

Increased IgA rheumatoid factor and V_H1 associated cross reactive idiotypic expression in patients with Lyme arthritis and neuroborreliosis

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Abstract

Objective—To investigate whether autoreactive mechanisms occur in Lyme disease (LD) by determining IgA, IgG and IgM rheumatoid factor (RF) concentrations and RF associated cross reactive idiotypic (CRI) expression in the serum of LD patients, with comparison to patients with rheumatoid arthritis (RA).

Methods—The RF isotype profiles were determined in 59 patients with LD; erythema migrans (EM) (n=19), neuroborreliosis (NB) (n=20) and Lyme arthritis (LA) (n=20). Mouse monoclonal antibodies (mAbs) G6 and G8 (V_H1 gene associated), D12 (V_H3 gene associated) and C7 (V_κIII gene associated) were then used to determine the RF associated CRI expression on IgM antibodies in 16 of these LD patients (eight seropositive for RF); (EM (n=3), NB (n=6), LA (n=7)).

Results—Seven (18%) patients with either NB or LA had increased concentrations of IgA RF compared with none with EM. Significant differences in the number of patients with raised concentrations of IgG RF or IgM RF were not found between the LD patient groups. Five (3NB, 1LA and 1EM) (31%) and three (2NB and 1LA) (19%) of LD patients had raised concentrations of the CRIs recognised by mAbs G6 and G8, respectively. These CRIs were detected in LD sera both with and without raised concentrations of RF and were not demonstrated on anti-*Borrelia burgdorferi* antibodies using ELISA. No LD sera tested had raised concentrations of the determinants recognised by mAbs C7 or D12.

Conclusion—Significantly raised concentrations of IgA RF and increased use of V_H1 germline gene associated CRIs are found on IgM antibodies in the serum of LD patients. These data indicate the recruitment of autoreactive B lymphocytes in some patients with the later stages of LD.

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Lyme disease (LD) is a multi-system disorder caused by the spirochaete *Borrelia burgdorferi* (Bb) *sensu lato* and transmitted by Ixodid ticks.¹ The initial manifestation of the disease is the characteristic skin lesion, erythema migrans (EM) (stage 1). This may be followed weeks to months later by lymphocytic meningitis, facial

palsy, radiculopathy, cardiac conduction disorders and migratory musculoskeletal pain (stage 2). Months to years later intermittent or continuous attacks of monoarthritis or oligoarthritis, chronic neurological manifestations and the rash, *acrodermatitis chronica atrophicans* may occur (stage 3).

In a North American study of patients with untreated EM, over half developed one or more episodes of intermittent monoarthritis or oligoarthritis lasting less than one year and 11% developed chronic synovitis in one to three large joints for more than one year, more than a third of whom had radiographic evidence of erosive disease.² Infection and autoimmunity are not mutually exclusive and there is some evidence to suggest that autoimmunity may occur in some patients with chronic Lyme arthritis (CLA). Firstly, in approximately 10% of American patients with Lyme arthritis (LA), the arthritis persists for months or even several years after the apparent eradication, by antibiotic therapy, of the spirochaete from the joint.³ These patients have cellular and humoral immunity to outer surface protein A (OspA) and increased frequency of HLA DR4,⁴⁻⁷ the haplotype associated with rheumatoid arthritis (RA) disease severity.⁸ Secondly, increased expression of the autoantibody associated idiotypic 16/6 occurs on IgA antibodies in patients with CLA⁹ and thirdly, MHC Class II restricted autoreactive T cells, (to irradiated, autologous peripheral blood lymphocytes) have also been found in LA.¹⁰ Furthermore, antibodies to cardiolipin and normal human axons occur in some patients with neuroborreliosis (NB).¹¹⁻¹²

Rheumatoid factors (RFs) are autoantibodies directed against the Fc region of IgG occurring in serum as IgM, IgA or IgG isotypes and are found in most patients with RA.¹³ RFs readily form immune complexes with autologous IgG and may be of central importance in the pathogenesis of RA synovitis.¹⁴ The clinical significance of the different RF isotypes is uncertain, however, several studies have found IgA RF in particular to be associated with bone erosion and disease activity.¹⁵⁻¹⁷ Two previous studies have reported raised concentrations of IgM RF in some patients with LA¹⁸⁻¹⁹ though other RF isotypes have not been determined in LD.

Idiotypes are serologically defined antigenic determinants expressed within the variable region of immunoglobulin molecules.²³ Cross reactive idiotypes (CRI) are expressed on

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different antibodies of the same or different specificities²¹ and are serological markers for immunoglobulin variable regions encoded by individual or a small set of highly related germline, or minimally mutated germline genes.²²⁻²³ Several CRIs have been found on IgM RFs including those recognised by D12, G6 and G8 mAbs. The determinant recognised by mAb C7 is expressed on all V_HIII light chains,²⁶⁻²⁷ mAb D12 recognises a V_HIII gene associated CRI and G6 and G8 recognise two CRIs encoded by genes from the V_H1 family. These CRIs are either absent or only expressed at very low levels in control subjects suggesting that highly conserved germline genes are important in the production of some autoantibodies.

In this study we have determined the IgA, IgG and IgM RF concentrations in the serum of LD patients and further examined the RF associated CRI expression using the panel of mouse monoclonal anti-idiotypic reagents. To provide further insight into potential LD auto-reactive mechanisms, these data are compared with those from patients with RA.

Methods

PATIENTS

Lyme disease patients

Sera were obtained from 59 well characterised American patients with LD: EM (n=19, 9 male, mean age 35 years, range 21–72), NB (n=20, 9 male, mean age 29 years, range 6–60) and LA (n=20, 13 male, mean age 37 years, range 12–61). The average disease duration at the time serum was obtained was three months (range 0.5–13 months) for patients with NB and 28 months (range 1–94 months) for patients with LA. Twelve patients had CLA, defined as continuous joint swelling for more than one year. Joint swelling was assessed at the time the sample was taken and graded zero to four.

Rheumatoid arthritis patients

Sera were obtained from 44 patients who met the revised ACR criteria for RA²⁰ (7 male, mean age 62 years, range 32–93). Average disease duration 15 years, range 5–50 years.

Control subjects

Control subjects consisted of patients with regional rheumatic disorders and osteoarthritis, who were otherwise healthy (n=40, 13 male, mean age 47 years, range 22–85).

MEASUREMENT OF IgG AND IgM ANTIBODIES TO BORRELIA BURGDORFERI AND ANTIBODIES TO OUTER SURFACE PROTEINS A AND B

This was carried out as previously described in the LD laboratory at New England Medical Center.²⁴ IgG and IgM anti-*Bb* antibodies were determined by ELISA and results expressed as a titre, antibodies to OspA were also determined by ELISA and results recorded as optical density (OD) values. Antibodies to outer surface protein B (OspB) were determined by immunoblotting and therefore expressed only as present or absent.

RHEUMATOID FACTOR ASSAYS

RF ELISA

One half of 96 well Immulon 1 immunoassay plates (Dynatech, UK) were coated with 100 µl of a 4 µg/ml (2 µg/ml for IgG RF measurement) solution of human IgG Fc (The Binding Site Ltd, Birmingham, UK) in carbonate-bicarbonate buffer (pH 9.6) by incubation overnight at 4°C. The plates were blocked with 3% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for one hour at 37°C. Serum samples were diluted 1 in 300 with PBS containing 0.05% Tween-80 (BDH, UK) and 1% BSA. One hundred µl of each sample was added in duplicate to both the antigen coated wells and uncoated control wells and incubated for one hour at 37°C. A positive control was also added in duplicate to each plate. IgG RF was detected using biotinylated sheep antihuman IgG Fd (The Binding Site Ltd, Birmingham, UK) and subsequently with streptavidin-HRP (Amersham, Buckinghamshire, UK). IgA and IgM RF were detected using biotinylated F(ab')₂ fragment of sheep antihuman α chain (Antibody binding site) or antihuman μ chain (Amersham). Results were recorded as OD values at 450 nm.

IDIOTYPE ASSAYS

Seventeen RA sera, 11 control sera and 16 LD sera (eight with the highest concentrations of IgA and/or IgM RF and eight without raised RF concentrations) were assayed for the presence of the RF associated CRIs as recognised by the mAbs G6, G8, C7 and D12 on IgM antibodies as previously described.²²⁻²⁵⁻²⁷ Briefly, ELISA plates were coated with the F(ab')₂ fragments of the mouse monoclonal anti-idiotypic antibodies in carbonate buffer (at G8: 2.5 µg/ml; C7, G6 and D12: 5 µg/ml) by incubation overnight at 4°C. The other half of the plate was coated with the same concentration of antibody G4, a control mouse IgG1 mAb.²⁵⁻²⁷ The plates were blocked with 3% BSA in PBS for one hour at 37°C and serum samples diluted 1 in 200 in PBS/Tween-80 and 1% BSA. One hundred µl of each sample was added in duplicate to both halves of the plates and incubated for one hour at 37°C. Bound IgM antibodies were revealed with biotinylated sheep antihuman IgM (Amersham) and streptavidin-HRP, as above. Background binding to the G4 coated control wells was subtracted from the readings and results expressed as a ratio of the mean OD of the test sample to the positive control.

DETECTION OF THE RF ASSOCIATED CRIs ON ANTI-B BURGDORFERI ANTIBODIES

One half of 96 well Immulon 1 immunoassay plates were coated with 100 µl of a 10 µg/ml suspension of sonicated *B burgdorferi* (multiple passaged strain of B31) in carbonate buffer by incubation overnight at 4°C, blocked and incubated with sera diluted 1/500 in PBS. Mouse monoclonal anti-CRI antibodies G6 and G8 diluted 1/32000 in PBS were added and bound antibodies revealed with biotinylated rabbit antimouse Ig and streptavidin-HRP. Background binding to the uncoated control wells

Table 1 Rheumatoid factor concentrations in patients with Lyme disease and rheumatoid arthritis (mean (SD) and range)

Subjects	IgA RF concentrations	IgG RF concentrations	IgM RF concentrations
Controls (n=40)	0.08 (0.04) (0.00–0.19)	0.38 (0.22) (0.04–0.88)	0.08 (0.10) (0.01–0.60)
Erythema migrans (n=19)	0.08 (0.06) (0.00–0.20)	0.17 (0.10) (0.01–0.37)	0.09 (0.12) (0.02–0.53)
Neuroborreliosis (n=20)	0.12 (0.17) (0.00–0.64)	0.19 (0.14) (0.00–0.47)	0.14 (0.17) (0.01–0.70)
Lyme arthritis (n=20)	0.18 (0.22) (0.00–0.92)	0.25 (0.17) (0.02–0.54)	0.14 (0.14) (0.02–0.45)
Rheumatoid arthritis (n=44)	0.85 (0.60) (0.02–2.16)	0.87 (0.48) (0.01–1.95)	0.76 (0.29) (0.09–1.06)

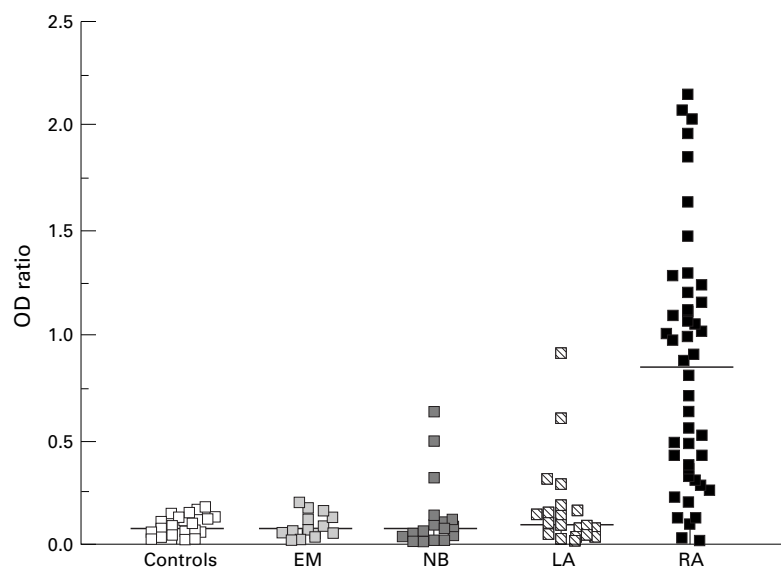


Figure 1 IgA RF concentration in controls and patients with erythema migrans (EM), neuroborreliosis (NB), Lyme arthritis (LA) and rheumatoid arthritis (RA). Bar represents median value.

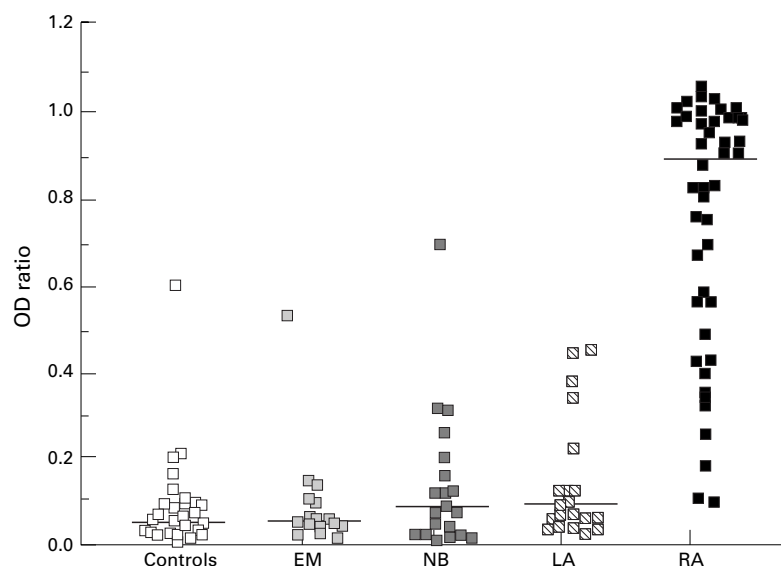


Figure 2 IgM RF concentration in controls and patients with EM, NB, LA and RA. Bar represents median value. Abbreviations as in figure 1.

was subtracted and results expressed as mean OD values.

STATISTICAL ANALYSIS

The coefficient of variation of the RF assays was IgM (5%), IgG (18%) and IgA (13%) and the idiotype assays G6 (2%), G8 (7%), C7 (9%), D12 (5%). Raised concentrations of IgA, IgG and IgM RF and CRI recognised by the G6, G8, C7 and D12 mAbs were defined as

OD ratios greater than three standard deviations (SD) above the mean OD ratio of the control population. Differences in the number of positives between groups were compared by a Fisher's exact test.

Results

ANTIBODIES TO BORRELIA BURGDORFERI

All patients with NB and LA had increased concentrations of IgG antibodies to *Bb* 81% had increased concentrations of IgM antibodies to *Bb*.

ANTIBODIES TO OSP A AND B

Antibody concentrations to OspA and B were determined in 13 patients with LA. Twelve (92%) had raised concentrations of anti-OspA antibodies and nine (69%) had raised concentrations of antibodies to OspB.

RHEUMATOID FACTOR ISOTYPES (TABLE 1, FIGS 1 AND 2)

Controls

No control had raised concentrations of IgA or IgG RF. One (2%) control had raised concentrations of IgM RF.

RA sera

IgA, IgG and IgM RF concentrations were raised in 39 (89%), 13 (30%) and 38 (86%) of RA patients sera respectively.

Lyme disease sera

Increased concentrations of IgA RF were found in seven (18%) patients with the later stages of LD (3 NB and 4 LA) compared with no patients with EM. No LD serum samples had raised concentrations of IgG RF. Five LD patients had increased concentrations of IgM RF; 1 EM, 1 NB and 3 LA. Neither IgA nor IgM RF concentrations correlated with duration of arthritis or degree of joint swelling.

EXPRESSION OF CRI (TABLE 2, FIGS 3 AND 4)

Rheumatoid arthritis sera

Eight (47%) of 17 sera had raised concentrations of G6 CRI, seven (41%) had raised concentrations of G8, two (12%) C7 and one (6%) D12. All of the patients with raised CRI concentrations had raised concentrations of IgA and IgM RF and seven (41%) had raised concentrations of IgG RF.

Lyme disease sera

Five (31%) of 16 sera had raised concentrations of the CRI recognised by mAb G6. Three of these were seropositive for IgA RF and/or IgM RF (2 NB, 1 LA) and two were seronegative for RF (1 NB, 1 EM). Three (19%) had raised concentrations of the CRI recognised by mAb G8: two of these patients had NB and were seropositive for IgA RF and one had LA and was seronegative for RF. None of the LD sera tested had raised concentrations of CRIs recognised by mAbs C7 or D12. The only LA patient with significantly raised concentrations of G8 (OD ratio 0.42) had very marked joint swelling (grade 4), in the three other patients with grade 3 or 4 joint swelling the CRI recognised by mAb G8 idiotype was not measured.

Table 2 Cross reactive idiotype concentrations in patients with Lyme disease and rheumatoid arthritis (mean (SD) and range)

Subjects	C7	D12	G6	G8
Controls (n=11)	0.60 (0.10) (0.39–0.78)	0.44 (0.15) (0.26–0.70)	0.06 (0.06) (0.00–0.143)	0.07 (0.09) (0.00–0.25)
Lyme disease (n=16)	0.59 (0.14) (0.15–0.78)	0.55 (0.22) (0.00–0.90)	0.21 (0.22) (0.01–0.90)	0.17 (0.29) (0.00–1.05)
Rheumatoid arthritis (n=17)	0.73 (0.14) (0.49–1.00)	0.56 (0.18) (0.36–1.00)	0.34 (0.32) (0.00–1.00)	0.41 (0.34) (0.00–1.10)

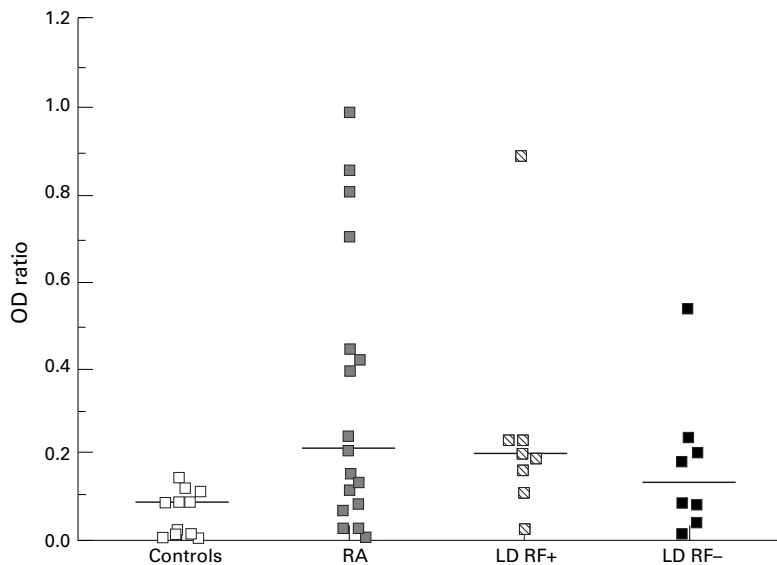


Figure 3 G6 CRI concentrations in controls, patients with RA and patients with LD seropositive (LDRF+) and seronegative (LDRF-) for RF. Bar represents median value.

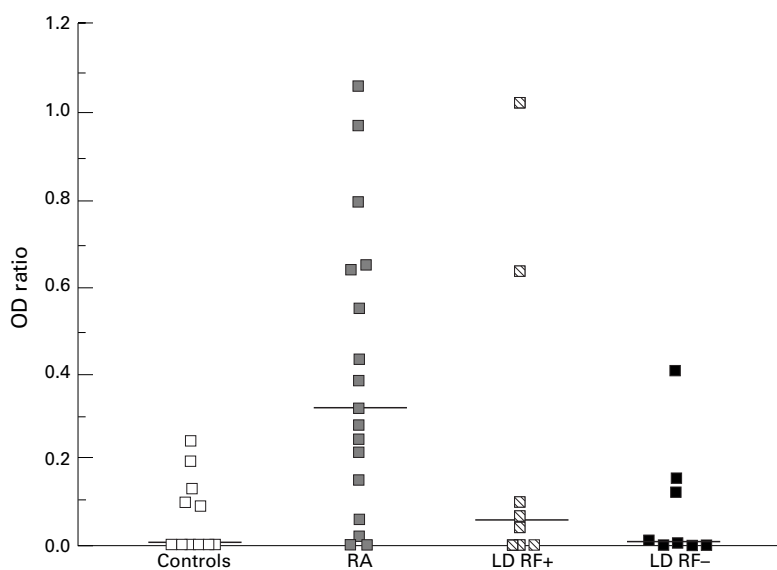


Figure 4 G8 CRI concentrations in controls, patients with RA and patients with LD seropositive (LDRF+) and seronegative (LDRF-) for RF. Bar represents median value.

EXPRESSION OF RFs AND CRIs MEASURED BY MABS G6 AND G8 AND B BURGENDORFERI ANTIBODIES
Antibodies expressing CRIs recognised by the G6 and G8 mAbs did not bind to *Bb*; and there was no difference in binding between CRI positive and CRI negative sera to *Bb*. RF concentrations did not correlate with either IgG or IgM anti-*Bb* antibody titres or anti-OspA or OspB antibody concentrations. One patient with NB who had the highest concentrations of both the CRIs measured by G6 and G8 (G6 ODR = 0.90, G8 ODR = 1.05) also had very high IgG anti-*Bb* antibody concentrations (titre

= 1/32 000) but not IgM anti-*Bb* antibodies. Anti-OspA and B antibodies were not measured in this patient. The number of patients in whom both CRI expression and anti-*Bb* antibody titres (eight in total) and/or anti-OspA and B antibody concentrations (five in total) were measured was too small to determine whether any correlation existed between these groups.

Discussion

In this study we have demonstrated, for the first time, raised concentrations of IgA RF in some patients with the later stages of LD. Concentrations of IgA RF were raised in 15% of patients with NB and 20% of patients with LA but not in early LD or control subjects. IgM RF was raised in 5% of patients with EM, 5% of patients with NB and 15% of LA patients and these differences were not significant. In some of the LD patients, serum RF concentrations were similar to the concentrations of RF in RA patients (figs 1 and 2). Further analysis demonstrated raised concentrations of the V_{H1} gene encoded RF associated CRIs recognised by G6 and G8 mAbs on IgM antibodies in the serum of some of the LD patients, but not of the V_{KIII} subgroup of light chains or the V_{H3} associated CRI recognised by the D12 mAb. Idiotype expression was not examined on IgA and IgG antibodies. The number of RA patients with raised concentrations of the studied CRIs was similar to that found in previous studies.^{25,26} The idiotypes were detected in LD sera both with and without raised concentrations of RF and were not demonstrated on anti-*Bb* antibodies in ELISA. There was no correlation between RF or CRIs and IgG or IgM anti-*Bb* antibodies or antibodies to OspA or B. We found no correlation between the presence of RF and duration or severity of LA. The only patient with LA in whom significantly raised concentrations of the CRI recognised by G8 mAb were found had very marked joint swelling. In the three other patients with grade 3 or 4 joint swelling concentrations of the CRI recognised by G8 mAb were not measured.

Two important points arise from these data. Firstly, this is the first study showing raised concentrations of IgA RF in some patients with stage II and III LD. There is evidence that in RA increased concentrations of IgA RF are associated with a worse prognosis^{15,17} and IgA antibodies bearing the 16/6 CRI are known to correlate with the development of LA.⁹ It may be, therefore, that IgA antibodies are particularly important in the pathogenesis of the later stages of LD. However, we found no correlation between IgA RF and duration or severity of arthritis in our LD patients. IgA RF concentrations did not correlate with anti-*Bb* antibodies and it therefore seems unlikely they were simply the result of polyclonal B cell activation. There is a possibility that IgA RF may be performing some form of normal physiological regulatory function. IgM RF is produced at low level and briefly in otherwise healthy persons after infections, however IgA RF have not been reported in these circumstances. In the subjects investigated in this instance there seems to have

been an immunoglobulin isotype switch to IgA RF and this has been sustained. What is driving the production of these antibodies may be open to speculation but B cell autoreactivity is a possible cause.

Secondly, our data demonstrated restricted expression of the RF associated CRIs recognised by G6 and G8, but not the V_KIII subgroup or CRI recognised by D12 on IgM antibodies in LD (CRIs were not determined on IgA or IgG antibodies). This suggests the use of these highly conserved V_H1 germline gene encoded CRIs, which are frequently associated with RF, in LD patients. The CRIs however, were detectable in sera with and without raised concentrations of RF showing that antibodies, other than RFs, encoded by the V_H1 genes may be produced in LD. Further analysis indicated that the CRIs were not present on antibodies to OspA or OspB and surprisingly, these CRIs were not demonstrated on anti-*Bb* antibodies in ELISA. This may indicate that the antibodies expressing these CRIs are not directed against *Bb*, however, there are three other possible explanations.

Firstly, the antibodies may have been directed against epitopes not present on the strain of *Bb* used in our experiments. This may have arisen as a result of loss of expression of the relevant antigen by the use of a multiple passaged strain of *Bb* or the antigens may only be expressed on genotypes of *Bb* other than *Bb sensu stricto* to which genogroup the strain we used belongs. Secondly, the antibodies that express these CRIs may be directed against non-protein components of *Bb* that are destroyed by the antigen preparation methods used.²⁹ Thirdly, the inability to detect CRI on antigen bound antibody may be attributable to steric hindrance as the CRI is involved directly in binding of antibody to the antigen.

In conclusion, we have found increased concentrations of IgA RF in patients with the later stages of LD compared with patients with EM. Neither the presence nor concentration of RF correlated with the duration or severity of LA and the relevance of these autoantibodies remains uncertain in the pathogenesis of the reactive arthritis that occurs in LD. We have also shown increased usage of the V_H1 germline gene associated CRIs recognised by the G6 and G8 mAbs in antibodies produced in LD. The genes that encode for protein products expressing these CRIs are associated with autoantibody production in RA and these data indicate that autoreactive B cells are expanded in some patients with LD.

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