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Suppression of experimental systemic lupus erythematosus (SLE) with specific anti-idiotypic antibody–saporin conjugate

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SUMMARY

The importance of the idiotypic network is represented in experimental SLE induced by active immunization of naive mice with an anti-DNA idiotype (Ab1) emulsified in adjuvant. The mice after 4 months of incubation generate Ab3 having anti-DNA activity. In addition, the mice develop other serological markers for SLE associated with clinical and histopathological manifestations characteristic of the disease. To confirm further the etiological role of the idiotype in this experimental model, the mice were treated with specific anti-idiotypic antibodies (anti-Id) which were also conjugated to a toxin–saporin (Immunotoxin (IT)). Pretreatment of hybridoma cell line producing the anti-anti-Id (anti-DNA = (Ab3)) for 48 h with the anti-Id MoAb (Ab2) reduced the production of anti-DNA by 58%, while pretreatment with the IT resulted in 86% decrease in anti-DNA secretion (saporin alone had only 12% effect). The anti-Id MoAb had no effect on the production of immunoglobulin by an unrelated cell line. *In vivo* treatment of mice with experimental SLE led to a significant decrease in titres of serum autoantibodies, with diminished clinical manifestations. The results were more remarkable when the IT was employed. These suppressive effects were specific, since an anti-Id treatment of experimental anti-phospholipid syndrome was of no avail. The anti-Id effect was mediated via a reduction in specific anti-DNA antibody-forming cells, and lasted only while anti-Id injections were given. Discontinuation of the anti-Id injection was followed by a rise in titres of anti-DNA antibodies. No immunological escape of new anti-DNA Ids was noted. Our results point to the importance of pathogenic idiotypes in SLE and to the specific potential of implementing anti-idiotypic therapy, enhanced by the conjugation of the anti-Id to an immunotoxin, in particular one with low spontaneous toxicity.

Keywords anti-idiotypic antibodies autoimmunity experimental systemic lupus erythematosus idiotype immunotoxin

INTRODUCTION

Experimental SLE, induced by active immunization with anti-DNA antibodies, is associated with raised titres of autoantibodies to DNA, Sm, Ro, La, histones and cardiolipin, in the serum. The disease is characterized by increased sedimentation rate, leukopenia, alopecia, proteinuria and deposition of immunoglobulins in the mesangium of the kidneys [1–4]. Recently, we have also reported on the induction of experimental anti-phospholipid syndrome associated with SLE ('secondary anti-phospholipid syndrome') following immunization with a different pathogenic human anti-DNA MoAb (MIV-7) [5]. The availability of these experimental models enabled us to evaluate various therapeutic modalities including hormonal manipulation, treatment with a drug that raises IL-2 levels (AS-101), cyclosporin-A, and infusion of specific idiotype T suppressor cells [6–9].

Since the idiotypic network is an important mechanism for controlling the immune repertoire [10–12], and autoimmune diseases may be attributed to the disturbance of the network [13,14], several groups decided to employ an anti-idiotypic modulation in experimental models.

Successful *in vitro* and *in vivo* manipulations of autoantibody production by anti-idiotypic (anti-Id) antibodies were previously described in murine lupus and lupus nephritis, autoimmune tubulointerstitial nephritis, collagen arthritis, experimental autoimmune myasthenia gravis, autoimmune uveoretinitis, autoimmune thyroiditis and experimental aller-

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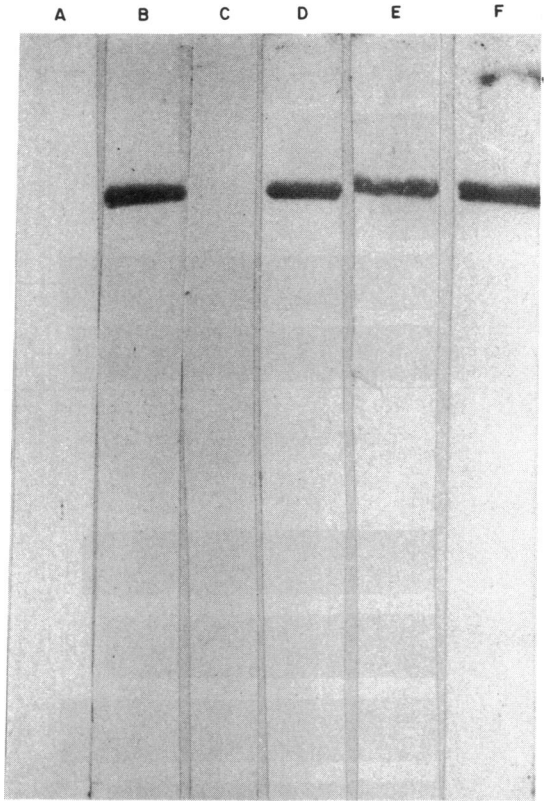

Fig. 1. Immunoblot analysis of anti-SA-1 MoAb. Lane A, binding of mouse IgG to human SA-1 (16/6 Id) MoAb; lane B, binding of anti-SA-1 to SA-1 MoAb; lane C, binding of mouse anti-SA-1 MoAb to human IgM; lane D, binding of mouse anti-16/6 to 16/6 MoAb; lane E, binding of mouse anti-SA-1 to 16/6 MoAb; lane F, binding of mouse anti-SA-1 to human MIV-7 (16/6 Id^+) MoAb.

gic encephalomyelitis [15–28]. To enhance the effect of the anti-idiotypic antibodies, cytotoxic agents such as neocarzinostatin (NCS) were conjugated to the anti-idiotypic antibodies [29]: the latter treatment resulted in a specific elimination of anti-DNA idiotypic positive cells with suppression of anti-DNA production [30,31]. The use of immunotoxin (IT) in other clinical setups was reported in the literature [32,33].

Saporin is one of the most widely used toxin compounds for IT preparation. It is a single chain (type 1) basic protein with a mol. wt of about 30 kD and has advantages over ricin, a type II toxic protein. Type I toxins are extremely stable proteins and do not bind spontaneously to cells [34,35], and are therefore non-toxic to intact cells, and safe to handle in the laboratory [36–38].

On the basis of the above studies and the importance of 16/6 Id in the pathogenesis of experimental SLE [39], we manipulated the mice with experimental SLE with an anti-16/6 Id MoAb conjugated to saporin.

MATERIALS AND METHODS

Human and mouse monoclonal antibodies

SA-1, a human MoAb IgM anti-DNA which carries the 16/6 Id, was generated by the human hybridoma technique from peripheral blood lymphocytes (PBL) of a patient with active polymyositis. The SA-1 antibody binds ssDNA and dsDNA and carries the 16/6 Id, and is able to induce experimental SLE disease in naive mice [3]. IgM was purified from the cell culture of the established hybridoma.

MIV-7, a human MoAb, was produced by the human hybridoma technique from normal PBL immunized *in vitro* with anti-idiotypic antibody to anti-mouse mammary tumour virus (MMTV). It is an anti-DNA antibody carrying the 16/6 idiotype, and was used to induce experimental SLE associated with anti-phospholipid syndrome (APLS) [5,40].

PEB, a human IgG MoAb, was produced by the human hybridoma technique from pleural effusion infiltrating lymphocytes, from a patient with breast cancer. The antibody binds to mouse and human mammary tumour virus (MMTV or HuMTV) [41].

CAM, mouse IgG anti-cardiolipin MoAb, was produced by the mouse hybridoma technique from mice with experimental SLE with secondary APLS [5].

N40, mouse IgG MoAb, does not bind to phospholipids or to DNA. This MoAb was produced from the same mice as CAM MoAb [5] and used as a control of irrelevant mouse IgG.

Preparation of the anti-idiotypic antibody (anti-SA-1)

The establishment of the anti-idiotype MoAb anti-anti-SA-1 was done by employing the hybridoma technique. Briefly, NSO myeloma cells were fused with spleen cells of BALB/c mice immunized with the purified SA-1 anti-DNA MoAb. The specificity of the anti-SA-1 MoAb to SA-1 was confirmed by an immunoblot assay (Fig. 1).

Immunoblotting of anti-SA-1 to various 16/6 MoAbs

To confirm the specific activity of anti-SA-1 to the 16/6 Id (SA-1), the immunoblotting technique was used by applying reduced anti-SA-1 MoAb into 10% SDS–PAGE. Samples of SA-1, MIV-7 (16/6 Id^+) MoAbs were reduced by 20 mM dithiothreitol (DTT). The reduced MoAbs were subjected to 10% SDS–PAGE at a concentration of 10 μg per lane. The gel was electrotransferred to nitrocellulose paper (NCP) (BS-85; Schleicher and Schull, Dassel, Germany; 0·45 μm). The lanes were blocked for 2 h at room temperature with 5% low fat milk in 0·05% PBS-Tween. Each lane was exposed separately to mouse MoAb IgG or mouse MoAb anti-SA-1 (IgG) or mouse MoAb-anti-16/6 (IgG). Following extensive washings, the strips were subjected to goat anti-rabbit IgG or goat anti-mouse IgG conjugated to alkaline phosphatase (Sigma, St Louis, MO), overnight at 4°C. The washed strips were exposed to nitro-blue-tetrazolin (NBT; Sigma) in TNM (0·1 μM Tris pH 9·5; 0·1 μM NaCl; 0·05 μM $MgCl_2$) and 5-bromo-chloro-3-indolyphosphate (BCIP, Sigma) in dimethyl formamide. After a few minutes the strips were rinsed with water and dried.

Purification of saporin [38]

Saporin was extracted and purified from the seeds of *Saponaria officinalis*. Briefly, a batch of 25 g of seeds was homogenized with 200 ml of 0·14 M NaCl in 5 mM sodium phosphate buffer pH 7·2. The homogenate was stirred overnight at 4°C, strained through a cotton gauze and centrifuged at 28 000 ***g*** for 30 min. The crude extract solution was dialysed against 5 mM sodium phosphate buffer pH 6·5. The dialysate was passed through a CN-Sepharose CL-6B column (Pharmacia, Brussels, Belgium)

equilibrated with the same buffer used in the dialysis procedure. The bound fractions were eluted with a 0–0·3 M NaCl gradient. The amount of protein was determined by the Bradford method.

Preparation of immunotoxin

Purified anti-SA-1 MoAb (1 mg/ml) was derivatized by N-succinimidyl pyridyl dithiopropionate (SPDP) treatment. The sulfhydryl groups were introduced to the purified saporin by 2-iminothiolana. The derivatized antibody and derivatized saporin were conjugated, leading to the IT product [38].

The in vitro *effects of the IT*

Anti-DNA-secreting hybridoma cells (MIV-7, 16/6/Id$^+$) were incubated with the anti-Id MoAb, with the IT, with the mouse IgG or with the purified saporin (SAP) for 48 h. Following extensive washes, the cells were incubated for 6 days. The effect of the mouse anti-Id MoAb, the IT and the other compounds on anti-DNA antibody production was assayed by an ELISA and by a spot ELISA.

Spot ELISA to determine anti-DNA secretion [42]

Hybridoma cells or mouse splenocytes (1×10^6 cells/ml) were assayed for their ability to secret *in vitro* anti-DNA, anti-SA-1 or anti-cardiolipin antibodies. The preparation of splenocytes was done by teasing the spleen and passing the splenocytes through 0·45 μM nylon wool. The erythrocytes were lysed with 0·83% Tris-buffered ammonium chloride. The cells were seeded in RPMI 1640, into 24-well tissue culture plates (Nunc, Roskilde, Denmark) precoated with DNA, 16/16 Id or cardiolipin with or without tested anti-Id or IT. Anti-human IgM alkaline phosphatase or anti-human or mouse IgG alkaline phosphatase was added for 2 h at 37°C. Following extensive washings, BCIP (Sigma) was added in 2-aminopropanol Triton X-405 $MgCl_2$ buffer to 3% Agar (type I, low electroendosmosis; Sigma) heated and diluted in BCIP buffer at 1 : 4 ratio, resulting in a 0·6% agar solution. Overnight incubation in 37°C resulted in blue spots.

Inhibition of antibody-forming cell activity

In order to confirm the specific binding of the antibodies which were secreted by the splenocytes, an inhibition assay was employed. The tested cells were preincubated with dsDNA, SA-1 or cardiolipin (CL) during 6 h at 37°C, and then the supernatant was added to the precoated plates with dsDNA, SA-1 or cardiolipin. The rest of the spot ELISA assay was unchanged.

Induction of experimental SLE and APLS

Human anti-DNA MoAb MIV-7 (16/6 Id$^+$) was used to induce SLE with a secondary APLS [5]. The mouse anti-cardiolipin MoAb CAM was employed to induce primary APLS in naive mice [5].

BALB/c mice were immunized intradermally in the hind footpads with 10 μg of MIV-7 MoAb, control human IgM, CAM mouse MoAb or with a control mouse IgG MoAb in Freund's complete adjuvant (FCA). Three weeks later a boost injection was given in PBS in the same manner. The mice were bled every month and autoantibody level titres were determined in the sera. We followed the parameters of SLE (increased erythrocyte sedimentation rate (ESR), leukopenia, proteinuria, anti-DNA antibodies, etc) and APLS (anti-CL antibodies, fetal loss, thrombocytopenia) as detailed by us previously [1–5].

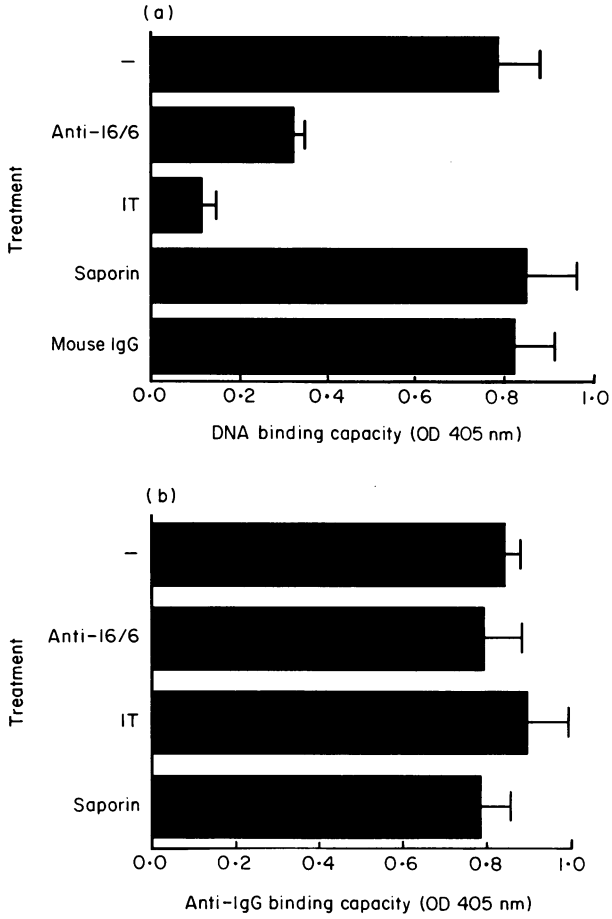

Fig. 2. (a) Effect of anti-16/6 Id immunotoxin (IT) on anti-DNA MoAb production *in vitro*. (b) Effect of anti-16/6 Id IT on IgG secretion *in vitro*.

Treatment of experimental SLE and APLS with IT

The treatment of both experimental models of SLE and APLS with the anti-idiotypic antibodies started 2 months after the boost injection, when the titres of the specific autoantibodies in the sera of the mice were already elevated and yet no 'clinical' findings were observed.

The mice were daily injected intraperitoneally with 200 μg of the MoAb anti-SA-1, or 200 μg of IT, or 200 μg of irrelevant IgG, or 50 μg of saporin alone, for 1 month.

Immunohistochemistry

Immunohistological studies were performed as previously described [1]. Briefly, kidneys were removed and frozen immediately in liquid nitrogen. Frozen cryostat sections (5–7 μm thick) were dried and fixed in acetone for 5 min and then washed in PBS. For detection of immunoglobulin deposits, FITC-conjugated goat anti-mouse immunoglobulin antibodies (Sigma) were applied for 60 min after extensive washing in PBS, and the specific binding was visualized by a fluorescent microscope.

Table 1. Autoantibody titres in the sera of mice treated with anti-16/6 MoAb or anti-16/6–SAP conjugate (IT)

Antibody to	Experimental model	Treatment with M-IgG ($n = 15$)	Saporin ($n = 15$)	Anti-16/6 ($n = 15$)	Anti-16/6 + SAP ($n = 15$)
dsDNA	SLE	1174 ± 217	901 ± 94	137 ± 29	94 ± 12
		(1103 ± 221)*	(1145 ± 216)	(1127 ± 281)	(1203 ± 264)
ssDNA		1038 ± 191	838 ± 99	145 ± 35	103 ± 21
		(1103 ± 204)	(994 ± 78)	(1208 ± 235)	(1143 ± 174)
Histones		805 ± 76	641 ± 81	214 ± 72	149 ± 23
		(791 ± 94)	(814 ± 92)	(847 ± 112)	(894 ± 103)
16/6 Id		745 ± 96	603 ± 94	273 ± 53	127 ± 12
		(772 ± 89)	(705 ± 85)	(943 ± 92)	(841 ± 69)
Anti-16/6 Id		898 ± 101	598 ± 86	203 ± 42	131 ± 39
		(798 ± 95)	(831 ± 82)	(912 ± 72)	(898 ± 75)
Cardiolipin		866 ± 95	625 ± 91	482 ± 71	403 ± 69
		(851 ± 79)	(791 ± 94)	(941 ± 85)	(848 ± 74)
Phosphatidylserine		649 ± 83	489 ± 75	407 ± 82	312 ± 62
		(654 ± 92)	(603 ± 85)	(782 ± 68)	(755 ± 88)
Cardiolipin	APLS	741 ± 92	604 ± 66	738 ± 84	642 ± 73
		(779 ± 89)	(752 ± 49)	(745 ± 59)	(757 ± 75)
Phosphatidylserine		684 ± 79	545 ± 79	702 ± 56	556 ± 95
		(701 ± 92)	(692 ± 87)	(692 ± 81)	(706 ± 92)
Phosphatidylinositol		596 ± 75	486 ± 88	621 ± 101	572 ± 84
		(554 ± 81)	(601 ± 95)	(598 ± 84)	(656 ± 102)

APLS, Anti-phospholipid syndrome.

* The sera were tested at dilution of 1 : 200. The values are expressed as mean ±s.d. OD at 405 nm × 10^3.

The titres of autoantibodies before treatment are presented in parentheses. The assay was performed 4 months after disease induction, 1 month after treatment. The levels of autoantibodies in the preimmune sera ranged between 97 ± 18 and 136 ± 46. Statistical analysis by Student's *t*-test of the results demonstrated above, before and after treatment with anti-16/6 or anti-16/6-SAP in SLE model gave $P < 0{\cdot}001$–$P < 0{\cdot}05$; in APLS model $P > 0{\cdot}5$. In all the control groups in both models, mice before and after treatment with non-relevant mouse IgG or saporin, $P > 0{\cdot}5$.

RESULTS

Characterization of the mouse anti-SA-1 (16/6) MoAb

Mouse anti-SA-1 (IgG) MoAb binds to the human anti-DNA antibodies which carry the 16/6 Id (e.g. SA-1, MIV-7 and 16/6 MoAbs), and does not bind to a control human IgM (Fig. 1). The binding of the anti-Id is specific to the heavy chain, as can be seen in the immunoblot performed in reduced conditions (Fig. 1).

In vitro *effect of the anti-16/6 Id antibodies or the IT on anti-DNA MoAb production*

The anti-dsDNA-secreting hybridoma cells (MIV-7) and anti-breast cancer hybridoma cell (PEB) [3] were preincubated with the toxin saporin, the IT, the anti-16/6 MoAb and mouse control IgG and left for 6 days in culture for antibody secretion.

Pretreatment of MIV-7 line cells for 48 h with the anti-16/6 MoAb reduced the anti-DNA MoAb production by 58%, while pretreatment of those cells with the IT reduced the anti-DNA secretion by 86% (saporin alone had reduced the production by 12%) (Fig. 2a). No significant effect on immunoglobulin production was noted in the PEB secreting cells (Fig. 2b). The data show a greater immune suppression was achieved when IT was employed in comparison with the mouse anti-MoAb alone.

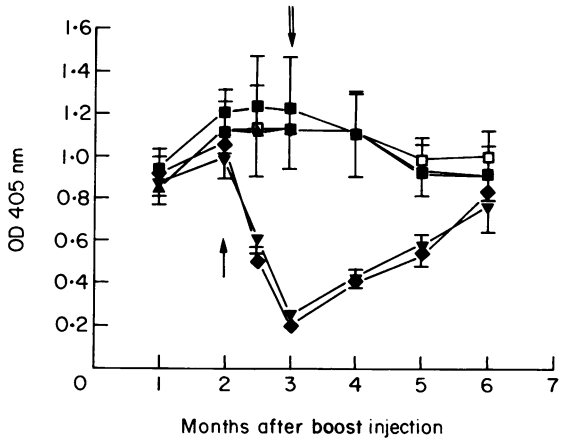

Fig. 3. Titres of anti-dsDNA antibodies in the sera of anti-16/6 and immunotoxin (IT) treated during 6 months of the experiment. ■, Not treated; ▲, saporin; ▼, anti-16/6; ◆, IT; □, irrelevant mouse IgG; →, start of treatment; ⇒, end of treatment. Each group contained 15 mice.

Table 2. SLE findings in the immunized mice treated with anti-16/6 MoAb or anti-16/6 + SAP conjugate (IT)

Findings	Experimental model	Treatment with M-IgG ($n = 15$)	Saporin ($n = 15$)	Anti-16/6 ($n = 15$)	Anti-16/6–SAP ($n = 15$)
ESR	SLE*§	11	10	3	2
(mm/6 h)		(12)†	(9)	(12)	(9)
Leucocyte count		2758 ± 489	2642 ± 382	5643 ± 751	6072 ± 814
(cells/mm^3)		(2531 ± 321)	(2437 ± 293)	(2543 ± 379)	(2984 ± 739)
Proteinuria		> 3	> 3	< 0·3	< 0·3
(g/dl)		(> 3)	(> 3)	(> 3)	(> 3)
APTT (s)	APLS*‡	87	71	63	76
Platelet count (cells/mm^3 × 10^3)		347 ± 79	457 ± 89	534 ± 96	379 ± 101
Per cent fetal resorption¶		56	42	47	59

* Statistical analysis by Student's *t*-test demonstrated that only the group of SLE mice treated with anti-16/6 MoAb or anti-16/6-SAP $P < 0{\cdot}001$–$P < 0{\cdot}02$; in all the other groups of experiments, $P > 0{\cdot}5$.

† The numbers in parentheses represent the values before treatment. The values of findings in mice immunized with non-relevant human IgM are as follows: erythrocyte sedimentation rate (ESR), 3 mm/6 h; leucocyte count, 6271 ± 703/mm^3; proteinuria < 3 g/dl.

‡ APLS, Antiphospholipid syndrome. The values before treatment were: APTT, 72 s; platelet count, 482 ± 93/mm^3 × 10^3; per cent fetal resorption 49%. The findings in mice immunized with control IgG were as follows: APTT, 27 s; platelet count, 987 ± 91/mm^3 × 10^3; per cent fetal resorption, 4%.

§ All the findings were observed 6 months after disease induction and 2 months after the end of treatment.

¶ Per cent fetal resorptions = (live fetuses − resorbed fetuses)/live fetuses.

In vivo *effect of IT treatment on experimental SLE*

Treatment of experimental SLE with anti-16/6 MoAb or with the IT resulted in suppression of anti-DNA, anti-16/6 and mouse 16/6 antibody levels in the sera of the mice (Table 1).

Mice which received the anti-16/6 MoAb or the anti-16/6-IT for 1 month had similar titres of anti-DNA, anti-16/6 Id antibodies or 16/6 Id levels to those recorded in sera of preimmunized mice; while the levels of the anti-phospholipid antibodies were reduced only by 50–70%, these data were statistically significant ($P < 0{\cdot}001$–$P < 0{\cdot}01$). The treatment with the anti-16/6 MoAb alone had less effect on autoantibody production in the sera of the treated mice (Table 1), in comparison with the treatment with IT. Both treatments had no effect on the primary experimental APLS model (Table 1), e.g. no decline was noted in the levels of anti-phospholipid antibodies in the sera of the treated mice (Table 1) ($P > 0{\cdot}05$). The kinetics of the immunosuppressive effect of the treatment showed a decline in the titres of the autoantibodies in the sera of the mice treated with anti-16/6 or IT, during the month of administration (Fig. 3). Discontinuation of the injections of the anti-16/6 MoAb or the IT was followed by recurrent raised titres of anti-DNA antibodies which 5 weeks later reached pretreatment levels (Fig. 3). No effect of injection of saporin or irrelevant IgG on the levels of autoantibodies was noted.

Data presented in Fig. 4 demonstrate that the effect of the anti-16/6 MoAb or the IT treatment on *in vivo* antibody production was mediated through reduction of the number of anti-dsDNA antibody-forming cells (Fig. 4a,b). Administration of the anti-16/6 MoAb to the mice with experimental SLE resulted in 64% reduction in anti-DNA antibody-forming cells (AFC) compared with 70% reduction recorded in AFC following IT treatment.

The mice which received either anti-16/6 MoAb or the anti-16/6-IT treatment did not develop the manifestations of SLE or APLS, such as accelerated ESR, leukopenia, proteinuria and immunoglobulin deposits in the kidneys (Table 2, Fig. 5). Comparison of the values before and after treatment with anti-16/6 MoAb or IT showed significance by Student's *t*-test of $P < 0{\cdot}001$–$P < 0{\cdot}02$. The mice which were immunized with anti-cardiolipin MoAb (CAM) and were treated with anti-16/6 MoAb or anti-16/6-IT conjugate, developed APLS (Tables 1 and 2) ($P > 0{\cdot}5$). No immunoglobulin deposits were observed in the kidneys following treatment with anti-16/6 MoAb or the anti-16/6-IT (Fig. 5a,b) compared with treatment with irrelevant IgG (Fig. 5c).

DISCUSSION

In previous studies [1–9] we have shown the potential pathogenic role of idiotype in inducing autoimmune disease. Following idiotypic immunization and 4 months of incubation the mice develop Ab3, which has identical binding capacities, idiotypic constellation and even a similar amino acid sequence [43] to the original human anti-DNA antibody. The mice develop the whole panoply of autoantibodies (anti-DNA, anti-Sm, anti-histone, anti-cardiolipin) seen in many individuals with SLE. The serological markers are associated with typical SLE findings such as accelerated ESR, leukopenia, thrombocytopenia, proteinuria and mesangial immune complex deposition. To confirm further the pathogenic importance of the idiotypes of anti-DNA in antibodies in this experimental model, we decided to manipulate the mice idiotypically. The manipulation was carried out both with the 'naked' anti-Id antibody and with the antibody conjugated to relatively

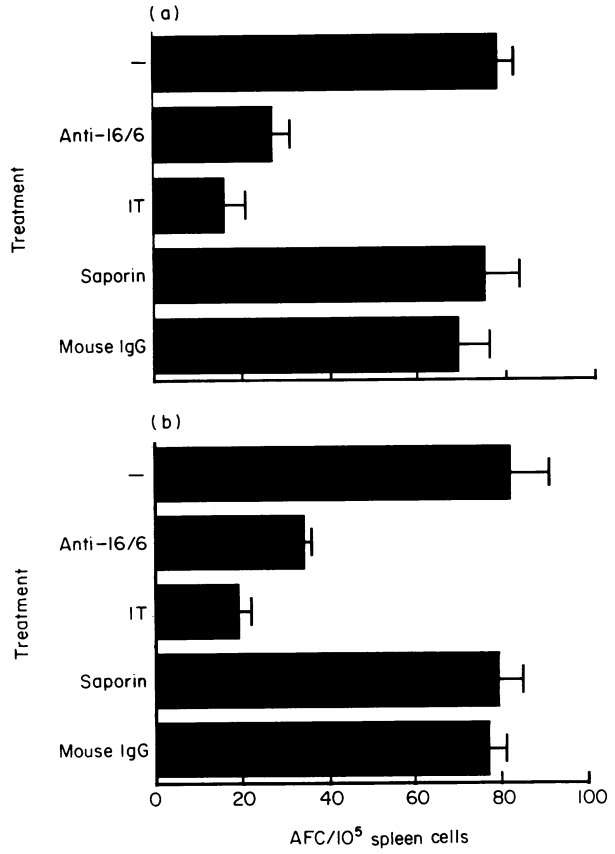

Fig. 4. (a) Anti-dsDNA antibody-forming cells in mice treated with anti-16/6 Id MoAb immunotoxin (IT). (b) Mouse 16/6 antibody-forming cells in mice treated with anti-16/6 Id MoAb. The data represent mean of three experiments.

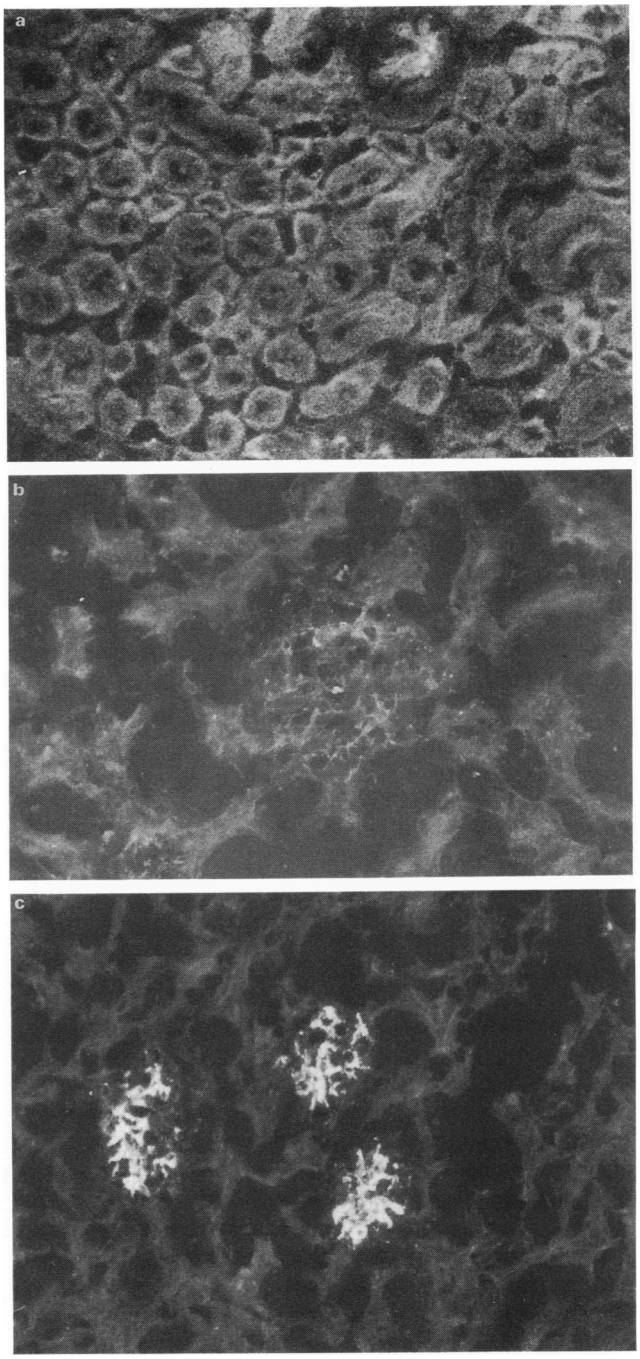

Fig. 5. Immunofluorescence study of representative kidneys from mice treated with anti-16/6 (a), anti-16/6 conjugated to saporin (IT) (b), or treated with irrelevant IgG (c).

innocent toxin, saporin. The effect of the anti-Ids was studied in both *in vitro* and *in vivo* experiments.

The anti-Id was specifically shown to reduce anti-DNA antibodies by a specific hybridoma cell line. The immunotoxin had a significantly superior result compared with the anti-Id itself. Yet, although impressive, the effect of the immunotoxin in reducing anti-DNA antibody production and abrogation of SLE manifestations was not better than the anti-Id alone.

The lack of difference in therapeutic effect between the anti-Id and the IT may be due to a susceptibility of the disulphide bond of the conjugate (IT) to an enzyme cleavage *in vivo* [44]. A more stable conjugate with a thioether bond (S–C), might be more effective, since there is no known enzyme in animals that can cleave thioether [45]. However, an IT prepared with such a linkage was reported to be much less active in killing target cells than comparable disulphide conjugates [46].

The results support the notion that in the proper circumstances (genetic background) an idiotype exposure in the presence of adjuvant or superantigen (provided by bacteria) may lead to the induction of autoimmune disease. Our results and those of others [19–28] also confirm the potential for idiotypic manipulation of autoimmune conditions. The treatment may require tailor-made anti-Ids, or the employment of a cocktail of anti-Id antibodies. Interestingly, we did not see an 'escape' from the Id suppression, only when the therapy was discontinued, suggesting that such a treatment may be effective in patients too. The human disease differs from the experimentally induced SLE by exacerbation and remissions. Thus, the idiotypic manipulation in patients will most probably be required until disease remission.

An alternative treatment may entail the use of high-dose intravenous gammaglobulin (IVIG). Recently we have shown that IVIG indeed contains anti-idiotypic antibodies to the 16/

6 Id [47]. The effectiveness of IVIG in some patients with SLE [48] may in part be explained by idiotypic suppression.

The effectiveness of anti-Id therapy (antibody and its IT) may be increased by: (i) using $F(ab')_2$ fragment instead of an intact antibody in preparing IT, since the presence of Fc parts may enhance the elimination of IT from blood circulation; (ii) entrapping both the antibody and its IT in a proper liposome system coated with target antibody, since this system has been reported to be one of the most effective drug delivery systems *in vivo* [49]; and (iii) using alternative cross-linkers in preparing IT, such as thioether cross-linkers, e.g. succiminyl 4-(N-malcimidomethyl) cyclohexane-1-carboxylate (SMCC).

The exact mechanism by which anti-Id suppressed experimental lupus-like disease awaits clarification. The simplest explanation is that the anti-Id which recognizes the specific Id, deletes specific Th cells and circulating Id in the sera. Indeed, our study shows a decrease in the number of anti-dsDNA-forming cells (Fig. 4a,b). Another alternative interpretation would be a stimulation of the regulatory Id network by the anti-Id, resulting in the regulation of the production of autoantibodies. This mechanism was previously suggested for SLE and myasthenia gravis [22,25].

Our results point to the importance of the idiotypic network in the pathogenesis of SLE, and to the effectiveness of anti-idiotypic manipulation of autoimmune states.

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