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Involvement in lupus disease of idiotypes Id.F-423 and Id.IV-228 defined, respectively, upon foetal and adult MRL/*Mp-lpr/lpr* DNA-binding monoclonal autoantibodies

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SUMMARY

The derivation of a monoclonal IgG3K autoantibody, designated F-423, from a foetal MRL/*Mp-lpr/lpr* mouse is described. It has immunochemical properties similar to DNA-binding monoclonal antibodies derived from adult mice with lupus disease in that it reacts with single-stranded DNA and, to a lesser extent, with double-stranded DNA and some forms of RNA. Its similarities to antibodies from adults extend further: it carries a public idiotype, Id.F-423, that can also be detected on antibodies from adult MRL and (NZB × NZW)F_1 mice, and F-423 itself expresses other idiotypes defined originally on antibodies from adult lupus mice of both strains. Its potential involvement in pathological processes is demonstrated by two observations: (i) immunization of young MRL/*Mp*-+/+ mice with antibody F-423 induced the nephritic and immunological changes associated with systemic lupus erythematosus; and (ii) heterologous rabbit anti-Id.F-423 anti-idiotypic antibodies suppressed the progression of lupus disease in adult MRL/*Mp-lpr/lpr* mice. Similar effects were found with monoclonal antibody IV-228, an antibody derived from an adult MRL mouse and previously known to be directly nephrotoxic, and with anti-Id.IV-228 antibodies. It is concluded that even during foetal life mice of lupus-prone strains have lymphocytes capable of making pathogenic autoantibodies long before symptoms of lupus disease appear.

INTRODUCTION

The immunopathology of systemic lupus erythematosus (SLE) is in part a function of autoantibodies, especially those reactive with DNA. Knowledge about the pathogenic mechanisms exerted by the antibodies is incomplete, but high affinity, net cationic charge and complement fixing ability appear to be important properties of nephrotoxic DNA-binding antibodies.[1–3] DNA-binding antibodies, whatever their properties, alone are clearly insufficient to induce the clinical expression of lupus, because they have been identified, for example, in the blood of normal humans and mice, as a consequence of infection,[4] and also in high titres in some patients with myeloma.[5]

Abbreviations: BEG-2, 16/6, F-423, I-402, II-28, IV-228, V-88 are trivial acronyms of mAb; BWF1 (NZB × NZW)F_1; DNA, deoxyribonucleic acid; dsDNA, double-stranded DNA; ELISA, enzyme-linked immunosorbent assay; FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; Id, idiotype; Ig, immunoglobulin; KLH, keyhole limpet haemocyanin; mAb, monoclonal antibody; MRL/lpr, MRL/*Mp-lpr/lpr*; MRL/n, MRL/*Mp*-+/+; PBS, phosphate-buffered saline; RNA, ribonucleic acid; SLE, systemic lupus erythematosus; ssDNA, single-stranded DNA.

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In foetal life, autoantibodies are probably part of an idiotypically connected autoreactive set that is a major part of the expressed repertoire. In adult life this becomes progressively diluted with other antibodies, some of them autoreactive, that show limited mutual connectivity.[6,7] It is not known whether pathogenic lupus antibodies are members of one or other, or both, sets. Immunochemically, DNA-binding antibodies from lupus mice, mice with graft-versus-host disease and normal mice[8] cannot be distinguished easily from each other.

It is possible that DNA-binding antibodies from many different cell sources and made at different times in the life of an individual could contribute to pathology. Here we describe the potential for the involvement in pathology of a DNA-binding monoclonal antibody (mAb) from a mouse foetus and compare it with an antibody derived from an adult MRL mouse and shown previously to be nephrotoxic.[3] The foetal antibody is indistinguishable from the adult antibody: in the terms studied here it has the same properties as those from adults.[9]

MATERIALS AND METHODS

Animals

Mice of the MRL/*Mp-lpr/lpr* (MRL/lpr) and MRL/*Mp*-+/+ (MRL/n) strains were kindly supplied by The Kennedy Institute for Rheumatology, Hammersmith, London, U.K. and were

maintained at King's College London. Inbred BALB/c and Tuck outbred mice (Tuck, Rayleigh, Essex, U.K.) and outbred adult New Zealand White rabbits (Hyline, Lymm, Cheshire, U.K.) were obtained commercially.

Monoclonal antibodies (mAb)
DNA-binding mAb from adult MRL/lpr and (NZB × NZW)F_1 (BWF1) mice, and their classification in five family groups according to their immunochemical properties, have been described previously.[9–11] Briefly, the classification deals with mAb that react respectively with: dsDNA alone (Group I); dsDNA and ssDNA with roughly equal functional affinity and with phospholipids (Group II); strongly with ssDNA and with nuclei but not nucleoli in immunofluorescence assay (Group III); ssDNA through interaction with unpaired bases (Group IV); and ssDNA and to a lesser extent dsDNA and RNA (Group V).

Monoclonal antibody F-423 is made by a hybridoma produced by the fusion of cells from a whole 16-day MRL/lpr foetus with NS-1 cells. Its properties are described below. Antibody IV-228 is an Ig2aK mAb derived from an adult MRL/lpr mouse. It is a member of Group IV of the classification above, it binds ssDNA and not dsDNA and it has been shown to be directly nephrotoxic when infused into MRL/lpr mice with early stage lupus nephritis.[3] Other mAb referred to include I-402, II-28 and V-88. Monoclonal antibodies were purified by salt precipitation and affinity chromatography on staphylococcal protein A-Sepharose 4B (Sigma Chemical Co, Poole, Dorset, U.K.).

Preparation of anti-idiotypic (anti-Id) reagents
Rabbits were immunized intradermally by repeated injection of individual mAb in Freund's complete and then incomplete adjuvants (FCA, FIA; Difco, Detroit, MI), and anti-Id antibodies from their sera were purified by absorption with pooled normal mouse immunoglobulin (Ig), and then by adsorption to and elution from homologous mAb.[12] Anti-idiotypic antisera were prepared against Id. F-423, Id.I-402, Id. II-28, Id.IV-228 and Id. V-88, which are the idiotypes defined on mAb F-423, I-402, II-28, IV-228 and V-88.

ELISA for ssDNA- and dsDNA-binding antibodies
As a direct assay, an ELISA was used to titrate mAb and serum antibodies. As a competition assay, with antigen as the competing analyte and a limiting amount of mAb in the fluid phase, it was used to establish the ligand binding profiles of individual antibodies. These and other assays below followed conditions described previously.[9,12,13] Units (arbitrary) of ssDNA and dsDNA antibody-binding activity were determined by comparing titrations of unknown sera against the linear parts of standard curves of mAb IV-228 and I-402, respectively, in the direct assay.

Inhibition ELISA for binding-site Id expression on mAb
The competition anti-DNA ELISA (above) was adapted to measure the activity of anti-Id antibodies in inhibiting the binding of a limiting concentration of mAb to immobilized ssDNA or dsDNA. Thus the inhibitory capacity of anti-Id antibodies on different mAb was established. Results here are given as percentage inhibition of antibody binding with 50 μg/ml anti-idiotype antibody.

Capture ELISA for Id expression by serum immunoglobulins
Purified rabbit anti-Id antibodies were adsorbed to the plastic wells and then incubated with serial dilutions of mouse serum. Captured Id^+ immunoglobulins were detected with a rabbit anti-mouse Ig peroxidase-linked reagent (Sigma) and quantified (arbitrary units/ml) against a standard curve of the appropriate reference mAb.[12]

Immunization of mice with mAb
Antibody was conjugated to an equal weight of keyhole limpet haemocyanin (KLH) with glutaraldehyde,[14] was emulsified in, and injected intradermally in FCA into the hind footpads (25 μg/footpad F-423-KLH or IV-228-KLH or KLH) of female MRL/n mice at 10 weeks of age. Subsequent injections were given in phosphate-buffered saline (PBS) at 14 and 21 weeks of age and in FIA at 31 weeks. Samples of urine were collected, and sera were prepared from blood taken from a tail vein when indicated in the text. Albuminuria and serum creatinine levels were determined by published methods.[3] Kidneys were removed at autopsy, snap-frozen and mounted in Tissuetek (Raymond Lamb, London, U.K.) for cryotomy.

Treatment of lupus mice with anti-Id antibodies
MRL/lpr mice were injected at 11 weeks of age, and thereafter at intervals of 3 weeks, with affinity-purified rabbit anti-Id antibodies (100 μg/dose in PBS) against either Id.F-423 or Id.IV-228 or pooled normal rabbit Ig absorbed on normal mouse Ig as a control. Sera from the mice treated with anti-Id antibodies were absorbed, in microwell trays, with normal rabbit Ig immobilized on Sepharose 4B to remove anti-rabbit Ig activity. In other respects the animals were handled and analysed in the same way as the MRL/n mice above.

Immunofluorescence
Kidney sections (4–5 μm thick) were prepared by cryotomy, air dried on glass slides and fixed in cold acetone. Sections were exposed for 45 min to a rabbit anti-mouse Ig-fluoresceinated reagent (Sigma), diluted 1/50 in PBS containing 10% normal rabbit serum, then washed three times in PBS and mounted in 50% glycerol/PBS containing Cityfluor retardant (Cityfluor Ltd, London, U.K.).

RESULTS

Ligand binding profile of F-423

Antibody F-423 bound to ssDNA with a higher affinity than to dsDNA in a manner similar to Group V DNA-binding mAb, especially mAb V-88, from adult lupus mice. Like these antibodies,[9] it was multi reactive and bound to tRNA and the synthetic DNA-like polynucleotide p[dA,dT] and weakly to the RNA-like p[U]. It reacted with guanine mononucleoside and GMP nucleotide but not other mononucleotides or nucleosides. In immunofluorescence assay it bound to the nucleoli of Hep-2 cells and weakly to cytoskeletal structures, as described for the adult mouse mAb V-88[9] and the foetal human antibody BEG-2 that shares many properties with V-88.[12] These properties of mAb F-423 will be described in detail elsewhere (manuscript in preparation).

Table 1. Expression of cross-reactive idiotypes by monoclonal DNA binding antibodies

	% inhibition of binding of mAb to DNA in the presence of anti-Id antibodies*		
mAb	Anti-Id.V-88	Anti-Id.IV-228	Anti-Id-F.423
II-28	< 1·00	1·4	< 1·0
V-88	92	6·2	71
I-402	40	< 1·0	60
IV-228	1·4	97	< 1·0
F-423	70	< 1·0	98

* The ELISA employed immobilized ssDNA and a limiting concentration (to give 75% maximum binding) of mAb mixed in fluid phase with 50 μg/ml anti-Id antibody reagent. Results are percentage changes in absorbance values in presence of anti-idiotypic antibodies.

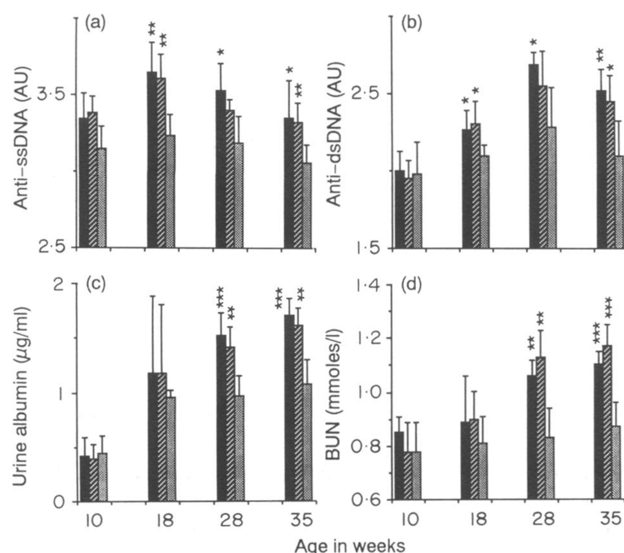

Figure 1. Effects of immunization of MRL/n mice with mAb IV-228-KLH (solid bars, $n=5$), F-423-KLH (hatched bars, $n=5$) or KLH (stippled bars, $n=6$). Sera and urine samples were collected monthly: only alternate data sets are shown for clarity but intermediate values reflected the trends illustrated. Changes (in mAb-immunized animals compared with control mice at each time interval) in levels of serum antibodies binding either ssDNA (a) or dsDNA (b), albumin in the urine (c) and blood urea nitrogen (BUN) (d) are consistent with the development of lupus disease. *$P<0·05$; **$P<0·01$; ***$P<0·001$ unpaired Student's *t*-test. The general increase in the measured parameters during the experiment in the MRL/n mice immunized with KLH is to be expected and is due to the natural insidious development of disease.

The idiotype of F-423

mAb F-423 was found to have a public idiotype, in that anti-Id.F-423 antibodies inhibited the binding of other mAb to DNA. Selected data are shown in Table 1, in which it can be seen that mAb F-423 itself also reacted in turn with antibodies against Id.V-88 but not those against Id.IV-228. An extended analysis of idiotype sharing between murine DNA antibodies will be published elsewhere, but these data illustrate that antibody F-423 shares public Id with mAb from adult BWF1 and MRL/lpr lupus mice. The close relationship of the idiotypes and specificity of antibodies 423 and 88 is notable, and will be returned to later.

Induction of lupus symptoms in MRL/n mice immunized with F-423 or with IV-228

Mice immunized with mAb conjugated to KLH (either 423-KLH or 228-KLH) produced antibodies reactive with ssDNA (Fig. 1a) and dsDNA (Fig. 1b). The mice were examined for the expression of five different Id on their serum immunoglobulins (Fig. 2), which in control mice remained appreciably unaltered during the experiment. As a consequence of immunization there was an elevation in the level of the homologous immunizing Id, although this was much more pronounced in the case of Id.423 and transient in the case of Id.228. Idiotypes 28 and 402 were not obviously affected, but there was a very clear enhancement of Id.V-88 expression following immunization with either mAb.

The serological changes in the mice immunized with mAb were accompanied by changes in kidney function that were inferred from elevated concentrations of albumin in the urine (Fig. 1c) and urea nitrogen in the blood (Fig. 1d). Creatinine in sera taken at the end of the experiment was not changed significantly (control mean ± SD = 1·56 ± 0·07; F-423 = 1·59 ± 0·14; IV-228 = 1·66 ± 0·12 μmoles/l, respectively).

This clinical picture characteristic of enhanced lupus disease in the MRL/n mice was reinforced by indirect immunofluorescence examination of kidney sections at autopsy. In every mouse immunized with 228-KLH there were granular or linear deposits of immunoglobulin along the capillary walls of the glomeruli, and granular deposits were seen in the kidney mesangium and capillary walls of every mouse immunized with 423-KLH. In the control group, one animal only had weakly stained glomerular deposits of Ig. On these criteria, MRL/n mice immunized with either the foetal antibody F-423 or the adult antibody IV-228 developed a form of SLE.

Suppression of lupus disease in MRL/lpr mice treated with anti-Id antibodies

Young MRL/lpr mice with early lupus disease were treated by the injection of anti-Id antibodies directed against either Id.F-423 or Id.IV-228. During these experiments, in contrast to those above, the antibody titres rose in control animals; however, the tendency was for the titres of serum antibodies reactive with either ssDNA (Fig. 3a) or dsDNA (Fig. 3b) to be depressed by the treatment. As shown in Fig. 4, treatment with anti-Id.IV-228 reduced titres of Id.228$^+$ and Id.423$^+$ immunoglobulins and treatment with anti-Id.F-423 reduced Id.423$^+$ and Id.228$^+$ immunoglobulins. Idiotypes Id.II-28 and Id.I-402 were unaffected or only mildly affected by either treatment but, as before, there were notable changes—this time a reduction—in the expression of Id.V-88.

The anti-Id treatment improved kidney function: levels of urine albumin (Fig. 3c), blood urea nitrogen (Fig. 3d) and terminal serum creatinine (control mean ± SD = 1·70 ± 0·18; anti-Id.F-423 = 1·65 ± 0·09; anti-Id.IV-228 = 1·58 ± 0·07

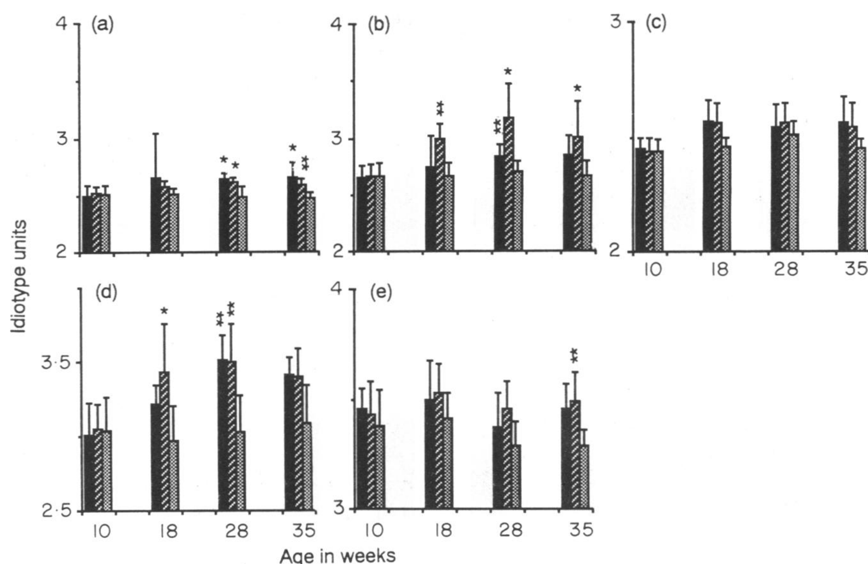

Figure 2. Effects of immunization of MRL/n mice with mAb IV-228-KLH (solid bars), F-423-KLH (hatched bars) or KLH (stippled bars) on the levels of idiotype expression in serum immunoglobulins measured in the capture ELISA. (a) Id.IV-228, (b) Id.F-423, (c) Id.I-402, (d) Id.V-88 and (e) Id.II-28 were measured and results are expressed as $\log_{10}$ units/ml of each individual idiotype calculated from a standard curve for the homologous reference mAb (see Fig. 1 for experimental details and probability values).

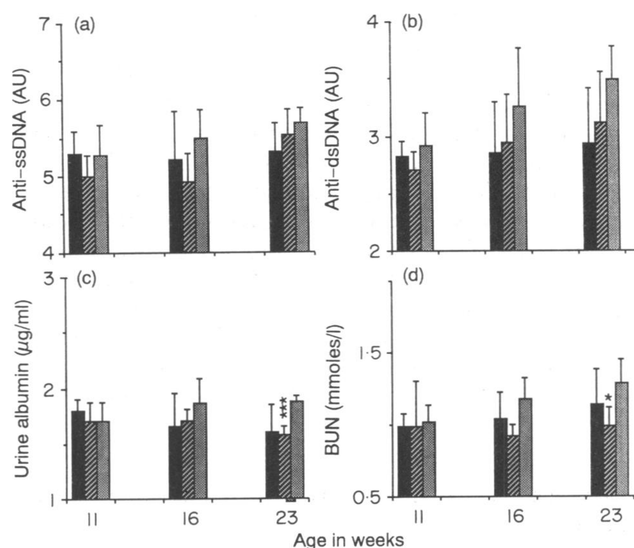

Figure 3. Effects of treating MRL/lpr mice either with anti-Id antibodies against Id.IV-228 (solid bars, $n = 6$) or Id.F-423 (hatched bars, $n = 6$) or with control rabbit Ig (stippled bars, $n = 8$). Sera and urine samples were collected every 3 weeks: only alternate data sets are shown but intermediate data reflected the trends illustrated. Reductions in the levels of serum antibodies reactive with ssDNA (a) or dsDNA (b), albumin in the urine (c) and blood urea nitrogen (d) are consistent with amelioration of disease (probability values, as in Fig. 1, are for comparisons between experimental and control groups at each time interval).

μmoles/l) tended to be lower than in control mice, but creatinine changes did not reach significance. Immunofluorescence examination did not reveal any objective reduction in immunoglobulin deposits in the kidneys, but, considering the poor differential sensitivity of this assay and the fact that high levels of autoantibodies persisted in all mice, this particular observation is unremarkable. However, the changes in blood and urine chemistry and serum antibodies and Id^+ immunoglobulins show that anti-Id antibodies against foetal and adult Id modified some parameters of lupus disease.

DISCUSSION

The mAb F-423 from a foetal MRL/lpr mouse has been shown to react with a number of natural and synthetic polynucleotides. It is very similar immunochemically to mAb BEG-2 derived from a human foetus[12] and to V-88 derived from an adult BWF1 mouse.[9]

The foetal antibody F-423 does not have a private Id, at least as defined with the anti-Id reagents used here, that distinguishes it from other DNA-binding antibodies derived from adults. The public Id of F-423 and the other monoclonals examined are clearly related. We show here that anti-Id.V-88 antibodies made against a mAb derived from an adult BWF1 lupus mouse also reacted with F-423: antibodies against Id.I-402 and Id.II-28 behaved in the same way (data not shown). In a similar fashion, anti-Id.F-423 antibodies reacted with two out of the other four mAb derived from adult mice that were tested here. This foetal mouse mAb F-423 is idiotypically close to the human foetal mAb, BEG-2,[12] which implies that the idiotypic structures or phenotypes of foetal antibodies, like the adult antibodies V-88 (mouse) and 16/6 (human),[15] are conserved. These experiments establish phenotypic associations between the idiotypes of different monoclonals that react with DNA. The frequency of Id sharing found here is reminiscent of the high interconnectivity of foetal antibodies described by others,[6,7] and will be described elsewhere in detail (manuscript in preparation).

Whether the F-423 idiotype identifies the ancestors of pathogenic antibodies that appear in later life is matter for conjecture: it is known that individual mice use different germ-line IgV genes to make DNA-binding autoantibodies of similar specificity,[16] but the critical information that would identify the

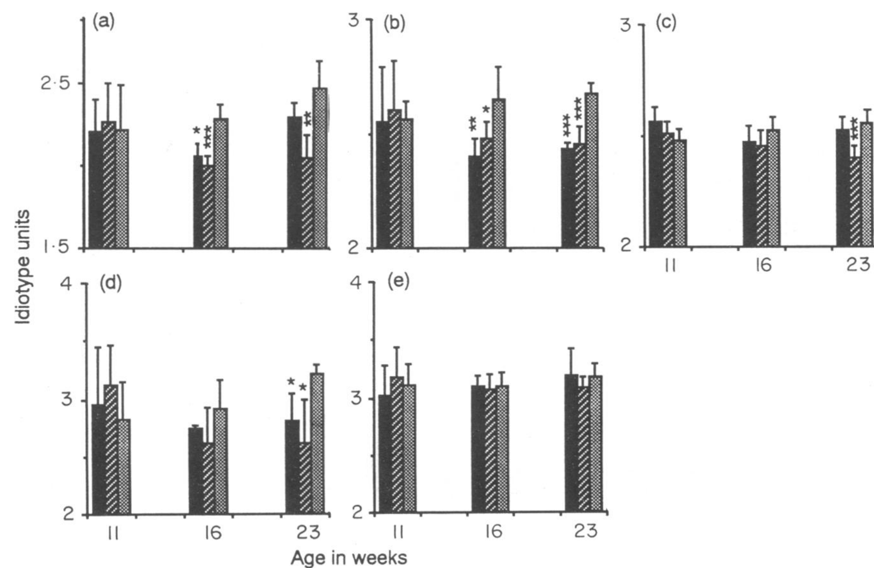

Figure 4. Effects of treating MRL/lpr mice either with anti-Id antibodies against Id.IV-228 (solid bars) or Id.F-423 (hatched bars) or with control rabbit Ig (stippled bars) on the levels of idiotype expression in serum immunoglobulins measured in the capture ELISA (see legend in Fig. 2 for details).

idiotypes of pathogenic antibodies is lacking. The results of the experiments in which immunization with mAb F-423 induced lupus symptoms and in which disease was suppressed by anti-Id.F-423 antibodies, respectively, imply that antibodies like F-423 are themselves pathogenic and/or are idiotypically related and functionally linked to other antibodies that are pathogenic. There was no direct evidence that F-423 was nephritogenic but the fact that it behaved similarly to the adult antibody IV-228 in the *in vivo* experiments reported here supports the former possibility because it has been shown that mAb IV-228 is nephritogenic and will directly augment kidney damage in MRL/lpr mice.[3]

The results of immunizing MRL/n mice with either the foetal antibody F-423 or the adult antibody IV-228 were similar and both treatments induced the symptoms of SLE. Induced lupus of this type was described originally in BALB/c mice and other non-lupus strains.[17,18] This report confirms these findings but it differs from them in that here murine IgG mAb were used, they were linked to a carrier protein (KLH), and MRL/n mice, which would develop SLE later anyway, were the hosts. The other experiments used human IgM mAb, no carrier, lower doses of mAb to immunize, and non-lupus mice as the hosts. Together these models demonstrate that idiotypic linkage between different lymphocyte clones may be implicated to the aetiology of idiopathic SLE.

The idiotypic and immunochemical relationship between F-423 and V-88 is particularly interesting. The antibody V-88 shares an idiotype as defined by a murine monoclonal antibody[15] with a DNA-binding antibody designated 16/6 from a human SLE patient. As we show elsewhere, Id.V-88 appears to relate to lupus pathogenesis in human patients with SLE or Sjögren's Syndrome.[12,19] The changes in the levels of Id.V-88$^+$ immunoglobulins in mice immunized with mAb or treated with their anti-Id antibodies emphasizes that the relatedness of the different Id of DNA-binding autoantibodies extends from the structural to the functional level. The structural homologies between these antibodies will be discussed elsewhere (manuscript in preparation).

The production of antibody following immunization with antibody itself probably represented an Ab3 response to the Ab1 antibody. The intermediate synthesis of Ab2 antibodies is postulated but was not confirmed because a reliable assay for Ab2 mouse–anti-mouse anti-Id antibodies was not available. The experiments on anti-Id therapy do, however, confirm that Ab2 antibodies against Id.F-423 or Id.IV-228 are able to modify both immune responses and the disease processes. In accord with other reports the suppression was incomplete and disease was not completely cured.[1,20-22] In this study, Id different from those of the immunizing mAb were also modulated, and the obvious changes in Id.V-88 expression in both the induction and suppression models was especially interesting. Thus the necessity to tailor anti-Id therapy to the individual may be avoidable if an idiotype, like Id.V-88, is commonly enough expressed to make it a target for universal, but specific, therapy.

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