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Immunosuppressive activity of 15-deoxyspergualin on normal and autoimmune peripheral blood mononuclear cells

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Abstract

Several experimental conditions were used in this study to evaluate the in vitro effects of 15-deoxyspergualin on the function of T lymphocytes, B lymphocytes and monocytes from healthy subjects and patients suffering from systemic lupus erythematosus. Whilst the secretion of polyclonal immunoglobulin (Ig) M and IgG from the B lymphocytes of the healthy subjects was diminished by 15-deoxyspergualin, neither the proliferative response of normal T and B cells to mitogenic stimulation nor the cytokine secretory capacity of these cells (e.g. interleukin-2, -4, -6 and γ-interferon) and monocytes (e.g. interleukin-1β and -6) were affected by the drug. In contrast, on the mononuclear cells obtained from the lupus patients not only did 15-deoxyspergualin inhibit the spontaneous production of polyclonal and anti-DNA IgG antibodies but also suppressed interleukin-1β secretion from the monocytes. Other functional responses of T and B cells and monocytes from lupus patients, including mitogenic activation and cytokine secretion, were not altered by the drug. These data suggest that 15-deoxyspergualin possesses a novel mechanism of pharmacological immunosuppression apparently different from that of other immunosuppressants, such as cyclosporin A, FK506 and corticosteroids, that seems to be primarily displayed at the level of autoreactive B cells and monocytes.

Keywords: 15-Deoxyspergualin; T cell; B cell; Monocyte; Cytokine

1. Introduction

15-Deoxyspergualin is a synthetic derivative of spergualin, an antibiotic with antitumor properties produced by *Bacillus laterosporus* (Takeuchi et al., 1981). Several studies conducted during the last years by ourselves and others provided clear evidence for the powerful immunosuppressive properties of 15-deoxyspergualin in experimental models of auto-, allo- and xenoimmunity (Takagishi et al., 1990; Ito et al., 1990; Mochizuku and Kawashima, 1990; Nicoletti et al., 1992, 1993, 1994; Starndler and Sandler, 1992; Lan et al., 1994; Jung et al., 1994; Reichenspurner et al., 1990; Nemoto et al., 1991). In agreement with these experimental observations, clinical studies in humans have demonstrated that 15-deoxyspergualin reverses kidney (Takahashi et al., 1990; Amemiya et al., 1991) and liver (Groth et al., 1990) allograft rejection. In a similar manner, short course treatment of type-1 diabetic patients with 15-deoxyspergualin suppresses rejection of transplanted pancreatic islets for greater than 9 months (Gores et al., 1993). However, in spite of these numerous studies, the precise mechanism of action of 15-deoxyspergualin is unsettled. Although it has been recently demonstrated that 15-deoxyspergualin binds to heat-shock cognate 70 of the human heat-shock protein family (Nadler et al., 1993), neither the cellular events which follow this interaction nor the cellular type targeted by the drug have been clearly identified. Some studies suggest that 15-deoxyspergualin interferes with the generation and differentiation of cyto-

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toxic T cells (Tepper et al., 1993; Nishimura and Tokunaga, 1989) and production of antibodies by B cells (Fujii et al., 1990); however, the action of 15-deoxyspergualin on T cells is controversial and inhibition of T cell proliferation or cytokine secretion has been reported in some (Nicoletti et al., 1993; Lan et al., 1994;Fujii et al., 1989) but denayed in other studies (Jiang et al., 1990; Yuh and Morris, 1993). While some of these controversial data may depend on methodological and technical differences, the susceptibility of 15-deoxyspergualin to in vitro degradation and generation of toxic compounds in the presence of serum (which hydrolizes 15-deoxyspergualin to toxic aldehydes) may have further confounded the elucidation of the precise mechanism of action of 15-deoxyspergualin. Moreover, other Authors suggest that the suppressive effect exhibited by 15-deoxyspergualin in vivo may be mediated through a specific and unique mechanism of action of the drug on monocytes/macrophages. In particular, this would include inhibition of superoxide radicals and lysosomal enzymes (Dickneite et al., 1987), expression of major histocompatibility class II antigens (Waaga et al., 1990) and secretion of interleukin-1 (Nemoto et al., 1987; Takasu et al., 1991). More recently, it has also been demonstrated that 15-deoxyspergualin interferes with antigen processing and/or presentation by monocytes (Hoeger et al., 1994).

These observations prompted us to perform further in vitro studies aimed at the better understanding of the immunosuppressive action of 15-deoxyspergualin. To prove this we presently evaluated the effects of 15-deoxyspergualin on circulating T cells, B cells and monocytes from healthy subjects and from lupus patients using different experimental conditions. Results demonstrate that 15-deoxyspergualin exhibit pleiotropic effects on the immune system, modulating antibody production from normal and lupus patients' B cells and specifically down-regulating interleukin-1β secretion by autoreactive lupus monocytes.

2. Materials and methods

2.1. Reagents

Ficoll-Isopaque (Lymphoprep) was provided from Nycomed (Oslo, Norway), phytohaemagglutinin, pokeweed mitogen and RPMI 1640 from Gibco-Life Technologies (Paisley, UK), anti-CD3 mAb (MCA 203) from Serotec (Oxford, UK), fetal calf serum from ICN (Costa Mesa, CA, USA), [^{3}H]thymidine from Amersham International (Buckingamshire, UK), phorbol-12-myristate-13-acetate and aminoguanidine from Sigma (St. Louis, MO, USA). The solid-phase ELISA kits for the detection of interleukin-1β, -2, -4 and -6 were provided from R&D Systems, (Minneapolis, MN, USA) and that for γ-interferon from Holland Biotechnology (Leiden, The Netherlands); they were used according to the manufacturer's recommendations.

2.2. Drug

15-Deoxyspergualin, provided by Behringwerke (Marburg, Germany), was dissolved in sterile distilled water at the concentration of 10 mg/ml and stored at −20°C for no longer than 5 days prior to use. On the basis of in vivo pharmacokinetic studies (Ohlman et al., 1994), we selected the doses of 10, 20 and 50 μg/ml for our experiments. In standard experimental conditions, the stock solution was diluted at the final concentrations tested in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 1% L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin.

2.3. Experimental design

The activity of 15-deoxyspergualin on human immune functions was studied in vitro on peripheral blood mononuclear cells obtained either from 13 healthy adult subjects or from 10 patients suffering from systemic lupus erythematosus diagnosed according to the American Rheumatology Association criteria (Tan et al., 1982) and selected as having active disease according to the European Consensus Lupus Activity Measurement (Vitali et al., 1992). The following parameters were investigated: (a) proliferative response by resting or mitogen-activated peripheral blood mononuclear cells; (b) polyclonal and anti-DNA immunoglobulin (Ig) M and IgG secretion by unstimulated or pokeweed mitogen-stimulated cells; and (c) cytokine production by activated or resting peripheral blood mononuclear cells. In the standard procedure, 15-deoxyspergualin was added for the whole culture period at the concentration of 10, 20 or 50 μg/ml. Moreover, to test the hypothesis that 15-deoxyspergualin may preferentially act on activated peripheral blood mononuclear cells, selected experiments were performed where the drug was added after a lag time from the beginning of the culture, thus allowing us to evaluate the effects of the drug both at the time of antigenic challenge and during ongoing immune responses. On the basis of kinetic studies carried out in our laboratory, the time of drug addition after the start of the cultures varied in the different tests, being 8–10 h for cytokine production, 24 h for cell proliferation and 72 h for Ig production.

2.4. Cell isolation and culture conditions

Peripheral blood mononuclear cells were obtained from heparinized venous blood by Ficoll-Isopaque density gradient centrifugation according to Boyum (1968) and resuspended in complete medium (RPMI 1640 plus 10% fetal calf serum, 1% L-glutamine and antibiotics).

2.5. Proliferation assays

Peripheral blood mononuclear cells were plated at the concentration of 10^5/well and stimulated by either phytohaemagglutinin (4, 1 and 0.25 μg/ml), anti-CD3 mAb (25, 5 and 1 ng/ml) or pokeweed mitogen (1% final dilution). Cell proliferation either in the absence or in the presence of different concentrations of 15-deoxyspergualin was assessed by [^{3}H]thymidine incorporation after 72-h cultures, as described (Meroni et al., 1982). Cell viability was evaluated by Trypan blue exclusion test.

2.6. Polyclonal Ig secretion

For in vitro polyclonal IgM and IgG synthesis, 10^6 peripheral blood mononuclear cells were cultured for 8 days in complete medium in a final volume of 1 ml, with or without pokeweed mitogen 1%, in the presence or absence of 15-deoxyspergualin (10, 20 or 50 μg/ml). To evaluate the role of serum-derived polyamine oxidase, in some cultures the enzyme was blocked by addition to the culture medium of 1mM aminoguanidine. Polyclonal Ig levels were determined by ELISA as previously reported (Meroni et al., 1987).

2.7. Anti-DNA IgM or IgG antibodies

The production of anti-DNA IgM or IgG antibodies in lupus peripheral blood mononuclear cell cultures was evidenced by a solid-phase ELISA (Barcellini et al., 1992) using ds-DNA coated plates; a reference curve was obtained with an anti-DNA antibody-positive serum (previously selected by using Farr technique).

2.8. In vitro cytokine production

Interleukin-1β and -6 production was measured in culture supernatants of adherent mononuclear cells. Isolation of monocytes from peripheral blood mononuclear cells often results in a population contaminated with other cells and different separation techniques can influnce morphological, biochemical and functional characteristics of this cell population. For instance, adherence itself may be an activating signal for monocytes and renders them temporarily refractory to other regulatory signals (Kurt-Jones et al., 1986). Moreover, isolation of mononuclear cells from lupus patients usually yields lower numbers of cells than healthy subjects. For these reasons, we used different culture conditions for cells from control subjects, in order to define the most suitable protocol for studies in lupus patients: (1) 10^6 peripheral blood mononuclear cells/sample were cultured in 75 × 12 mm tubes in serum-free medium for 24 h, with or without 20 ng/ml phorbol-12-myristate-13-acetate in a final volume of 1 ml; (2) monocytes, isolated by plastic adherence (90% pure population with B lymphocytes contaminating) in 24-well flat-bottomed plates (Sozzani et al., 1988) were stimulated with phorbol-12-myristate-13-acetate or left unstimulated for 24 h in serum-free medium; (3) monocytes were fractionated from peripheral blood mononuclear cells using Percoll 46% (285 mOs) density gradient centrifugation; as determined by cytofluorimetric analysis, the purity of the obtained population varied from 70 to 85% and most of the contaminating cells were lymphocytes. Monocytes were seeded in 24-well flat-bottomed plates at the concentration of 10^6/ml in serum-free medium; 15-deoxyspergualin was added to unstimulated and phorbol-12-myristate-13-acetate-stimulated cultures and plates were incubated for 24 h at 37°C with 5% CO_2. Thereafter, regardless of the experimental condition considered, supernatants were collected at the end of the culture period, centrifuged at 2500 rpm for 15 min, filtered (0.22 μm) and stored at −20°C until cytokine dosage.

To measure the levels of interleukin-2, -4, -6 and γ-interferon, peripheral blood mononuclear cells (10^6/ml) were cultured either with or without phytohaemagglutinin (4 μg/ml) in the presence or absence of the different concentrations of the drug for 48 h, both in 10% fetal calf serum and serum-free medium.

The amounts of the different cytokines were determined in culture supernatants by the above indicated solid-phase ELISA kits.

2.9. Statistical analysis

Statistical evaluation of the results was performed by using Student's t-test for paired data.

3. Results

3.1. 15-Deoxyspergualin does not affect mitogen activation of normal T lymphocytes

The effect of 15-deoxyspergualin on in vitro mitogen-induced activation of T lymphocytes was evaluated in peripheral blood mononuclear cells obtained from healthy donors and stimulated by either phytohaemagglutinin (4, 1 and 0.25 μg/ml), anti-CD3 mAb (25, 5 and 1 ng/ml) or pokeweed mitogen (1% final dilution), either in the absence or in the presence of different concentrations of 15-deoxyspergualin. The drug does not affect proliferation induced either by optimal or suboptimal concentrations of the stimuli (data not shown).

Comparable results were observed by adding 15-deoxyspergualin after cell preactivation or blocking polyamine oxidase present in fetal calf serum-containing culture media with the enzyme inhibitor aminoguanidine 1 mM (data not shown).

Table 1
15-Deoxyspergualin activity on polyclonal immunoglobulin secretion by normal peripheral blood mononuclear cells

	IgM	IgG
Resting cells		
Medium	288 ± 375 [a]	194 ± 290
+ 15-Deoxyspergualin 10 μg/ml	175 ± 146	174 ± 269
+ 15-Deoxyspergualin 20 μg/ml	172 ± 231	156 ± 250
+ 15-Deoxyspergualin 50 μg/ml	162 ± 227 [b]	151 ± 224
Stimulated cells		
Pokeweed 1%	3541 ± 3506	1206 ± 1134
+ 15-Deoxyspergualin 10 μg/ml	2801 ± 3598	875 ± 784
+ 15-Deoxyspergualin 20 μg/ml	2991 ± 4106	763 ± 692 [b]
+ 15-Deoxyspergualin 50 μg/ml	2782 ± 3243	750 ± 651 [b]

[a] Values are expressed as ng/ml, mean ± S.D., of 11 healthy controls.
[b] $P < 0.05$ (Student's t-test).

Table 3
15-Deoxyspergualin activity on cytokines secretion by activated normal peripheral blood mononuclear cells

	15-Deoxyspergualin (μg/ml)			
	–	10	20	50
Phytohaemagglutinin-induced [a]				
Interleukin-2	510 ± 322 [b]	408 ± 249	433 ± 291	457 ± 303
γ-Interferon	2878 ± 2796	2747 ± 2492	2420 ± 2438	2557 ± 2774
Interleukin-4	21 ± 15	27 ± 20	28 ± 20	29 ± 23
Interleukin-6	8487 ± 722	8757 ± 877	8877 ± 966	9242 ± 1140
Phorbol-12-myristate-13-acetate-induced				
Interleukin-6	851 ± 682	1029 ± 753	1021 ± 782	942 ± 690

[a] Background values of cytokine production are: interleukin-2, 3.8 ± 3.2; γ-interferon, not detectable (lower than kit minimum sensitivity limit); interleukin-4, 0.8 ± 0.9; and interleukin-6, 515 ± 622.
[b] Values are expressed as pg/ml, mean ± S.D., of 10 healthy controls.

3.2. 15-Deoxyspergualin decreases polyclonal Ig secretion by normal peripheral blood mononuclear cells

The effect of 15-deoxyspergualin on in vitro polyclonal IgM and IgG synthesis is shown in Table 1. The presence of the drug during the whole culture period induces a slight dose-dependent reduction of polyclonal IgM and IgG production by resting peripheral blood mononuclear cells. Even if a statistical significance is reached for IgM levels with the highest dose of 15-deoxyspergualin, the difference between values in the absence or in the presence of the drug seems not to be biologically relevant. A more evident decrease of Ig content is seen in pokeweed mitogen-stimulated cultures and the effect acquires statistical significance for IgG secretion in the presence of 20 and 50 μg/ml 15-deoxyspergualin. No significant difference in the suppressive effects of 15-deoxyspergualin could be found when aminoguanidine was added to the cultures (data not shown).

3.3. 15-Deoxyspergualin does not interfere with the cytokine secretory capacity of normal peripheral blood mononuclear cells in vitro

To evaluate whether the inhibitory action of 15-deoxyspergualin on B lymphocytes could be mediated by an action of the drug on monocyte and/or T cell-derived cytokines, we examined the ability of the drug to modulate cytokine secretory capacity by both adherent and non-adherent mononuclear cells from the healthy subjects. As shown in Tables 2 and 3, respectively, 15-deoxyspergualin did neither modify interleukin-1β secretion by phorbol-12-myristate-13-acetate-activated monocytes in any of the different culture conditions, nor it affected mitogen-induced secretion of interleukin-2, -4, -6 and γ-interferon in standard cultures. A comparable lack of drug effect was obtained in serum-free cultures (data not shown). The different concentrations of 15-deoxyspergualin were equally uneffective in modulating cytokine secretion from unstimulated cultures, as it was the addition of 15-deoxyspergualin after cell preactivation (data not shown).

3.4. 15-Deoxyspergualin inhibits B lymphocytes and monocytes from lupus patients in vitro

In agreement with the results obtained in normal peripheral blood mononuclear cells, 15-deoxyspergualin neither influenced [^{3}H]thymidine uptake of lupus peripheral blood mononuclear cells regardless of whether resting or mitogen-activated (phytohaemagglutinin, anti-CD3 mAb, pokeweed mitogen) T and B cells were considered (data not

Table 2
15-Deoxyspergualin effect on phorbol-12-myristate-13-acetate-induced interleukin-1ß production by adherent mononuclear cells in different culture conditions

	Unfractionated cells [a]	Adherent monocytes [b]	Density isolated monocytes [c]
Phorbol-12-myristate-13-acetate	527 ± 281 [d]	557 ± 314	595 ± 322
+ 15-Deoxyspergualin 10 μg/ml	463 ± 223	492 ± 290	580 ± 304
+ 15-Deoxyspergualin 20 μg/ml	471 ± 194	396 ± 300	576 ± 267
+ 15-Deoxyspergualin 50 μg/ml	484 ± 262	412 ± 206	600 ± 246

[a] Whole peripheral blood mononuclear cell population.
[b] Monocytes isolated by plastic adhesion.
[c] Monocyte fraction obtained by Percoll gradient.
[d] Values are expressed as pg/ml, mean ± S.D., of 6 healthy subjects.

shown). As previously reported by others, lupus B cells showed a spontaneous production of polyclonal IgG higher than that induced by pokeweed mitogen, and this phenomenon was reduced in a clear dose-dependent fashion by 15-deoxyspergualin (Fig. 1A). Although, to a lesser extent, the inhibitory effect was also observed when 15-deoxyspergualin was added after a lag time from the beginning of the culture. 15-Deoxyspergualin also exerts a suppressive activity on spontaneous polyclonal IgM secretion, though basal values were lower than IgG, as well as on pokeweed mitogen-induced IgM and IgG production, independently of the different experimental conditions (data not shown). We also tested the effects of 15-deoxyspergualin on the in vitro production of anti-DNA antibodies by lupus B cells. As shown in Fig. 1B, a substantial decrease in the spontaneous production of anti-DNA IgG was achieved both when 15-deoxyspergualin was present during the whole culture period and in the preactivation model. No clear effect is found on spontaneous IgM or pokeweed mitogen-induced anti-DNA Ig even though a trend toward reduction could be observed (data not shown).

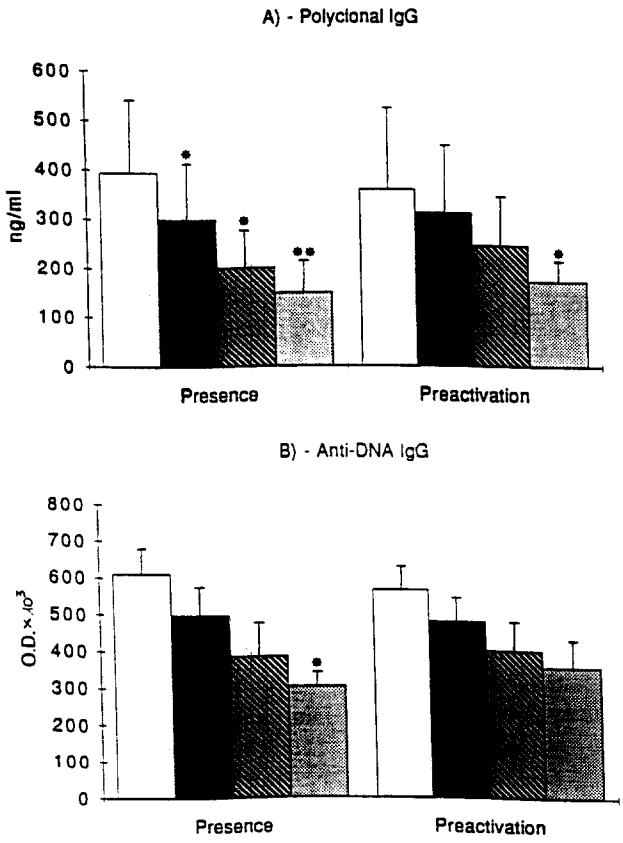

Fig. 1. (A) Spontaneous polyclonal IgG production by lupus peripheral blood mononuclear cells in the presence of 15-deoxyspergualin (presence) or after addition of the drug on preactivated cells (preactivation). Results are expressed as ng/ml, mean ± S.E., of 10 lupus patients. (B) Anti-DNA IgG production by resting PBM from SLE subjects, in the presence of different concentrations of DSG or by adding the drug in the preactivation model. Results are expressed as O.D., mean ± S.E., of 10 lupus patients. White bar, medium; dark grey bar, 15-deoxyspergualin 10 μg/ml; hatched bar, 15-deoxyspergualin 20 μg/ml; pale grey bar, 15-deoxyspergualin 50 μg/ml. * $P < 0.05$; ** $P < 0.005$ (Student's *t*-test for paired data).

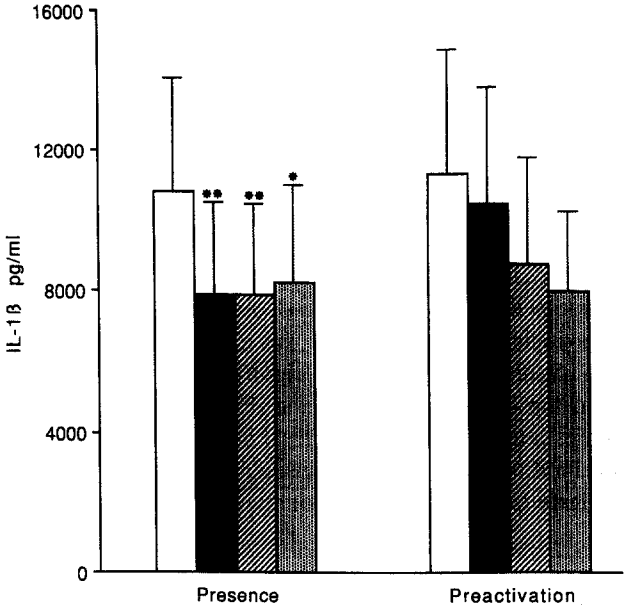

Fig. 2. 15-Deoxyspergualin effect on interleukin-1ß secretion by phorbol-12-myristate-13-acetate (20 ng/ml)-stimulated adherent mononuclear cells from lupus patients. *Presence*: 15-deoxyspergualin at the different concentrations tested was present during the whole culture period. *Preactivation*: the different doses of 15-deoxyspergualin were added to the cells after a lag time from the beginning of the culture. White bar, medium; dark grey bar, 15-deoxyspergualin 10 μg/ml; hatched bar, 15-deoxyspergualin 20 μg/ml; pale grey bar, 15-deoxyspergualin 50 μg/ml. Results are presented as pg/ml, mean ± S.E., of 10 lupus patients. * $P < 0.01$; ** $P < 0.005$ (Student's *t*-test for paired data).

Concerning cytokine secretion, lupus patients turned out to produce significantly lower amounts of Th1-derived cytokines, such as interleukin-2 and γ-interferon, and larger amounts of monocyte-derived interleukin-1β and -6 than did normal individuals; this finding accords with the well-known imbalance of cytokine secretion observed both in murine and human systemic lupus erythematosus (Tables 2

Table 4
15-Deoxyspergualin activity on cytokine secretion by activated lupus peripheral blood mononuclear cells

	15-Deoxyspergualin (μg/ml)			
	–	10	20	50
Phytohaemagglutinin-induced [a]				
Interleukin-2	116 ± 97 [b]	90 ± 70	106 ± 84	105 ± 89
γ-Interferon	907 ± 680	892 ± 630	987 ± 786	933 ± 743
Interleukin-4	25 ± 42	24 ± 41	28 ± 48	25 ± 37
Interleukin-6	8346 ± 2209	8471 ± 2305	8580 ± 1653	8851 ± 2126
Phorbol-12-myristate-13-acetate-induced				
Interleukin-6	2825 ± 2665	3037 ± 2990	2700 ± 3266	2762 ± 3276

[a] Background values of cytokine production are: interleukin-2 and γ-interferon, not detectable (lower than kit minimum sensitivity limit); interleukin-4, 0.2 ± 0.4; and interleukin-6, 1363 ± 993.
[b] Values are expressed as pg/ml, mean ± S.D., of 7 lupus patients.

and 3, Fig. 2; for a review, see Via and Handwerger, 1992). However, as it was previously observed with peripheral blood mononuclear cells from healthy individuals, 15-deoxyspergualin did not affect mitogen-induced interleukin-2, -4, -6 and γ-interferon secretion by lupus peripheral blood mononuclear cells at any of the doses and experimental conditions considered (Table 4). In contrast, 15-deoxyspergualin exerted a strong suppressive activity on interleukin-1β secretion by phorbol-12-myristate-13-acetate-activated unfractionated lupus peripheral blood mononuclear cells. The effect was evident in all the conditions tested, with statistical significance being achieved when the different doses of the drug are present for the whole culture period (Fig. 2). 15-Deoxyspergualin does not modify spontaneous interleukin-1β, -2, -4, -6 and γ-interferon secretion (data not shown).

4. Discussion

The immunosuppressive properties of 15-deoxyspergualin were studied in vitro under several experimental conditions allowing us to investigate the action of this drug on peripheral T cells, B cells and monocytes obtained from both normal individuals and selected lupus patients. 15-Deoxyspergualin neither interferred with the mitogenic activation nor with the cytokine secretory capacity of T cells from both normal subjects and lupus patients. Neither the capacity of monocytes from the healthy subjects to secrete interleukin-1β and -6 was inhibited by 15-deoxyspergualin, whilst on the normal B cells the drug reduced in a dose-dependent fashion the spontaneous polyclonal IgM secretion and, more evidently, the pokeweed mitogen-induced secretion of IgG. The main outcome of the study was the ability with which 15-deoxyspergualin acted on autoimmune B cells and monocytes obtained from lupus patients, where it reduced the spontaneous production of polyclonal and anti-DNA IgG and inhibited the phorbol-12-myristate-13-acetate-induced interleukin-1β secretion.

Our study confirms the inhibitory activity of 15-deoxyspergualin on antibody production from human B lymphocytes (Fujii et al., 1990) and also demonstrates for the first time the down-regulatory activity displayed by the drug on spontaneous production of anti-DNA IgG antibody from lupus patients' B lymphocytes. This latter finding accords with in vivo studies on murine models of lupus where 15-deoxyspergualin suppresses the increment of IgG producing cells in the spleen and diminishes the serum titres of anti-DNA antibodies (Ito et al., 1990). Further support to the action of 15-deoxyspergualin on B lymphocytes hyperreactivity during autoimmune pathologies comes from animal models of collagen-induced arthritis (Takagishi et al., 1990), autoimmune uveitis (Mochizuku and Kawashima, 1990), Goodpasture's syndrome (Lan et al., 1994) and experimental autoimmune thyroiditis (Nicoletti et al., 1994) where autoantibody production is inhibited by 15-deoxyspergualin.

The monocyte/macrophage system has also been considered as a potential target for 15-deoxyspergualin (Dickneite et al., 1987; Waaga et al., 1990; Nemoto et al., 1991; Takasu et al., 1991) and Hoeger et al. (1994) recently demonstrated that 15-deoxyspergualin only inhibited the proliferation of human peripheral blood mononuclear cells to antigens whose processing from monocytes is required for them to be presented to T cells, such as tetanus toxoid and diphteria toxoid. In contrast, 15-deoxyspergualin did not affect the proliferation of human peripheral blood mononuclear cells to mitogens that stimulate T cells directly without being processed by monocytes, such as phytohaemagglutinin, phorbol-12-myristate-13-acetate/ionomycin or superantigens (Hoeger et al., 1994). Our present observation that 15-deoxyspergualin suppressed interleukin-1β production from monocytes obtained from lupus patients substantiates, confirms and extends the action of 15-deoxyspergualin on the monocyte/macrophage system and underlines a new concept on the specific down-regulatory activity of this drug on activated autoimmune monocytes. Interestingly, the action of 15-deoxyspergualin on the cytokine secretory capacity of monocytes appeared not to include all the pattern of macrophage-derived cytokines, as the compound did not affect the secretion of interleukin-6, another cytokine produced by monocytes. The reasons why 15-deoxyspergualin was only acting on the monocytes from the lupus patients but not from the healthy subjects is not known. Since mononuclear cells obtained from patients with autoimmune diseases can be preactivated in vivo (Kroemer and Wick, 1989), one possible explanation would be that this condition can render monocytes more prone to the action of 15-deoxyspergualin through yet unidentified immunopharmacological pathways. Note however that, regardeless of the biological mechanism underlying the inhibitory effect of 15-deoxyspergualin on interleukin-1β secretion from lupus patients' monocytes, this action could account for by some of the immunosuppressive properties of 15-deoxyspergualin. Interleukin-1 has repeatedly been reported to play a role in the pathogenesis of several immunoinflammatory diseases (Bendtzen, 1989), and the inhibitory effect of 15-deoxyspergualin on interleukin-1 secretion might thus contribute to the beneficial actions of the drug in autoimmune models and possibly anticipates its beneficial effect in the correspective pathologies in humans.

The potential effect of 15-deoxyspergualin on T cell function has been controversial. As already discussed, both our own and previous studies (Tepper et al., 1993) failed to demonstrate any interference of 15-deoxyspergualin with several T cell responses. In contrast, the drug significantly inhibits response of both human and murine lymphocytes to alloantigens during a one way mixed lymphocytic reaction and also suppresses interleukin-2 receptor expression on CD4 and CD8 T cells (Tepper et al., 1993). The

generation of cytotoxic T cells has also been reported to be affected by 15-deoxyspergualin, in vitro, possibly through a γ-interferon-dependent mechanism (Tepper et al., 1993; Nishimura and Tokunaga, 1989). Moreover, that 15-deoxyspergualin might either directly or indirectly interfere with T cell function accords with experimental studies where the drug suppresses development of T cell-dependent autoimmune pathologies in animal models of insulin-dependent diabetes mellitus (Nicoletti et al., 1992, 1993; Starndler and Sandler, 1992), autoimmune thyroiditis (Nicoletti et al., 1994) and multiple sclerosis (Jung et al., 1994). The action of 15-deoxyspergualin on T cells might occur through a modulatory activity on the production of macrophage- and/or T cell-derived cytokines regulating T cell differentiation and functions, including interleukin-1β, -2, -4, -6 and γ-interferon. However, as mentioned above, the only significant action of 15-deoxyspergualin we found was the substantial reduction of interleukin-1β secretion by monocytes of lupus patients. Anyhow, as for the activity of 15-deoxyspergualin on T cell function, its action on cytokine production has also been controversial. For example, in our hands, 15-deoxyspergualin significantly suppressed γ-interferon secretion both from autoimmune (cyclophosphamide-treated) NOD/Whei mice and normal CBA/J mice (Nicoletti et al., 1993) and the preventative effect of the drug on a rat model of Goodpasture's syndrome was found to be associated with a reduced production of TNF-α (Lan et al., 1994). 15-Deoxyspergualin did not reduce the ex vivo production of either interleukin-1 or interleukin-2 in normal mice (Makino et al., 1987) and it had no effect on LPS-induced monocyte interleukin-1β, TNF-α and interleukin-6 mRNA and protein production in rat renal allograft rejection (Kerr et al., 1994). The reasons for these discrepancies are not clear. The different experimental conditions used and the difficulty to extrapolate studies in animal models to the human counterpart must be certainly considered. Moreover, the proposed mechanism of action of 15-deoxyspergualin at the level of antigen processing and presentation by monocytes could also be involved. It seems possible that how 15-deoxyspergualin modulates immune responses may depend on whether it inhibits monocytic processing and presentation of an antigen which stimulates either Th1 or Th2 cells. Accordingly, while 15-deoxyspergualin blockage of Th1-mediated immune responses may conceivably lead to diminished production of Th1-derived cytokines (e.g. γ-interferon and interleukin-2) and DTH-like events, the inhibition of Th2-mediated phenomena may result in reduced secretion of Th2-derived cytokines (e.g. interleukin-4) and humoral-mediated immune reactions.

In conclusion, our study further suggests that 15-deoxyspergualin possesses a new and more selective mean of pharmacological immunosuppression which seems to occur through a down-regulatory action displayed at the level of autoreactive cells. Although not yet completely understood, the mechanism of action of 15-deoxyspergualin seems to be largely different from that of other immunosuppressants, such as FK506 and cyclosporin A, two compounds that inhibit T cell activation in a reversable but unspecific fashion through binding to their specific intracellular cytosolic receptors (for a review, see Thomson, 1992). It will be particularly important for the clinical setting to evaluate whether a synergistic action of 15-deoxyspergualin with cyclosporin A and/or with FK506 will follow their different immunopharmacological properties. The combined use of these drugs during immunoinflammatory conditions may allow lowering dosages of each compound therefore reducing dose-dependent side-effects while maintaining or possibly enforcing the therapeutic action.

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