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Cross-reactive idiotypes in sera from patients with leprosy, lupus and Lyme disease and from healthy individuals

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SUMMARY

Monoclonal IgM autoantibodies have previously been generated from a patient with lepromatous leprosy. Polyclonal anti-idiotypes raised against two of these monoclonal antibodies (8E7 and TH9) were used in an immunoassay to detect the presence of idiotype in human serum. The anti-idiotypes recognize different but overlapping sets of idiotypic determinants, some of which are present on antibodies which bind to *Mycobacterium leprae*. Sera were tested from 16 individuals with leprosy, 45 with systemic lupus erythematosus, 20 with Lyme disease, and 80 healthy subjects. Positive sera were detected in all groups (seven, two, three, and four, respectively). In most cases the serum bound to both anti-idiotypes, the idiotype being present in the IgM and/or IgG fraction. Levels of the two idiotypes varied independently of total serum IgG concentration and, in serial serum samples from one patient, independently of each other. The results indicate that 8E7 and TH9 may be representative of serum antibodies which are commonly expressed in leprosy, but may also be expressed in other diseases and in health; and they suggest that such serum antibodies are encoded by a widely shared set of variable region genes.

Keywords cross-reactive idiotypes leprosy systemic lupus erythematosus Lyme disease

INTRODUCTION

The study of monoclonal autoantibodies has played a major part in the past few years in the investigation of autoimmunity. An important question is whether such monoclonal antibodies represent autoantibodies in the serum of patients. One approach to this problem has been through the study of idiotypes. Cross-reactive idiotypes defined on monoclonal anti-DNA antibodies have been detected in the serum of patients with systemic lupus erythematosus (SLE), and their levels have been shown to parallel disease activity (Isenberg *et al.*, 1984) Moreover, antibodies bearing such idiotypes have been eluted from the kidneys of humans with SLE (Isenberg & Collins, 1985) and mice with lupus-like disease (Gavalchin & Datta, 1987). They have also been detected in a small proportion of normal human sera (Isenberg *et al.*, 1985).

Recently a panel of human monoclonal autoantibodies has been generated from the peripheral lymphocytes of a patient with lepromatous leprosy (Duggan *et al.*, 1988). Despite having a wide range of ligand-binding specificities, a selection of these antibodies has been found to show a considerable degree of idiotypic cross-reactivity; they also share similar idiotypic determinants with certain monoclonal anti-DNA antibodies derived from patients with SLE (Mackworth-Young, Sabbaga & Schwartz, 1987). Two of the leprosy-derived antibodies are representative of the group: 8E7, which binds to mitochondria, and TH9, which binds to the acetylcholine receptor (Duggan *et al.*, 1988).

In order to determine the possible relevance of these leprosy-derived monoclonal antibodies to serum antibodies in patients with leprosy and other conditions, an ELISA has been established to detect the idiotypes of 8E7 and TH9 in human serum. the assay employs two antisera: RId8E7 (raised against 8E7), which recognizes private idiotopes on 8E7, and public idiotypic determinants on several leprosy-derived monoclonal antibodies, including TH9 and antibodies which bind to the *M. leprae* organism itself; and RIdTH9 (raised against TH9) which shows less cross-reactivity, and recognizes predominantly private idiotopes on TH9 (Mackworth-Young *et al.*, 1987).

Patients with leprosy, SLE and Lyme disease were tested, as well as healthy controls. Nearly half of the individuals with leprosy were positive for both idiotypes, as were small numbers of healthy controls and patients with Lyme disease and lupus. These results indicate that the monoclonal antibodies 8E7 and TH9 may be representative of serum antibodies which are expressed commonly in leprosy and relatively infrequently in these other diseases and in health; and they suggest that such serum antibodies may be encoded by a conserved set of variable region genes.

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MATERIALS AND METHODS

Patients

Sera from 16 patients with leprosy (13 men, three women) were tested. They were collected in India, and were a kind gift from Dr J. Ivanyi (MRC Tuberculosis and Related Infections Unit, London). They represented all types of disease: tuberculoid (two), borderline tuberculoid (nine), borderline (one), borderline lepromatous (one), and lepromatous (three). Seven matched sera from healthy individuals living in India were used as controls (also a gift from Dr Ivanyi).

Twenty sera from patients with Lyme disease were tested. Seven had the early manifestation of the condition, erythema chronicum migrans; six had more advanced disease, characterized by neurological abnormalities (mostly meningoencephalitis); and seven had the late manifestation of arthritis. These sera were collected in New Haven, CT, and were a gift from Dr A. C. Steere, New England Medical Center, Boston, MA.

Forty-five sera were obtained from patients attending the Hammersmith Hospital Rheumatology Unit, all of whom fulfilled the American Rheumatology Association criteria for the diagnosis of SLE.

As controls, 73 sera from normal healthy individuals were used. These were mostly employees at the Hammersmith Hospital/Royal Postgraduate Medical School, and were age- and sex-matched with the SLE patients. Most of these (68) were Caucasian, and this group is hereafter called Caucasian controls.

Sera were stored at −20°C until use.

Anti-idiotypes

The generation of IgM monoclonal antibodies 8E7 and TH9 from the peripheral lymphocytes of a patient with lepromatous leprosy has been previously described (Duggan *et al.*, 1988); as has the generation of rabbit antisera RId8E7 and RIdTH9, which recognize idiotypic determinants on 8E7 and TH9 respectively (Mackworth-Young *et al.*, 1987). Both antisera had been purified by extensive absorption on a Sepharose column bearing pooled human myeloma protein. 8E7 binds to mitochondria, while TH9 binds to the acetylcholine receptor (Duggan *et al.*, 1988). However, both monoclonals share at least one idiotope present on other monoclonal antibodies which bind to the *M. leprae* organism itself (Mackworth-Young *et al.*, 1987; and unpublished data).

Preparation of F(ab′)$_2$ fragments

F(ab′)$_2$ fragments of RId8E7 and RIdTH9 were prepared using a modification of a previously described method (Stanworth & Turner, 1986). Briefly, immunoglobulin was purified from the antiserum by absorption onto a protein A–Sepharose column, followed by elution with 0·1 M glycine HCl, (pH 2·8). After dialysis against 20 mM sodium acetate buffer (pH 4·5), the eluate was incubated with immunobilized pepsin at 37°C for 18 h. The enzyme was removed by centrifugation and the remaining solution was neutralized. This was then passed over a protein A–Sepharose column, and the flow-through fraction collected and stored at −20°C or at 4°C in 0·02% azide. A F(ab′)$_2$ fraction of normal rabbit immunoglobulin (NRIg: Sigma Chemical Company, St Louis, MO), previously extensively absorbed on normal human immunoglobulin, was also generated for use as a control.

Measurement of immunoglobulin and F(ab′)$_2$ fragment concentration

The concentration of IgM monoclonal antibody culture supernatants was measured by direct-binding ELISA as previously described (Mackworth-Young *et al.*, 1987). An estimate of the absolute concentration of F(ab′)$_2$ fragment preparation was obtained by optical density (OD) measurement. The relative concentrations were also measured by ELISA. The F(ab′)$_2$ fragments (and NRIg as a control) were coated onto microtitre plates (Flow Laboratories, Irvine, UK) in serial dilutions in 0·05 M borate buffer (pH 8·6) and incubated at 4°C overnight. Unoccupied sites were blocked with 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) (1 h at room temperature), and then alkaline phosphate-linked goat anti-rabbit IgG (Sigma) in 3% BSA–PBS/0·1% Tween 20 (Sigma) was added (overnight at room temperature). Substrate (Sigma) was added in the usual way and the OD was read in a Titertek Multiskan reader at 405 mm. The plates were washed with PBS 1% Tween and PBS between each step. The relative concentrations of the F(ab′)$_2$ fragments were compared using the steep portion of each OD curve. These comparative results obtained by ELISA agreed closely with those obtained by optical density measurement. This confirmed that similar amounts of the F(ab′)$_2$ preparations bound to the ELISA plate when applied at the same concentration (determined by OD measurement).

Anti-idiotype ELISA

In order to detect idiotypes borne by 8E7 (Id8E7) and TH9 (IdTH9) in the serum of patients and controls, a direct binding ELISA was set up. Microtitre wells were coated with F(ab′)$_2$ fragments of RId8E7, RIdTH9 or NRIg at the same concentration in 0·05 M borate buffer (overnight at 4°C). The concentration used was 1 μg/ml as defined by OD. Unbound sites were blocked with 5% BSA–2·5% fetal calf serum (FCS) (Flow) in PBS (1 h at room temperature). Test serum samples were then added at a dilution of 1:1000 in 2·5% BSA–1·25% FCS–PBS/0·2% Tween, and incubated for 2 h at room temperature. In preliminary experiments it had been found that a serum dilution of 1:1000 gave an optimum balance between detectable specific idiotype activity and non-specific background binding. Alkaline phosphatase-linked goat anti-human IgM or anti-human IgG (Sigma) were then added at 1:1000 in the same diluent (overnight at room temperature), and the plates developed and read as above.

In each experiment serial dilutions in 2·5% BSA–1·25% FCS–PBS/0·2% Tween of the culture supernatants of 8E7 and TH9 were used as controls, using alkaline phosphatase-linked rabbit anti-human IgM as a second step. Positive curves were obtained with the homologous anti-idiotypes, i.e. 8E7 binding to the F(ab′)$_2$ fraction of RId8E7 and TH9 to the F(ab′)$_2$ fraction of RIdTH9 (not shown). Neither monoclonal bound to the F(ab′)$_2$ fraction of NRIg, and there was no significant binding to wells coated with borate buffer but no anti-idiotype. Nor was there significant binding to any of the F(ab′)$_2$ reagents by an irrelevant human monoclonal IgM (Serotec, Oxford, UK)

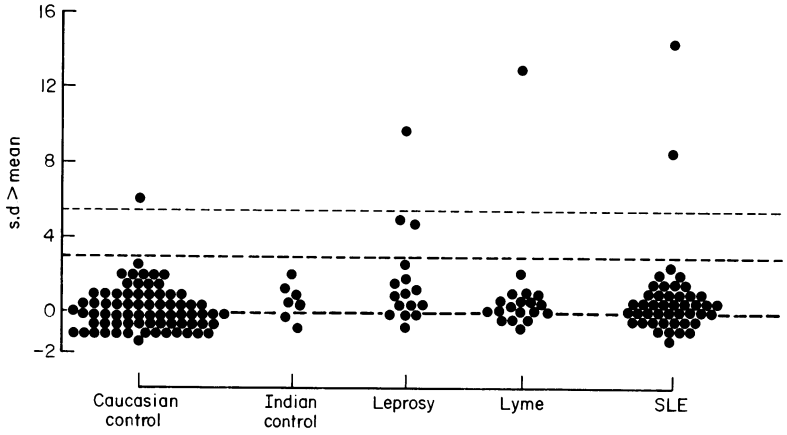

Fig. 1. IgG-specific assay for Id8E7. Scattergram showing results obtained using this assay to test all patient and control groups. Dashed lines indicate (from bottom) mean, 3 s.d., and 5 s.d. of the Caucasian control group.

diluted in culture supernatant of the GM4672 lymphoblastoid cell line used in the generation of the 8E7 and TH9 hybridomas (Duggan *et al.*, 1988).

Serum IgM and IgG concentration

The concentration of total IgM and IgG in serum samples was measured by immunoturbidometry using a Technicon RA-1000 system.

RESULTS

Anti-idiotype ELISA

Control monoclonal antibodies. A solid-phase direct-binding ELISA was developed to detect binding to the $F(ab')_2$ fragments of RId8E7 and RIdTH9. In these assays, 8E7 and TH9 showed weak cross-reactivity with the heterologous anti-idiotype: thus, in a typical assay, the OD produced by the binding of TH9 to $F(ab')_2$ of RId8E7 was 8·0% of that produced by 8E7, while the OD produced by the binding of 8E7 to $F(ab')_2$ of RIdTH9 was 8·3% of that produced by TH9. These values were significantly greater than the binding to the $F(ab')_2$ of NRIg. They were also significantly greater than the binding of an irrelevant human monoclonal IgM (Serotec) to RId8E7 or RIdTH9.

The sensitivity of the assays was determined using 8E7 and TH9 in serial dilutions of a normal human serum. Using the homologous anti-idiotypes, levels down to approximately 20 ng/ml were detected for both monoclonals.

Control sera. Sera from the 73 Caucasian controls were tested for the presence of Id8E7 and IdTH9 by ELISA. This was preformed using alkaline phosphatase-linked anti-human reagents specific for IgM or IgG. For all four of these assays a range of OD measurements was obtained. Weakly positive values were defined as being between 3 and 5 s.d. above the mean; values >5 s.d. were defined as strongly positive. In subsequent experiments control sera were used to enable comparison between assays. The intra- and inter-assay variation was consistently less than 15%.

No Caucasian control sera were positive in IgM assays for either Id8E7 or IdTH9; however, one serum gave a value between 2 and 3 s.d. above the mean for Id8E7, and a fourth gave a similar result for IdTH9. One serum was found to be strongly positive in the IgG assay for Id8E7 (5·6 s.d. above the mean) and for IdTH9 (5·1 s.d.). Another was weakly positive in the IgG assay for IdTH9 only. (Fig. 1; Table 1). In no assay did any of the control sera show significant binding to the $F(ab')_2$ fraction of NRIg, or to wells coated with borate buffer but no anti-idiotype.

Table 1. Positive results for serum samples from patients with leprosy, Lyme disease, and systemic lupus erythematosus (SLE) and from two control groups, tested for the presence of Id8E7 and IdTH9 in IgG- and IgM specific assays

Patient group	Patient no.	IgM		IgG	
		Id8E7	IdTH9	Id8E7	IdTH9
Caucasian control	21	—	—	5·6	5·1
($n=73$)	36	—	—	—	3·4
Indian control	1	4·3	6·0	—	—
($n=7$)	7	7·8	12·6	—	—
Leprosy	2	—	—	4·6	3·8
($n=16$)	6	—	—	9·8	13·9
	8	—	3·4	—	—
	9	3·2	5·1	—	—
	12	4·7	9·0	—	—
	13	—	—	4·5	6·7
	14	3·8	4·3	—	—
Lyme disease	3	7·1	10·9	—	—
($n=20$)	11	7·0	8·2	12·9	14·8
	18	9·4	12·9	—	—
SLE	47	—	—	8·5	10·1
($n=45$)	57	—	—	14·3	13·1

Mean ± s.d. for 73 Caucasian controls.

Values between 3 and 5 s.d. over mean, weakly positive; values >5 s.d., stongly positive.

Test sera. Three of the 16 sera from patients with leprosy were positive in the IgM assays for both Id8E7 and IdTH9, two of them strongly so for IdTH9; a fourth was positive for IdTH9

only. Three further leprosy-derived sera were positive in the IgG assays for both Id8E7 and IdTH9, one strongly for IdTH9, one strongly for both. Six of these patients had borderline tuberculoid leprosy while the seventh (no. 14) had tuberculoid disease.

By comparison, two of the sera from healthy Indian controls were positive for both idiotypes in IgM assays, but none was positive in either IgG assay.

Three of the 20 Lyme disease sera were strongly positive for both idiotypes in IgM assays. The patients were in different clinical groups. One (no. 3) had erythema chronicum migrans, and another (no. 18) had arthritis. The third (no. 11) who had meningocephalitis, was also strongly positive for both idiotypes in the IgG assays.

Of the 45 sera from patients with SLE, none was positive in the IgM assays, while two were strongly positive in IgG assays for both idiotypes. These two individuals had active disease, which included arthritis and nephritis in one (No. 47), and arthritis and anti-phospholipid syndrome in the other (No. 57).

No other test sera were positive in any assay, although there was a wide spread of values within the normal range. No test serum showed significant binding to the $F(ab')_2$ fraction of NRIg, or to wells coated with borate buffer but no anti-idiotype.

Further experiments on selected positive sera showed that IgG binding to $F(ab')_2$ of RId8E7 and RIdTH9 could be inhibited by 8E7 and TH9 respectively, but not by an irrelevant human monoclonal IgM (Serotec) (not shown).

The results of testing the control and test sera in the IgG assay for Id8E7 are shown in Fig. 1, and the positive results of all four assays are summarized in Table 1.

Total immunoglobulin levels

There was no correlation between the degree of positivity in the idiotype assays and the total IgM or IgG concentration in the serum.

Serial idiotype levels

Seven serial serum samples were available from the single individual with Lyme disease (no. 11) whose serum was positive in the IgG idiotype assays. These were obtained over a period of nine months during which the patient initially had meningocephalitis and later developed arthritis, and were a kind gift from Dr A. C. Steere. The patient's case has been previously reported (Mackworth-Young *et al.*, 1988). Most of these samples were strongly positive on the $F(ab')_2$ of both RId8E7 and RIdTH9 when tested in the IgG assays; however, two were negative on RId8E7 and a third was only weakly positive on RIdTH9. The results are shown in Fig. 2 which illustrates that the levels of the 8E7 and TH9 idiotypes varied independently of each other. They also varied independently of the total IgG concentrations (data not shown).

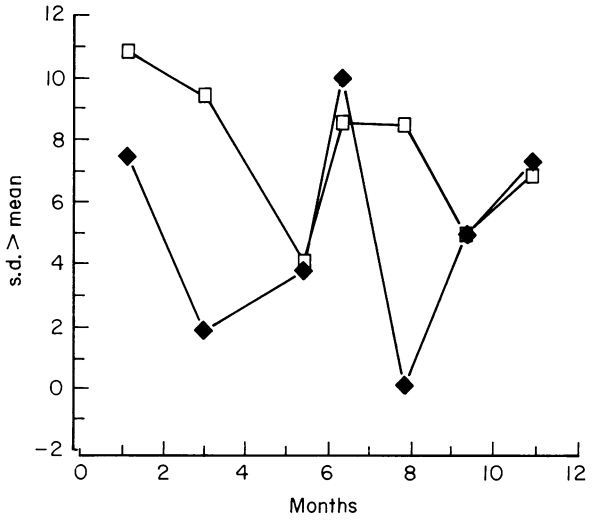

Fig. 2. Idiotype variation with time. Results of testing sequential serum samples from a patient with Lyme disease (No. 11) for the presence of Id8E7 (■) and IdTH9 (□) in an IgG-specific assay. Results are expressed as s.d. above the mean of the Caucasian controls.

DISCUSSION

These experiments have demonstrated the presence of two related idiotypes in human sera of different origin. The idiotypes (Id8E7 and IdTH9) had originally been defined on two monoclonal autoantibodies of different ligand-binding specificities derived from a patient with leprosy, and had been shown to share at least one idiotope (Mackworth-Young *et al.*, 1987; Duggan *et al.*, 1988). The sera were derived from patients with leprosy, Lyme disease and SLE, as well as from healthy normal controls. The results suggest that the monoclonal antibodies 8E7 and TH9 are idiotypically representative of serum antibodies commonly expressed in leprosy, but that this is not disease-specific.

Previous studies of 8E7 and TH9 have shown that they do not bind to the *M. leprae* organism in a solid-phase assay, but they bind certain autoantigens—mitochondria and the acetylcholine receptor respectively. Indeed TH9 has been shown to share idiotypic determinants with other anti-acetylcholine receptor antibodies (Duggan *et al.*, 1988). 8E7 and TH9 share idiotopes with each other, and with other leprosy-derived monoclonal antibodies, including several which bind to *M. leprae* itself (Mackworth Young *et al.*, 1987; and unpublished data). They also share idiotopes with monoclonals derived from patients with SLE of different geographical and racial origin (Mackworth-Young *et al.*, 1987). Taken together these results suggested that 8E7 and TH9 are representative of a subset of B cells which are expanded in both leprosy (possibly in direct response to the organism) and in lupus, and that they are encoded by conserved immunoglobulin variable region genes (*V* genes).

The present experiments are consistent with this. They confirm that idiotypes on 8E7 and TH9 are indeed present in certain human sera, and they suggest that the genes encoding them are present in both Caucasian and Indian populations, and are thus likely to be widely shared. RId8E7 recognizes idiotypic determinants present on antibodies which bind the *M. leprae* organism. However, this study demonstrates that expression of Id8E7 and IdTH9 in serum is not confined to patients with leprosy: patients with Lyme disease and lupus may express them, as may healthy individuals. There was a trend towards greater expression among the Indian leprosy patients (seven out of 16 for IgM and/or IgG positivity) compared with Indian controls (two out of seven) but the numbers are too small for statistical comparison. Compared with the Caucasian (unmatched) healthy subjects (two out of 73 positive) and lupus patients (two out of 45 positive) there was a significant increase

in positivity in the leprosy group ($p<0{\cdot}001$ and $P<0{\cdot}01$, respectively), but the populations were largely of different racial and geographical background. The preponderance of individuals with borderline tuberculoid leprosy who were idiotype positive probably reflects patient selection. The present results in leprosy patients and normals compare with the expression of the related idiotype 16/6, which shares idiotopes with both 8E7 and TH9 (Mackworth-Young *et al.*, 1987). The 16/6 idiotype has been found in 44% of lupus sera and 4% of normal sera; however, this was determined using a different assay system (Isenberg *et al.*, 1985).

The close concordance of positivity for Id8E7 and IdTH9 raised the possibility that the assay may not be detecting true idiotypic interactions. The following evidence suggests that it is: (i) 8E7 and TH9 showed only a small degree of cross-reactivity with their heterologous anti-idiotypes, and an irrelevant human monoclonal IgM was not bound by either; (ii) there was no significant binding to the $F(ab')_2$ fraction of NRIg or to wells coated with borate buffer only; (iii) individual sera showed consistent differences between the degree of positivity for Id8E7 and that for IdTH9; (iv) measurements on serial serum samples from the patient with Lyme disease showed independent variation between levels of positivity for the two idiotypes; (v) binding of selected sera to the $F(ab')_2$ fractions of RId8E7 and RIdTH9 was inhibitable by the homologous monoclonal antibodies; and (vi) there was no correlation between idiotype positivity and total serum IgG levels. However, the possibility has not been formally excluded that the assay detects certain sub-group or allotypic determinants.

It seems likely that the concordance between Id8E7 and IdTH9 positivity is due to the idiotypic relatedness of 8E7 and TH9. Although there are at least three idiotopes on 8E7 (defined by monoclonal antibodies) which are not present on TH9, one or more shared idiotopes are recognized by four separate antisera in cross-idiotype competition assays (Mackworth-Young *et al.*, 1987). These idiotopes are shared by other monoclonal antibodies of different ligand binding specificity and racial origin, and may represent conserved amino acid sequences.

The finding that positivity for Id8E7 and IdTH9 in serum is present in the IgG fraction supports the idea that these idiotypes may be present on serum immunoglobulins of physiological or pathological significance. Ligand binding and other studies of these immunoglobulins, including comparison between the disease and control populations, should help to clarify this question.

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REFERENCES

DUGGAN, D.B., MACKWORTH-YOUNG, C.G. LEFVERT, A.K. ANDRE-SCHWARTZ, J., MUDD, D., MCADAM, K.P.W.J. & SCHWARTZ, R.S. (1988) Polyspecificity of human monoclonal antibodies reactive with *Mycobacterium leprae*, mitochondria, ss DNA, cytoskeletal proteins and the acetylcholine receptor. *Clin. Immunol. Immunopathol.* **49,** 327.

GAVALCHIN, J. & DATTA, S.K. (1987) The NZB × SWR model of lupus nephritis. II. Autoantibodies deposited in renal lesions show a restricted idiotypic diversity. *J. Immunol.* **138,** 138.

ISENBERG, D.A. & COLLINS, C. (1985) Detection of cross-reactive antibody idiotypes on renal tissue-bound immunoglobulins from lupus patients. *J. clin. Invest.* **76,** 287.

ISENBERG, D.A., SHOENFELD, Y., MADAIO, M.P., RAUCH, J., REICHLIN, M., STOLLAR, B.D. & SCHWARTZ R.S. (1984) Anti-DNA antibody idiotypes in systemic lupus erythematosus. *Lancet*, **ii,** 417.

ISENBERG, D.A., SHOENFIELD, Y., WALPORT, M. MACKWORTH-YOUNG, C.G., DUDENEY, C., TODD-POKROPEK, A. BRILL, S., WEINBERGER, A. & PINKAS, J. (1985) Detection of cross-reactive anti-DNA antibody idiotypes in the serum of systemic lupus erythematosus patients and their relatives. *Arthritis Rheum.* **28,** 999.

MACKWORTH-YOUNG, C.G., SABBAGA, J. & SCHWARTZ, R.S. (1987) Idiotypic markers of polyclonal B cell activation: public idiotypes shared by monoclonal antibodies derived from patients with systemic lupus erythematosus or leprosy. *J. clin. Invest.* **79,** 572.

MACKWORTH-YOUNG, C.G., HARRIS, E.N., STEERE,. A.C., RIZVI, F., MALAWISTA, S.E., HUGHES, G.R.V. & GHARAVI, A.E. (1988) Anti-cardiolipin antibodies in Lyme disease. *Arthritis Rheum.* **31,** 923.

STANWORTH, D.R. & TURNER, M.W. (1986) Immunochemical analysis of human and rabbit immunoglobulins and their subunits. In *Handbook of Experimental Immunology* Volume I (ed by D.M. Weir, L.A. Herzenberg, C. Blackwell & L.A.Herzenberg) ch.12 Blackwell Scientific Publications, Oxford.