from R&D Systems Europe Ltd. (Abingdon, UK). The hIL-10 COS cell supernatant was used at a 1 : 100 dilution. In order to evaluate the concentration corresponding to this dilution, serial dilutions of crude COS cell supernatant were assayed in a classical sandwich ELISA (see *infra*) in parallel with the ELISA kit hIL-10 standard. When tested at a 1:100 dilution, absorbance was equivalent to that observed for a concentration of 6 ng/ml, as indicated by the dilution curve of the hIL-10 standard provided in the ELISA kit. Human rIFN-γ was purchased from R&D Systems and used at a 100 U/ml concentration. Its biological activity was tested on an oat-cell carcinoma line (Ludwig Institute for Cancer Research, Brussels Branch, Belgium) expressing MHC class I antigens upon IFN-γ stimulation.

Goat blocking anti-hIFN-γ polyclonal antibodies were purchased from R&D Systems and used at a concentration of 30 μg/ml.

PBMC cultures

PBMC were isolated by centrifugation on a cushion of Lymphoprep (Nycomed AS, Oslo, Norway), washed twice and frozen at −80°C. For measurements of immunoglobulin and cytokine productions, PBMC were thawed and 3×10^6 cells were cultured for 6–13 days in tissue culture plates (Nunclon Multidish plates; Nunc, Roskilde, Denmark) in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS; 56°C, 30 min). Supernatants were harvested on days 6 and 13.

Immunoglobulin measurements

Polystyrene microtitre plates (Nunc) were coated with 50 μl of anti-human Fcγ goat IgG (Tago, Camarillo, CA) diluted to 5 μg/ml in glycine-buffered saline (GBS) (50 mmol/*l*, pH 9·2) and incubated overnight at room temperature. The plates were washed with PBS–Tween (0·1%) and a 50-μl aliquot of the sample diluted with PBS–bovine serum albumin (BSA; 0·5%) was added in duplicates. After 2 h at room temperature, plates were washed and the binding of IgG antibody was quantified by addition of 50 μl peroxidase-conjugated anti-human Fcγ goat IgG (Sigma Chemical Co., St Louis, MO) diluted 1 : 1000 in Tris-casein (0·5%). Two hours later, the plates were washed and 50 μl of orthophenylene diamine (OPD; 800 mg/*l* in citrate-phosphate buffer pH 5) were added. Optical density (OD) was read at 492 nm in a spectrophotometer. A similar assay was used to measure IgM antibody, except that plates were coated with an anti-human Fcμ goat IgG (Tago) and the binding of IgM was quantified by addition of a biotin-labelled anti-human Fcμ mouse MoAb produced in our laboratory, followed by peroxidase-conjugated avidin. For the determination of IgG1, IgG2, and IgG3 subclasses, plates were coated with 50 μl of a rat anti-mouse monoclonal (m) IgG (Experimental Immunology Unit, Louvain Medical School, Belgium), diluted to 5 μg/ml in GBS, and incubated overnight at room temperature. After washing, 50 μl of a mouse MoAb (0·5 μg/ml in PBS–BSA) specific for either human IgG1 (Oxoid Ltd., Basingstoke, UK), IgG2, or IgG3 (both from Calbiochem-Novabiochem Corp., La Jolla, CA) were then added and incubated for 2 h at 4°C. Plates were washed and 50 μl of the sample were added and incubated for 2 h at room temperature. After washing, 50 μl of a peroxidase-conjugated anti-human Fcγ goat IgG (Sigma) (1 : 1000 in Tris-casein) were added, followed by washing and addition of OPD. For the evaluation of IgG4, 50 μl of an IgG3 mouse MoAb towards human IgG4 (Calbiochem-Novabiochem) (5 μg/ml in GBS) were bound directly onto the plate, followed by the sample and the detection system.

Cytokine measurements

IL-6 was measured using the 7TD1 hybridoma cell line, which is specific for the cytokine [12]. IFN-γ and IL-10 titres were measured by specific ELISA: anti-human IFN-γ MoAb (1-DIK for coating and biotinylated 7-B6-1 for detection) and anti-human IL-10 MoAb (JES3-9D7 for coating and biotinylated JES3-12G8 for detection) were purchased from Chromogenix (Mölndal, Sweden) and Pharmingen (San Diego, CA), respectively. Tumour necrosis factor (TNF) titres (TNF-α and -β) were measured as described elsewhere [13], using WEHI-164 clone 13, a mouse fibrosarcoma cell line sensitive to TNF.

Assays for counting specific anti-dsDNA antibody- and immunoglobulin-secreting cells (Elispot assays)

Nitrocellulose Milliliter HA plates (Mahans 4550; Millipore, Eschborn, Germany) were coated overnight at 4°C in a humid chamber with 100 μl of methylated (m) BSA (Boehringer, Mannheim, Germany) in PBS (100 μg/ml). Plates were then flicked dry, and 100 μl of salmon sperm dsDNA (Sigma; 100 μg/ml in PBS) were added. After overnight incubation at 4°C, plates were washed, and non-specific binding sites were blocked by a 2-h incubation at 37°C with PBS containing 1% BSA, 2% bovine milk powder, and 10% FCS. After washing, 100 μl of different concentrations of thawed PBMC suspensions (from 2×10^5 down to $2{\cdot}5 \times 10^4$) were dispensed into the coated wells. The plates were incubated in 5% CO_2 for 16 h at 37°C in the presence or absence of IL-12 (2 ng/ml).

Before being processed in the Elispot plates, PBMC were incubated in polypropylene tubes during 4 h at 37°C with or without IL-12. One aliquot was used to determine the percentage of B lymphocytes by flow cytometric analyses using an anti-CD19 MoAb. After incubation of the PBMC, plates were washed with PBS–Tween (0·25%). Biotinylated goat anti-human IgG (Southern Biotechnology, Birmingham, AL; 100 μl) diluted 1 : 750 in PBS–Tween (0·05%)–BSA (0·5%) was then added to the wells. After 3 h incubation at 37°C and after washing, extravidine horseradish peroxidase (Sigma; 2 μg/ml in PBS–Tween 0·05%) was added for 1 h. After washing, the enzyme-substrate solution (3-amino-9-ethylcarbazole; Sigma) was added. Spots appeared after a few minutes and the reaction was stopped by briefly washing the plates with tap water. Spots were counted under low magnification ($\times$40) using an inverted microscope and recorded as the number of spot-forming cells per 10^4 $CD19^+$ cells. The spot count in nitrocellulose-bottomed wells coated with mBSA, but not with dsDNA, was always <5% of the spot count obtained on dsDNA-coated wells. The same technique was used to detect the number of IgG-secreting cells, except that plates were coated with $F(ab')_2$ fragments of goat anti-human IgG γ-chains (Tago).

RESULTS

IL-12 inhibits spontaneous immunoglobulin production by lupus PBMC

We measured *in vitro* spontaneous immunoglobulin production by lupus and control PBMC and tested whether it was influenced by IL-12. As expected, IgG titres measured by ELISA were much higher in supernatants from unstimulated lupus PBMC than in those from control PBMC (mean ± s.e.m. IgG concentration on day 13, 2862 ± 721 ng/ml *versus* 243 ± 70 ng/ml). Most interestingly, IL-12 strongly inhibited IgG production by lupus PBMC (61% and 78% mean inhibition at days 6 and 13, respectively), while it had no effect on IgG production by control cells (Fig. 1). A similar inhibitory activity of IL-12 was observed on the production

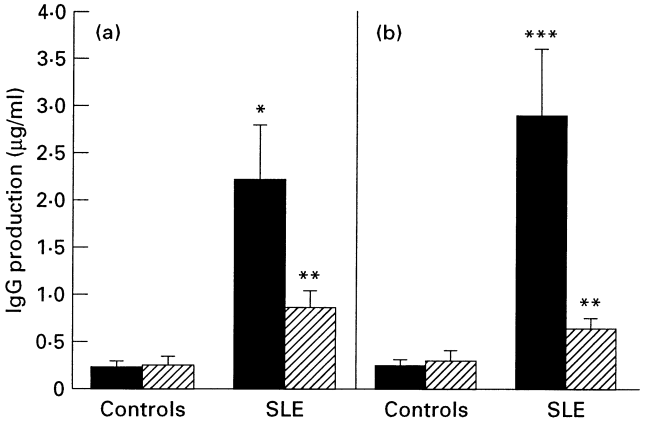

Fig. 1. Inhibitory activity of IL-12 on IgG production. PBMC from lupus patients and from controls were cultured for 6 days (a) or for 13 days (b) in the absence (■) or in the presence (▨) of IL-12. IgG titres were measured by ELISA, as described in Patients and Methods. Results are expressed as mean ± s.e.m. Five controls and 14 systemic lupus erythematosus (SLE) patients were tested on day 6, while 10 controls and nine SLE patients were tested on day 13. *$P < 0·005$ *versus* controls (Mann–Whitney *U*-test); **$P < 0·005$ *versus* no stimulation (Wilcoxon signed rank test); ***$P < 0·0005$ *versus* controls (Mann–Whitney *U*-test).

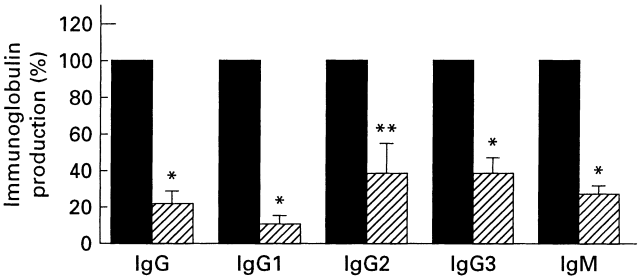

Fig. 2. Inhibitory activity of IL-12 on IgG subclasses and IgM production. PBMC from five systemic lupus erythematosus (SLE) patients were cultured for 13 days in the absence (■) or in the presence (▨) of IL-12. IgG subclasses and IgM antibodies were measured by ELISA, as described in Patients and Methods. Results are expressed as mean ± s.e.m. Mean '100%' ± s.e.m. IgG, IgG1, IgG2, IgG3 and IgM concentrations (μg/ml) were 3·3 ± 1·7, 4·9 ± 2·0, 0·7 ± 0·3, 0·2 ± 0·1, and 0·7 ± 0·2, respectively. *$P < 0·05$ *versus* no stimulation; **NS ($P = 0·068$) *versus* no stimulation; Wilcoxon signed rank test. IgG4 antibodies were not detected in supernatants.

of IgG1, IgG2, IgG3 and IgM antibodies by lupus PBMC (Fig. 2). IgG4 antibodies were not detected in supernatants. Titres of IgG subclasses measured in supernatants from control PBMC were too low to interpret an effect of IL-12.

IL-12 inhibits anti-dsDNA antibody production

We investigated whether the specific anti-dsDNA antibody response associated with SLE was also inhibited by IL-12. Attempts to detect anti-dsDNA antibody by ELISA or radioimmunoassay (RIA) in supernatants from unstimulated lupus PBMC were unsuccessful, probably because of the low levels of antibody present. Therefore, we chose another approach based on the detection of anti-dsDNA-secreting cells using a sensitive enzyme-linked immunospot (Elispot) assay. After a 4-h incubation in tubes in the presence or absence of IL-12, PBMC from SLE patients and controls were cultured for 16 h with or without IL-12 in microtitre plates coated with dsDNA. The number of anti-dsDNA antibody-secreting cells was evaluated by counting the microscopic spots resulting from the imprints of dsDNA-binding antibodies produced by individual anti-dsDNA-secreting cells and detected by immunochemical methods. Elispot assays for the detection of IgG-secreting cells in PBMC from lupus patients and controls were run in parallel on anti-human IgG-coated plates. As anticipated, anti-dsDNA-secreting cells could not be retrieved from control PBMC (data not shown). Moreover, the number of IgG-secreting cells was much lower in controls compared with lupus patients (128 ± 25 *versus* 4992 ± 1282 spots/10^4 $CD19^+$ B cells (mean ± s.e.m.); $P < 0·005$ by Mann–Whitney *U*-test). As indicated in Table 1, addition of IL-12 to lupus PBMC cultures significantly inhibited the number of anti-dsDNA- and IgG-secreting cells. No significant effect of IL-12 was observed on the number of IgG-secreting cells in control patients.

The inhibitory effect of IL-12 is independent of IFN-γ and IL-10 modulation

In order to investigate the mechanisms whereby IL-12 inhibited immunoglobulin production by lupus PBMC, we tested whether IL-12 modulated the production of certain cytokines. As indicated in Fig. 3, TNF and IL-6 concentrations measured in supernatants from lupus PBMC were not modified by addition of IL-12. By

Table 1. Inhibition of IgG and anti-dsDNA-secreting cells by IL-12

	dsDNA Elispots*			IgG Elispots*		
	IL-12			IL-12		
Patient	−	+	Inhibition (%)	−	+	Inhibition (%)
1	1142	868	24	12 402	8362	33
2	288	210	27	4146	2296	45
3	17	10	61	1410	611	57
4	1007	862	14	10 595	7126	33
5	62	36	44	2762	1520	45
6	92	81	13	8363	5945	29
7	53	19	64	1127	1328	Stim.
8	88	59	34	8963	7976	11
9	62	41	34	1825	508	72
10	34	30	12	2574	1842	28
11	60	51	15	746	679	9
Mean	264	206**	32	4992	3472***	33
S.e.m.	123	99	5	1282	958	6

Elispot assays for the detection of IgG- and anti-dsDNA-secreting cells were performed with systemic lupus erythematosus (SLE) PBMC cultured with and without IL-12, as described in Patients and Methods.

*Results are expressed as numbers of spots/10^4 $CD19^+$ B cells.

**$P < 0{\cdot}005$; ***$P < 0{\cdot}01$ *versus* without IL-12; Wilcoxon signed rank test.

Stim. Stimulatory activity.

contrast, IFN-γ titres were consistently up-regulated by IL-12, while those of IL-10 were significantly down-regulated.

We next investigated whether the inhibitory activity of IL-12 on immunoglobulin production was mediated either by up-regulation of IFN-γ production or by down-regulation of endogenous IL-10 production by lupus PBMC. We cultured lupus PBMC (i) without any additive, (ii) with IL-12, (iii) with IFN-γ, and (iv) with IL-12 in the presence of blocking anti-IFN-γ antibody. We measured IgG (Fig. 4a) and IL-10 (Fig. 4b) titres in the supernatants of the same cultures. Addition of IFN-γ to lupus PBMC cultures did not down-regulate immunoglobulin production but, as expected, reduced IL-10 production. Conversely, addition of anti-IFN-γ blocking antibody to PBMC stimulated with IL-12 did not suppress the inhibition exerted by IL-12 on immunoglobulin synthesis, but restored the production of IL-10. Taken together, these results indicate that: (i) IFN-γ did not mediate the inhibitory effects of IL-12 on immunoglobulin production; (ii) IFN-γ mediated the inhibition exerted by IL-12 on endogenous IL-10 production; and (iii) down-regulation of endogenous IL-10 production by IL-12 did not explain the inhibition exerted by IL-12 on immunoglobulin production. Two other lines of evidence further supported the latter conclusion. First, we did not find a positive correlation between the individual rates of IL-10 and immunoglobulin down-regulation by IL-12 ($r = 0{\cdot}04$; data not shown). Second, IL-12 still displayed its inhibitory activity in the presence of exogenously added IL-10. As indicated in Fig. 5, addition of IL-10 to lupus PBMC increased their IgG production, but even under these experimental conditions—namely with IL-10 concentrations 20 times higher than those measured in unstimulated SLE PBMC supernatants—immunoglobulin production could be down-regulated by addition of IL-12.

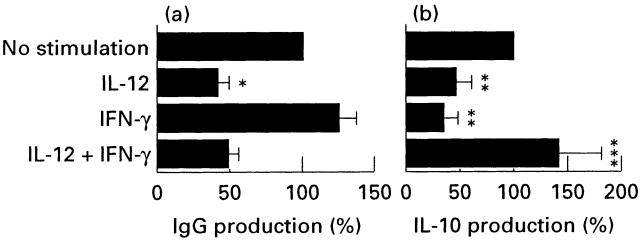

Fig. 4. Effect of IFN-γ and anti-IFN-γ antibodies on immunoglobulin and IL-10 production. PBMC from seven systemic lupus erythematosus (SLE) patients were cultured for 6 days with the indicated additives, at concentrations described in Patients and Methods. IgG (a) and IL-10 (b) production was measured by ELISA. Results are expressed as mean ± s.e.m. Mean '100%' ± s.e.m. IgG and IL-10 concentrations were $1{\cdot}3 \pm 0{\cdot}3$ μg/ml and $0{\cdot}7 \pm 0{\cdot}25$ ng/ml, respectively. *$P < 0{\cdot}05$ *versus* no stimulation; **$P < 0{\cdot}05$ *versus* no stimulation; ***$P < 0{\cdot}05$ *versus* IL-12; Wilcoxon signed rank test.

DISCUSSION

The data presented here demonstrate that: (i) IL-12 strongly inhibits *in vitro* IgG and IgM production by lupus PBMC; (ii) the specific anti-dsDNA response characteristic of SLE is also

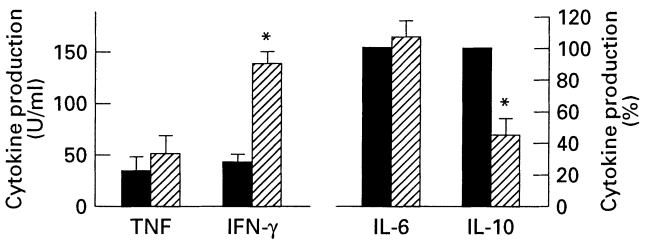

Fig. 3. Cytokine regulation by IL-12. PBMC from systemic lupus erythematosus (SLE) patients were cultured for 6 days in the absence (■) or in the presence (▨) of IL-12. Titres of tumour necrosis factor (TNF; $n = 11$), IFN-γ ($n = 13$), IL-6 ($n = 14$) and IL-10 ($n = 13$) were measured as described in Patients and Methods. Results are expressed as mean ± s.e.m. *$P < 0{\cdot}0005$ *versus* no stimulation; Wilcoxon signed rank test. Maximal mean (± s.e.m.) IL-6 and IL-10 concentrations were $10{\cdot}4 \pm 2{\cdot}0$ ng/ml and 385 ± 152 pg/ml, respectively.

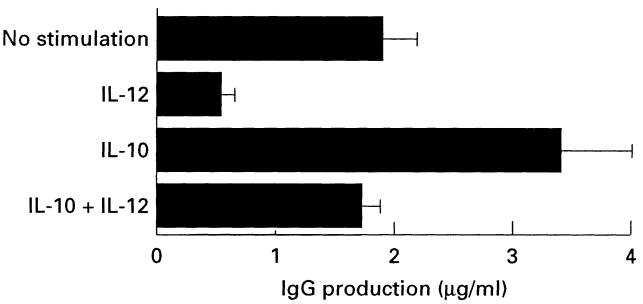

Fig. 5. Inhibition by IL-12 of IL-10-induced immunoglobulin production. PBMC from three systemic lupus erythematosus (SLE) patients were cultured for 13 days with the indicated additives, at concentrations described in Patients and Methods. IgG production was measured by ELISA. Results are expressed as mean ± s.e.m.

down-regulated by IL-12; and (iii) the inhibition exerted by IL-12 on immunoglobulin production by lupus PBMC is independent of IFN-γ up-regulation and IL-10 down-regulation.

The pivotal role played by IL-12 in promoting cell-mediated immunity is well established. Thus, IL-12 triggers the development of Th1-type cells *in vitro* [14,15] and *in vivo* [16], stimulates T and natural killer (NK) cell proliferation, induces IFN-γ production and enhances cytotoxic T lymphocyte (CTL) differentiation [10]. These properties explain why cell-mediated autoimmune diseases such as insulin-dependent diabetes mellitus in NOD mice [17], collagen-induced arthritis [18], and experimental allergic encephalomyelitis [19] are aggravated by *in vivo* treatment with IL-12. On the other hand, it was recently reported that IL-12 inhibited *in vivo* humoral immune responses. Thus, IL-12 was found to suppress the polyclonal immunoglobulin response induced in mice by treatment with anti-IgD antibody [7]. Similarly, the specific IgG1 antibody response against antigens was shown to be inhibited by IL-12 in different murine immunization models [5,8,9]. In one such model, namely peritoneal immunization with phosphorylcholine conjugated with keyhole limpet haemocyanin (KLH) or with Freund's complete adjuvant (FCA), *in vivo* treatment with IL-12 led to the disappearance of peritoneal B1 lymphocytes, which are purportedly involved in autoimmunity. Moreover, addition of IL-12 to peritoneal lymphocytes cultured *in vitro* significantly inhibited their proliferation in response to IL-5 [5]. Finally, serum immunoglobulin and anti-DNA titres in 'parent into F_1' mice suffering from chronic GVHD were strongly inhibited by *in vivo* IL-12 treatment [6]. While this effect may be essentially due to stimulation of a host-reactive CTL response (acute GVHD) that rejects the host immune system, the data also suggest that lower doses of IL-12 are capable of inhibiting B cell responses without inducing an acute GVHD [6].

Our results, demonstrating an inhibitory activity of IL-12 on *in vitro* immunoglobulin production by lupus PBMC, are in line with these experiments, but contrast with the recently reported stimulating effects of IL-12 on normal human B cells. Thus, the proliferation and immunoglobulin production of Staphylococcus A Cowan (SAC)-stimulated human B cells in response to IL-2 were found to be further up-regulated by IL-12, in an IFN-γ-independent manner [20]. Interestingly, IL-12 had no effects on its own but synergized with IL-2, possibly by increasing CD25 expression. Although these results might appear inconsistent with those reported here, it should be stressed that the experimental systems differed in many ways, in particular the cells studied (normal *versus* diseased cells; B cells *versus* PBMC) and their state of activation (*in vitro* stimulation with SAC *versus ex vivo* unstimulated cultures).

The mechanisms whereby IL-12 inhibits immunoglobulin production by lupus PBMC could be only partially addressed in our study design, in particular because identification of the cellular subset(s) responding to IL-12 required amounts of PBMC that could not be obtained from critically ill patients. The reported absence of IL-12R on human resting and SAC-stimulated human B cells [21] suggests that the inhibitory effect exerted by IL-12 on immunoglobulin production is indirect, although the possibility that lupus B cells express IL-12R *in vivo* should not be ruled out. On the other hand, our data indicate that IFN-γ, the cytokine whereby T cells usually mediate the effects of IL-12 *in vivo* [7,9,17,18], is not implicated in the inhibition exerted by IL-12 on lupus B lymphocytes. Finally, the critical role played by IL-10 in SLE B cell activation [1,3] prompted us to test the possibility that the negative effects of IL-12 on immunoglobulin production were due to down-regulation of endogenous IL-10 production, the more so as IL-10 production by $CD4^+$ allergen-specific human T cells was shown to be inhibited by IL-12 [22]. Interestingly, IL-10 production was significantly down-regulated in lupus PBMC cultures stimulated with IL-12, an effect that was attributed to IFN-γ up-regulation by IL-12. However, our data strongly argue against a causal relationship between IL-10 inhibition and immunoglobulin down-regulation, the best evidence being that anti-IFN-γ antibody restored IL-10 production without suppressing the inhibitory activity of IL-12 on immunoglobulin production, and that IL-12 still displayed its inhibitory activity in the presence of exogenously added IL-10.

Taken together, our results indicate that modulation of excessive immunoglobulin production by lupus PBMC can be achieved *in vitro* by IL-12. The relevance of our data is further strengthened by the recently reported impaired IL-12 production by stimulated lupus lymphocytes and monocytes [23], thereby suggesting that treatment with IL-12 might be beneficial to SLE patients. In this respect, our observation that IL-12 inhibits immunoglobulin production independently of IL-10 modulation suggests that treatment with IL-12 could offer a second and independent level of blockade against the consequences of the overt B cell activation associated with SLE.

ACKNOWLEDGMENTS

The authors are most grateful to Alain Crusiaux for performing the ELISA, to Geneviève Depresseux for the statistical analyses, and to the Fonds National de la Recherche Scientifique and the Belgian Lupus Patient Aid Group ('Association Lupus Erythémateux') for providing financial support. The authors are indebted to Dr S. Wolf (Genetics Institute Inc., Cambridge, MA) and to Dr K.W. Moore (Dnax Research Institute of Molecular and Cellular Biology, Palo Alto, CA) for kindly providing human rIL-12 and the human IL-10 cDNA, respectively. J.-C.R. is research associate with the Fonds National de la Recherche Scientifique, Belgium.

REFERENCES

1 Llorente L, Richaud-Patin Y, Wijdenes J *et al.* Spontaneous production of interleukin-10 by B lymphocytes and monocytes in systemic lupus erythematosus. Eur Cytokine Netw 1993; **4**:421–30.

2 Houssiau FA, Lefebvre C, Vanden Berghe M *et al.* Serum interleukin 10 titers in systemic lupus erythematosus reflect disease activity. Lupus 1995; **4**:393–5.

3 Llorente L, Zou W, Levy Y *et al.* Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. J Exp Med 1995; **181**:839–44.

4 Ishida H, Muchamuel T, Sakaguchi S *et al.* Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F_1 mice. J Exp Med 1994; **179**:305–10.

5 Vogel LA, Lester TL, Van Cleave VH *et al.* Inhibition of murine B1 lymphocytes by interleukin-12. Eur J Immunol 1996; **26**:219–23.

6 Via CS, Rus V, Gately MK *et al.* IL-12 stimulates the development of acute graft-vs.-host disease in mice that normally would develop chronic, autoimmune graft-vs.-host disease. J Immunol 1994; **153**:4040–7.

7 Morris SC, Madden KB, Adamovicz JJ *et al.* Effects of IL-12 on *in vivo* cytokine gene expression and Ig isotype selection. J Immunol 1994; **152**:1047–56.

8 McKnight AJ, Zimmer GJ, Fogelman I *et al.* Effects of IL-12 on helper T cell-dependent immune responses *in vivo*. J Immunol 1994; **152**:2172–9.

9 Gracie JA, Bradley JA. Interleukin-12 induces interferon-γ-dependent switching of IgG alloantibody subclass. Eur J Immunol 1996; **26**:1217–21.

10 Trinchieri G. Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. Blood 1994; **84**:4008–27.
11 Tan EM, Cohen AS, Fries JF *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982; **25**:1271–7.
12 Van Snick J, Cayphas S, Vink A *et al.* Purification and NH_2-terminal amino acid sequence of a T-cell-derived lymphokine with growth factor activity for B-cell hybridomas. Proc Natl Acad Sci USA 1986; **83**:9679–83.
13 Traversari C, van der Bruggen P, Van den Eynde B *et al.* Transfection and expression of a gene coding for a human melanoma antigen recognized by autologous cytolytic T lymphocytes. Immunogenetics 1992; **35**:145–52.
14 Manetti R, Parronchi P, Giudizi MG *et al.* Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. J Exp Med 1993; **177**:1199–204.
15 Hsieh CS, Macatonia SE, Tripp CS *et al.* Development of T_H1 $CD4^+$ T cells through IL-12 produced by *Listeria*-induced macrophages. Science 1993; **260**:547–9.
16 Afonso LCC, Scharton TM, Vieira LQ *et al.* The adjuvant effect of interleukin-12 in a vaccine against *Leishmania major*. Science 1994; **263**:235–7.
17 Trembleau S, Penna G, Bosi E *et al.* Interleukin 12 administration induces T helper type 1 cells and accelerates autoimmune diabetes in NOD mice. J Exp Med 1995; **181**:817–21.
18 Germann T, Szeliga J, Hess H *et al.* Administration of interleukin 12 in combination with type II collagen induces severe arthritis in DBA/1 mice. Proc Natl Acad Sci USA 1995; **92**:4823–7.
19 Leonard JP, Waldburger KE, Goldman SJ. Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12. J Exp Med 1995; **181**:381–6.
20 Jelinek DF, Braaten JK. Role of IL-12 in human B lymphocyte proliferation and differentiation. J Immunol 1995; **154**:1606–13.
21 Desai BB, Quinn PM, Wolitzky AG *et al.* IL-12 receptor. II. Distribution and regulation of receptor expression. J Immunol 1992; **148**:3125–32.
22 Marshall JD, Secrist H, DeKruyff RH *et al.* IL-12 inhibits the production of IL-4 and IL-10 in allergen-specific human $CD4^+$ T lymphocytes. J Immunol 1995; **155**:111–7.
23 Horwitz DA, Rengaraju M, Behrendsen SC *et al.* Decreased interleukin-12 production by mitogen-stimulated lymphocytes and monocytes in untreated patients with SLE. Arthritis Rheum 1995; **38**:S212 (abstr.).