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Isoelectric focusing characterization of IgM-V_{Kiiib} immunoglobulin light chains and their association with anti-IgG autoantibodies in essential mixed cryoglobulinaemia, Sjögren's syndrome and rheumatoid arthritis

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SUMMARY

Previous studies have demonstrated that the IgM monoclonal anti-IgG autoantibodies (AGAs) characteristic of essential mixed cryoglobulinaemia (EMC) display preferential use of κ light chains of the V_{Kiiib} sub-subgroup. In order to gain insights as to the possible basis for this V region selection, IgM-V_{Kiiib} immunoglobulin was affinity purified from normal human serum, analysed by dissociating two-dimensional gel electrophoresis and compared to the two-dimensional gel patterns of IgM-V_{Kiiib} anti-IgG autoantibodies (AGAs) from patients with essential mixed cryoglobulinaemia (EMC). The results suggest that only part of the available V_{Kiiib} light chain repertoire is selected by EMC AGAs. When AGAs from EMC, rheumatoid arthritis (RA) and primary Sjögren's syndrome (SS) patients were analysed by ELISA, it was found that the association of V_{Kiiib} light chains with anti-IgG autoantibodies differed significantly among the three diseases. In fact, in RA there appeared to be a negative selection against the use of V_{Kiiib} in AGAs. Clearly, the V_{Kiiib} determinant is not required for anti-IgG autoreactivity. The possibility emerges, therefore, that the genesis and perpetuation of AGA synthesis in these diseases may follow quite different pathways.

INTRODUCTION

Anti-IgG autoantibodies (AGAs) are found at high serum titres in patients with many diseases, and occasionally at low levels in normal, healthy individuals (Bartfeld, 1969). The mechanisms that activate the B-cell subset(s) responsible for the synthesis of these AGAs are not well understood; however, the transient appearance of AGAs in a variety of acute and chronic infections and following immunization suggests that they may be a potential component of the normal immune response (Welch *et al.*, 1983; Tarkowski, Czerkinsky & Nilsson, 1985; Nemazee & Sato, 1982). Indeed, their production during polyclonal B-cell activation of both cord and adult peripheral blood lymphocytes provides further evidence to suggest that they are a natural and frequently induced antibody (Slaughter *et al.*, 1978; Rodriquez, Ceuppens & Goodwin, 1982). Thus, during normal immune homeostasis the production of AGAs may be tightly regulated. In the sera of most patients with Sjögren's syndrome (SS), essential mixed cryoglobulinaemia (EMC) and rheumatoid arthritis (RA) there are persistently high titres of circulating AGAs, which may contribute to the pathophysiology of these diseases. In these disorders it is not known whether the production of these antibodies is induced by an unrecognized antigenic stimulus, by an antigen-independent stimulus, or by a basic defect in the immune regulatory networks.

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Due to their monoclonal character and high serum concentrations, the best-characterized anti-IgG autoantibodies are those found in EMC. These AGAs are uniformly IgM and almost exclusively have kappa light chains. The majority of these belong to the V_{Kiiib} sub-subgroup, which partially if not completely overlaps the Wa cross-reactive idiotypic group (Kunkel *et al.*, 1974; Ledford *et al.*, 1983). Primary amino-acid sequence analysis of these V_{Kiiib} light chains reveals that the V regions from different patients can be virtually identical, suggesting the utilization of a germ-line V gene (Goni *et al.*, 1985; Pons-Estel *et al.*, 1984), a concept supported by the recent isolation and DNA sequencing of a V_{Kiii} germ-line gene (Radoux *et al.*, 1986).

In contrast to the findings in EMC, AGAs in RA and SS are polyclonal or oligoclonal IgM; frequently, IgG and IgA anti-IgGs are also present (Ziff, 1985). Although preferential selection of kappa light chains also occurs in these AGAs (Fong *et al.*, 1981; Carson & Lawrance, 1978), the distribution and selection of the V_K subgroups have not been studied extensively. If polyclonal or oligoclonal AGAs in RA and primary SS preferentially associate with V_{Kiiib}, then it may be acceptable to view the monoclonal AGAs in EMC as a model for anti-IgG

autoantibodies and their generation. Alternatively, EMC AGAs may represent an unique autoantibody and may not support extrapolation to the much more common AGAs found in RA and related diseases.

Thus, the purpose of this paper is to examine V_{Kiiib} sub-subgroup selection in AGAs from different diseases. Two approaches have been used. First, we compared the isoelectric focusing (IEF) heterogeneity of light chains of affinity-purified IgM-V_{Kiiib} isolated from the serum of normal young adults, and from cryoprecipitated EMC AGAs. Then, we determined the association of V_{Kiiib} light chain with AGAs from the serum of patients with EMC, RA and SS. The results of these experiments suggest that very different mechanisms are stimulating and perpetuating AGA production in these diseases.

MATERIALS AND METHODS

Antibodies

Murine monoclonal antibody (MoAb)-producing clones were grown intraperitoneally in CAF_1 mice, ascites were collected and used at dilutions of 1:500 to 1:1000. Anti-kappa containing ascites was produced from ATCC clone HB-61, and anti-mu containing ascites was from ATCC clone HB-57 (ATCC, Bethesda, MD). MOPC 141 ascites was a gift from Dr Clark Anderson (Ohio State University, Columbus, OH). A description and characterization of MoAb IgG2b anti-V_{Kiiib} (JG-B1) has been published (Greenstein, Solomon & Abraham, 1984). All antibodies covalently linked to Sepharose 4B were purified from ascites by DE-52 chromatography before coupling to CNBr pre-activated Sepharose 4B according to the manufacturer's instructions (Pharmacia, Piscataway, NJ).

Affinity-purified $F(ab')_2$ sheep anti-mouse IgG antibody was purchased from Cappel Inc. (Cochranville, PA) and conjugated to alkaline phosphatase with glutaraldehyde for use in the enzyme-linked immunosorbent assay (ELISA) (Engvall & Perlman, 1972). Affinity-purified $F(ab')_2$ goat anti-human IgM (mu-chain specific) was purchased from TAGO Inc. (Burlingame, CA) for use in the IgM affinity capture ELISA.

IgG1/lambda (MIT) and IgG2/lambda (UPD) human myeloma proteins, used as antigen in the anti-IgG capture ELISA, were purified from serum by sodium sulphate precipitation followed by DE-52 chromatography. The IgG fractions were monoclonal and free of kappa light chain by ELISA. The IgM/V_{Kiiib} EMC AGA, TEH (Mathison *et al.*, 1971) was purified from the plasma by cryoprecipitation at 4°, washed three times with chilled 0·015 M phosphate buffer, pH 7·4, redissolved at 37° and applied to a DE-52 column equilibrated in 0·03 M phosphate buffer, pH 7·4. After extensive washing with 0·03 M phosphate buffer at 37°, the IgM was eluted with 0·15 M phosphate buffer, pH 7·4, and showed strong anti-IgG activity by slide latex agglutination. Two-dimensional gel electrophoresis demonstrated the monoclonality of the IgM and absence of IgG antigen. EMC AGAs DON, GRE, GLO, and MAT were purified from patients' sera by cryoprecipitation and extensive cold 0·1 M borate/0·15 M NaCl, pH 7·4, (BBS) washings. After the last cold wash the cryoprecipitate was redissolved in warm BBS and allowed to recryoprecipitate at 4°.

ELISA

The ratio of V_{Kiiib} light chains to kappa light chains in total IgM, (V_{Kiiib}/κ)IgM, was determined in normal volunteers and RA patients with an IgM affinity-capture ELISA. Standard 96-well polyvinyl assay plates (Costar 2595, Cambridge, MA) were coated with affinity-purified $F(ab')_2$ goat anti-human IgM, 10 μg per ml coating buffer (15 mM Na_2CO_3/35 mM $NaHCO_3$/3 mM NaN_3 pH 9·6), followed by post-coating with 2% BSA. Then, doubling dilutions of TEH were added for the construction of standard curves; or, alternatively, dilutions of test sera were pipetted into the wells. Subsequent steps were the addition of mouse monoclonal anti-μ, anti-κ, or anti-V_{Kiiib}; alkaline phosphatase-labelled $F(ab')_2$ sheep anti-mouse IgG; and disodium *p*-nitrophenyl phosphate in diethanolamine substrate buffer. After the addition of substrate, colour development was quenched after a set time by the addition of 5 N NaOH and optical densities recorded at 405 nm on an automated ELISA reader. For each test serum, triplicate determinations of μ, κ, and V_{Kiiib} were quantified by comparison with the three separate μ, κ, and V_{Kiiib} standard curves derived from TEH. All antibody–antigen reaction steps were at 37° for 2 hr, and each step was followed by at least four washes with BBS.

The ratio of V_{Kiiib} light chains to kappa light chains in the anti-IgG autoantibodies, (V_{Kiiib}/κ)AGA, was determined by an anti-IgG affinity-capture ELISA. This ELISA was identical to the previous one, except that wells were coated with the IgG myeloma proteins MIT and UPD (20 μg IgG/ml coating buffer, 1:1), to which the anti-IgG autoantibodies bind. Subsequently, the amounts of μ, V_{Kiiib}, and κ were quantified by ELISA with the specific MoAbs anti-μ, anti-V_{Kiiib}, and anti-κ. In both ELISAs the linear correlation coefficients for the standard curves (OD_{405} *vs* dilution) were at least 0·98.

Affinity purification of proteins

IgM-V_{Kiiib} was obtained by first fractionating normal serum from two healthy, young adults on a BBS Sephadex G200 gel filtration column. The high molecular weight peak was conservatively pooled, absorbed on a mouse MOPC 141 (IgG2b) myeloma protein coupled Sepharose 4B column, and then absorbed onto an anti-V_{Kiiib} antibody (IgG2b)-coupled Sepharose 4B column. IgM kappa from the serum of a normal, healthy volunteer was similarly obtained by Sephadex G200 column chromatography followed by adsorption on the mouse IgG2b column, and finally affinity adsorption on a murine MoAb anti-kappa antibody-coupled Sepharose 4B column. After adsorption, the anti-V_{Kiiib}, anti-κ and sham mouse IgG2b columns were washed extensively with 0·1 M borate/0·5 M NaCl, pH 7·2, followed by 0·015 M phosphate, pH 7·2. The bound material was eluted with 0·1 M glycine/HCl, pH 2·5, dried under vacuum and stored at −20° until analysis. In neither purification could material be eluted from the sham mouse IgG2b-coupled Sepharose 4B column. The specificity of these columns is further demonstrated by the lack of lambda light chains after two-dimensional gel electrophoretic analysis. In addition, non-V_{Kiiib} human myeloma IgGs and IgMs did not adsorb to the anti-V_{Kiiib} column.

Two-dimensional gel electrophoresis

Two-dimensional gel electrophoresis was performed in 5× 100 mm cylindrical IEF gels in the first dimension at room temperature, and on 10% SDS–PAGE slab gels at 4° in the second dimension (O'Farrell, 1975). The samples were dissolved in sample buffer containing 8 M urea/5% 2-mercaptoethanol/2% NP-40/and a 2% ampholine blend, pH 3·5–9·5 (LKB, Rockville,

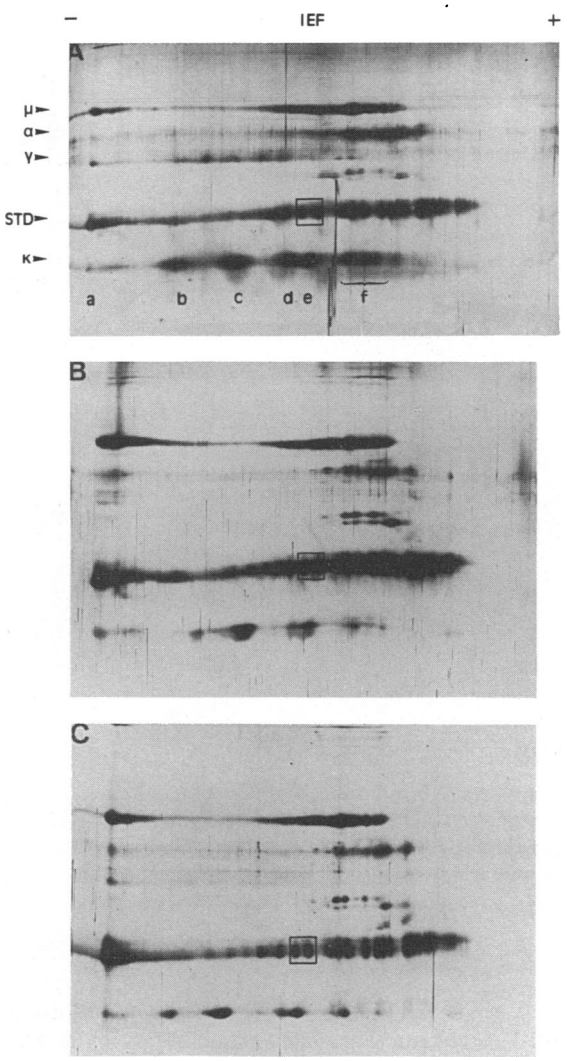

Figure 1. Two-dimensional gel electrophoretic analysis of affinity-purified IgM-κ from serum of a normal volunteer (A) and IgM-V_{Kiiib} isolated from serum of each of two normal volunteers (B and C). Included in each sample is an internal isoelectric focusing standard, carbamylated GAPDH. The open square identifies a doublet within the IEF internal standard train, which is consistently discernible in all gels and serves as an easily identifiable reference spot. Letters a–f identify the pH regions of the gel that correlate with peaks a–f in Fig. 2.

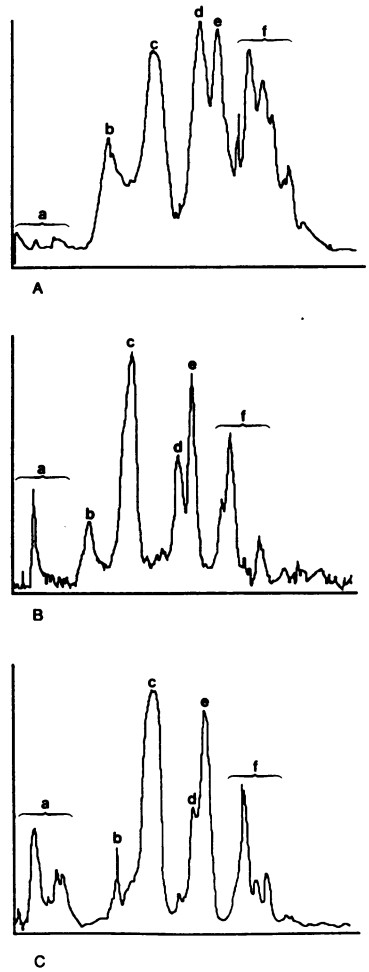

Figure 2. Densitometer scans of the light chains from the isoelectric focusing profiles shown in Fig. 1. (A) Affinity-purified kappa light chains from the IgM of a normal volunteer. (B and C) Affinity-purified V_{Kiiib} light chains from the IgM pools of two normal volunteers. The peak designations, a–f, correspond to the pH regions, a–f, indicated in Fig. 1. (B) and (C) demonstrate that regions (c), (e), and a small segment in (f) are markedly rich in V_{Kiiib}, which demonstrates that the monoclonal reagent used in affinity purification recognizes different isoforms.

MD). To each sample was added carbamylated glyceraldehyde-3-phosphate dehydrogenase, GAPDH (graciously supplied by Pharmacia, Piscataway, NJ) as an internal reference isoelectric focusing standard. After the second dimension the gels were silver stained (Gorg *et al.*, 1985).

Statistics

All statistical calculations were done with the aid of a Texas Instruments TI-55 programable calculator.

RESULTS

Two-dimensional gel analysis

The two-dimensional gel electrophoretic patterns of affinity-purified IgM-κ and IgM-V_{Kiiib} (from two individuals) are shown in Fig. 1. Although all gels show some IgA, the preparations are free of IgG, as evidenced by the lack of gamma heavy chains. In none of the gels is there evidence of lambda light chains, which have a slightly heavier molecular weight and are readily distinguishable from the kappa light chains by this technique. Thus, there appears to be very little or no non-specific adsorption of proteins. The presence of IgA is due to incomplete resolution of IgM and IgA by G200 gel filtration chromatography. Although a previous study (Moynihan, Looney & Abraham, 1985) failed to detect V_{Kiiib} in association with the IgA or IgG immunoglobulin classes, we have revealed both IgA-V_{Kiiib} and IgG-V_{Kiiib} immunoglobulins using two independent methods that are significantly more sensitive than the previously used ELISA assay. Both methods rely on prior affinity purification of V_{Kiiib} before analysing for association with mu, alpha and gamma immunoglobulin heavy chains (J. W. Williams and G. N. Abraham, manuscript in preparation). Thus, the presence

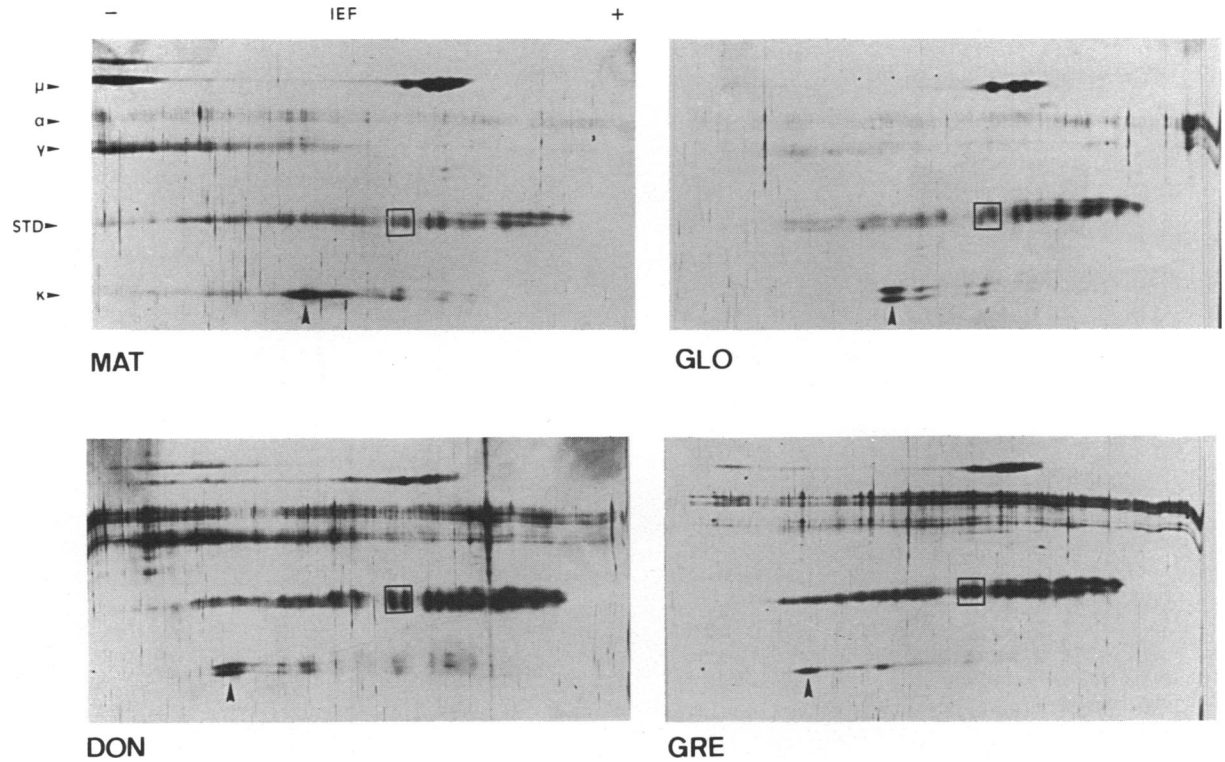

Figure 3. Two-dimensional gel electrophoretic analysis of cryoprecipitated IgM-V_{Kiiib} AGAs from four EMC patients. The arrows indicate the light chain position associated with the IgM AGA. The fainter spots observed in the kappa light chain lane are attributed to any IgG antigen that copurified with the IgM AGA. The open square is part of the internal IEF standard focusing train which serves as a readily identifiable pH reference point.

Table 1.

Normal volunteers	(V_{Kiiib}/κ)IgM	κ/μ
1 JMW	0·11	0·68
2 IB	0·17	0·77
3 EW	0·14	0·84
4 RJL	0·14	0·79
5 PL	0·13	0·87
6 PG	0·15	0·68
7 RL	0·14	0·86
8 DI	0·15	0·58
Mean (SE) for eight normal volunteers	0·14 (0·006)	0·76 (0·037)
Mean (SE) for six RA patients	0·16 (0·012)*	—

The (V_{Kiiib}/κ)IgM and the κ/μ serum ratios were determined in eight normal volunteers and six patients with RA using an IgM affinity-capture ELISA assay. There was no significant difference between the fractions 0·14 and 0·16.

*Not significantly different from normal volunteers ($P < 0·05$).

of IgA in gels B and C is not due to non-specific adsorption to the anti-V_{Kiiib} column. The slight amount of IgG in Fig. 1A may be due to high molecular weight IgG aggregates co-migrating with the IgM during Sephadex G200 column chromatography. To further control for the specificity of the anti-V_{Kiiib} column, a sham mouse IgG2b-coupled Sepharose 4B column was prepared and used for adsorption. Under identical conditions this column failed to absorb any immunoglobulin or other proteins.

Figure 2 shows the isoelectric focusing pattern of the light chains from gels A, B and C by densitometric scan. The letters a–f indicate light chain isoelectric focusing peaks that have the same pI relative to the internal isoelectric focusing standard, carbamylated glyceraldehyde-3-phosphate dehydrogenase. Thus these peaks, a–f, correspond to the IEF regions labelled in Fig. 1A. Visual comparison of the IEF profiles for affinity-purified V_{Kiiib} in Fig. 2B and C *vs* the affinity-purified kappa light chains in Fig. 2A indicate that the V_{Kiiib} light chain IEF profiles are much more restricted than those of kappa light chains, and that the IEF regions (c), (e), and a small segment of (f) are markedly rich in V_{Kiiib}. These data are consistent with V_{Kiiib} light chains representing a distinct kappa light-chain subset.

Figure 3 shows the two-dimensional gel patterns of four EMC AGAs, purified by extensive washing of the cryoprecipitates. Each gel pattern consists of one major light chain spot (arrowed) attributed to the IgM AGA, and faint spots representing light chains from the IgG antigen. The continuous heavy staining at the approximate positions of alpha and gamma

Table 2.

Patient	Anti-V_{Kiiib} reactivity
1 GRE	++
2 DEG	+
3 FOW	++
4 BUS	++++
5 DIA	++++
6 DON	++++
7 ANT	++
8 GLO	++++
9 SAU	++
10 TEH	+++
11 MAT	++
12 ZYW	+
13 SOR	−
14 VID	−
15 PLA*	−

The reactivity of the monoclonal anti-V_{Kiiib} antibody with AGAs in EMC serums was determined using an anti-IgG capture ELISA assay. Although the majority of patients are reactive with the monoclonal reagent, the degree of reactivity can vary over a large range.

*By primary amino-acid sequence analysis the associated AGA light chain is V_{Kiiib}.

heavy chains is predominantly an artifact sometimes observed in two-dimensional gel electrophoresis (Marshall & Williams, 1984). By comparison to the internal isoelectric focusing standard (using the doublet within the open square as a reference point), it is apparent that MAT and GLO V_{Kiiib} light chains have similar pIs that are different from those obtained for DON and GRE V_{Kiiib}. However, neither these nor the V_{Kiiib} light chains from five other EMC AGAs have pIs as anodal as those represented by the V_{Kiiib} peaks d–f in Fig. 2B and C. Thus, only a subset of the V_{Kiiib} repertoire is found in these EMC AGAs.

Determination of (V_{Kiiib}/κ) IgM in whole serum

This fraction was determined using the IgM affinity-capture ELISA. Table 1 shows that in eight normal volunteers the fraction of the IgM kappa population that contains V_{Kiiib} light chains ranges from 0·11 (11%) to 0·17 (17%), with a mean of 0·14 (14%). The fraction of IgM that contains kappa light chain ranges from 0·58 to 0·87, with a mean of 0·76. In six patients with RA the mean V_{Kiiib}/κ in the total IgM pool is 0·16 and is not significantly different from the value of 0·14 ($P < 0·05$) found for the normal volunteers.

Determination of (V_{Kiiib}/κ) AGA in whole serum

For patients with EMC, RA and SS, the ratio of V_{Kiiib}/κ in the anti-IgG autoantibodies in serum was determined using the

Table 3.

Patient	(V_{Kiiib}/κ)AGA
1 AUG	0·21
2 SHE	0·10
3 GAR	≥0·03
4 DEN	0·06
5 STO	≤0·03
6 HAN	≤0·03
7 PIE	≤0·03
8 MAR	≤0·03
9 BEN	≤0·03
10 BOH	≤0·03
11 WIL	≤0·03
12 BRO	≤0·03
13 ENN	0·09
14 ARN*	≤0·03
15 LYT	≤0·03
16 KIN	0·06
17 SPR	≤0·03
18 SON*	≤0·03
19 WIS†	≤0·03
20 GIL*	≤0·03

The (V_{Kiiib}/κ) AGA ratio in each RA patient's serum was determined using an anti-IgG capture ELISA assay. In patients with (V_{Kiiib}/κ)AGA ≤ 0·03 a value of 0·03 was assigned for the calculation of the group's mean value (see Table 5).

*Patients with RA and Felty's syndrome.

†Patient with RA and Sjögren's syndrome.

Table 4.

Patient	(V_{Kiiib}/κ)AGA
1 JAC	0·15
2 WAL	0·29
3 PG4	0·26
4 PG5	0·42
5 PG6	0·20
6 NIC	0·12
7 DAG	≤0·03
8 HAY	0·18

The (V_{Kiiib}/κ) AGA ratio in each primary Sjögren's patient's serum was determined using the anti-IgG capture ELISA assay described in the Materials and Methods. The value 0·03 was used in the calculation for this group's mean (see Table 5).

Table 5. Statistical summaries of data obtained for the three patient groups

Patient group	Mean (SE) (V_{Kiiib}/κ)AGA	% patients (V_{Kiiib}/κ)AGA $\geq 0{\cdot}10$
EMC	1·21 (0·249)	80
Primary SS	0·21 (0·042)	88
RA	0·05 (0·010)	10

The value of (V_{Kiiib}/κ)AGA $> 1{\cdot}00$, noted for the EMC group, reflects that the monoclonal anti-V_{Kiiib} antibody has a greater affinity for some monoclonal EMC V_{Kiiib} AGA light chains than for the V_{Kiiib} determinant in the monoclonal EMC V_{Kiiib} AGA TEH used in construction of the standard curves, which is described in the Materials and Methods.

anti-IgG-capture ELISA. Table 2 lists the reactivity of serum AGA in 15 EMC patients with the monoclonal anti-V_{Kiiib} antibody. Twelve, or 80%, of the patients have monoclonal AGA recognized as V_{Kiiib}. Of the three that are negative, PLA has a V_{Kiii} light chain by primary amino-acid sequence analysis (Johnston, Abraham & Welch, 1975). The light chain subgroups of SOR and VID are not known, nor is it known whether these carry the Wa or Po cross-reactive idiotype (Kunkel *et al.*, 1974).

The reactivity of anti-V_{Kiiib} with AGAs from 20 patients with RA with or without Felty's syndrome or SS is listed in Table 3. Only two out of 20 patients have (V_{Kiiib}/κ) AGA values greater than 0·10. For patients with values $\leqslant 0{\cdot}03$, 0·03 is used for statistical calculations. The mean value for this group is 0·05, a value lower than the mean serum (V_{Kiiib}/κ)IgM for the RA group. Such a low value in RA AGAs may suggest negative selection of the V_{Kiiib} light chains or loss of the determinant by somatic mutation.

Table 4 lists the (V_{Kiiib}/κ)AGA value for eight patients with primary Sjögren's syndrome. Seven have a value greater than 0·10, with a mean of 0·21. None had clinical or laboratory evidence of EMC or rheumatoid arthritis.

A summary of the data is given in Table 5. EMC and primary SS patients are significantly different from the RA group in terms of both the mean (V_{Kiiib}/κ)AGA value and the percentage of patients having (V_{Kiiib}/κ) AGA $\geqslant 0{\cdot}10$. The SS and EMC patients have significantly different ($P < 0{\cdot}05$) mean (V_{Kiiib}/κ) AGA values, but the percentage of patients having values greater than 0·10 does not differ. Thus, these three groups of patients can be clearly differentiated based on (V_{Kiiib}/κ) AGA values.

DISCUSSION

Previous studies of the protein sequence of V_{Kiiib} light chains isolated from monoclonal IgM anti-IgG autoantibodies in essential mixed cryoglobulinaemia suggest that these light chains may be encoded by a germ-line V_K gene sequence. The very prominent selection of V_{Kiiib} light chains that occurs in the EMC AGAs combined with the germ-line origin of these light chains is consistent with a hypothesis suggesting that V_{Kiiib} is the primordial V_L gene that is most concordant with anti-IgG autoreactivity.

In an attempt to understand this unique selection, we used a mouse monoclonal anti-V_{Kiiib} antibody to study the V_{Kiiib} from normal individuals, and also its association with polyclonal anti-IgG autoantibodies found in the serum of patients with RA and primary SS. The monoclonal anti-V_{Kiiib} reagent has the advantages of specificity for a single epitope and availability in large quantity from ascites for use in affinity purifications. Furthermore, the monospecificity of this reagent allows it to be sensitive to V-region somatic mutations, which may potentially affect the recognized determinant. Thus, failure of the monoclonal anti-V_{Kiiib} antibody to recognize a kappa light chain can be explained by several possibilities. The V-region may be encoded by a somatically mutated V_{Kiiib} gene that no longer encodes for the determinant recognized by the monoclonal reagent. Alternatively, a small group of V_{Kiiib} germ-line genes may exist that do not encode for the determinant recognized by the monoclonal antibody. Lastly, the V-region may be encoded by either V_{Ki}, V_{Kii} or V_{Kiv} subgroup genes.

Densitometer scans of V_{Kiiib} light chains from normal serum IgM clearly demonstrate that the monoclonal anti-V_{Kiiib} reagent recognizes multiple V_{Kiiib} isoforms which, as a group, are much more restricted in IEF heterogeneity than that obtained for the entire pool of IgM kappa light chains. The discreteness of the V_{Kiiib} peaks is not unexpected since previous primary amino-acid sequence analyses of EMC AGA V_{Kiiib} light chains have shown a remarkable lack of diversity within the first 94 residues of the V region. In these previous studies almost all protein sequence differences occurred at residue 95 or 96, or from residues 97–108, and were attributed to V–J junctional joining diversity or to the utilization of different J_K genes, respectively (Goni *et al.*, 1985). Alternative sources that can contribute to the heterogeneity observed in V_{Kiiib} include the existence of a limited number of other germ-line genes not used by EMC AGAs, and occurrence of V-region somatic mutations. It should be emphasized that the true diversity within each scan peak is unknown since IEF is most sensitive to charged functional groups. Thus, this technique only provides a minimum estimate of V_{Kiiib} diversity. However, since kappa light chains are not glycosylated (Anderson, 1981), all the diversity can be attributed to differences in amino-acid composition.

The two-dimensional gel patterns observed in V_{Kiiib} light chains of AGAs from four cases of EMC are shown in Fig. 3. The cathodal restriction of these light chains suggests that only a subset of the V_{Kiiib} repertoire is associated with EMC AGAs. Possible explanations for such restriction include unsuitability of anodal V_{Kiiib} for anti-IgG autoreactivity or loss of cryoprecipitability with anodal V_{Kiiib} AGAs. Alternatively, anodal V_{Kiiib} light chains may be somatically mutated and derived from older, mature B cells that are not specifically activated by mechanisms stimulating the germ-line gene expressing autoantibody-producing clones present in EMC.

In contrast to EMC, there is a paucity of V_{Kiiib} association with polyclonal AGAs in rheumatoid arthritis. Although much less pronounced, primary SS also differs from EMC in terms of the percentage of anti-IgG antibodies that have V_{Kiiib} light chains. Despite this, the fraction of SS patients who have an

increased percentage of AGA containing V_{Kiiib} light chains does not differ significantly from patients with EMC. It is interesting that both EMC and primary SS patients have a high association with V_{Kiiib} anti-IgG autoantibodies, and that both patient populations have been described to be at increased risk of malignant lymphoid transformation.

The significant differences in (V_{Kiiib}/κ)AGA in RA and SS *vs* EMC may indicate that selection of the V_{Kiiib} sub-subgroup in EMC is independent of antibody specificity, i.e. anti-IgG autoreactivity. Consequently, stimulation of the B cells that produce the AGA in EMC may occur through an antigen-independent mechanism. Alternatively, the paucity of this sub-subgroup in RA AGAs may be due to either the 'recruitment' of non-V_{Kiiib} genes, the loss of the V_{Kiiib} determinant in these AGAs via somatic mutation (Siekevitz *et al.*, 1987; Cumano & Rajewsky, 1986; Radbruch, *et al.*, 1985), or the use of germ-line V_{Kiiib} genes that do not encode the determinant recognized by the monoclonal anti-V_{Kiiib} antibody.

V-gene AGA selection preferences have also been described in mice. Interestingly, in these experiments the B cells were stimulated with the polyclonal activator LPS (Shlomchik *et al.*, 1986; Manheimer-Lory *et al.*, 1986; Painter *et al.*, 1986). Other mechanisms capable of the antigen-independent stimulation of B cells include viral infection, other B-cell mitogens, and possibly defective T-cell idiotype interfacing (Male, 1986; Rosen *et al.*, 1977). Some of these mechanisms are thought to act more specifically on subsets of B cells, a property that could account for the selection of a germline gene expressing B cells in EMC.

In summary, the data show that normal IgM V_{Kiiib} light chains have limited diversity, and that only a subset of the V_{Kiiib} repertoire is represented in the EMC AGAs. The ELISA data indicate that V_{Kiiib} selection in EMC is not essential for anti-IgG autoreactivity. Further, the variable association of V_{Kiiib} with RA, EMC, and SS AGAs suggests that different mechanisms may cause stimulation and perpetuation of the autoantibody-producing cells in these diseases.

Technical note

Three lines of evidence suggest that the continuous heavy staining at the approximate molecular weight positions of alpha and gamma heavy chains (Fig. 3, DON and GRE) is predominantly an artifact. Firstly, this artifact has been studied and noted to occur at approximate molecular weight positions 68,000 and 54,000 (Marshall & Williams, 1984). Secondly, alpha heavy chains isoelectric focus in a relatively narrow region as appropriately demonstrated in Fig. 1A, B and C. Thirdly, the quantity of light-chain staining would be disproportionately small if the continuous heavy staining in the alpha and gamma molecular weight range was caused by actual immunoglobulin heavy chains.

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