JCI The Journal of Clinical Investigation

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J Clin Invest. 1988;82(2):469-475. https://doi.org/10.1172/JCI113620.

Research Article

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Idiotypic and Subgroup Analysis of Human Monoclonal Rheumatoid Factors

Implications for Structural and Genetic Basis of Autoantibodies in Humans

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Abstract

Rheumatoid factors (RFs) in humans have been studied intensively because of their association with autoimmune and lymphoproliferative diseases. Many human IgM-RFs express cross-reactive idiotypes (CRIs) and have homologous light chains, some of which are encoded by a single V_κ gene, termed $V_{\kappa 325}$. However, although antibody activity generally requires the interaction between heavy and light chain variable regions, much less is known about structural relationships among RF heavy chains. To delineate further the structural and genetic basis of RF autoantibody synthesis, we generated "sequence-dependent" reagents specific for the human heavy and kappa light chain subgroups, and used them to analyze a panel of 27 monoclonal RFs. In addition, these proteins were tested for the expression of a heavy chain–associated CRI (G6), and a light chain–associated CRI (17.109). The results showed that most 17.109-reactive RFs contain heavy chains of the V_{HI} subgroup, which bear the G6 idiotypic marker. However, among the 14 17.109-reactive RFs, two have heavy chains of the V_{HII} subgroup, and another two contain heavy chains of the V_{HIII} subgroup. Previously, we have shown that 17.109 is a phenotypic marker of the human $V_{\kappa 325}$ gene. Accordingly, these results demonstrate that the same human V_κ gene can combine with several V_H genes from different V_H gene subgroups to generate RF activity.

Introduction

Kunkel and co-workers reported that at least 60% of human monoclonal IgM rheumatoid factor (RF)[1] cryoglobulins reacted with an absorbed, polyclonal rabbit antiidiotypic antiserum, termed anti-Wa (1). The anti-Wa reagent recognized determinants that are dependent on the interaction between RF heavy and light chains; however, the exact structural basis for the Wa cross-reactive idiotype (CRI) has not been determined (2, 3).

IgM-RFs with the Wa CRI have structurally similar light chains that belong to the $V_{\kappa III}$ subgroup (4–7). Many of these light chains express a CRI identified by the monoclonal anti-idiotypic antibody 17.109 (8). Recently, it has been established that the 17.109 CRI is a phenotypic marker of a human germline V_κ gene that has been cloned, sequenced, and designated either $V_{\kappa 325}$ or $V_{\kappa RF}$ (9, 10). The 17.109 CRI is expressed by ~ 2% of circulating IgM from normal adults (11, 12), and higher levels of expression occur after EBV infection (13). Furthermore, this marker is present on ~ 40% of circulating RFs from some patients with Sjogren's syndrome (14), and on 25% of kappa positive chronic lymphocytic leukemia cells (11). Taken together, the results suggest that the $V_{\kappa 325}$ gene (or very closely related genes), is common in the human population, and may be associated with certain B cell lymphoproliferative diseases.

Much less is known about the variable regions used by RF heavy chains. Andrews and Capra sequenced the complete heavy chain variable regions of two Wa CRI-positive RFs, Sie and Wol (15). Both proteins belong to the V_{HI} subgroup, and have J_{H4} segments. However, they share only 60% homology and have divergent sequences in all three complementarity-determining regions, which suggests that they derive from different germline V region genes. More recently Capra and co-workers have determined the heavy chain sequences of two additional IgM-RFs of the Wa idiotype group, Bor and Kas (16). These two heavy chains are very similar to each other, yet differ extensively from Sie and Wol heavy chains, suggesting that they probably derive from a different V_{HI} gene. Heavy chain heterogeneity was also suggested when antibodies prepared against synthetic peptides, and corresponding to the second complementarity-determining regions of Sie and Wol heavy chains, failed to identify any other RF proteins (17).

The limited amino acid sequence data suggest that a human IgM-RF may result when a $V_{\kappa 325}$ gene-encoded light chain combines with heavy chains that derive from the V_{HI} subgroup. To verify this hypothesis more generally, we have used heavy chain specific reagents to analyze a large series of RF proteins. The subgroup-specific reagents were generated by immunization with synthetic peptides that corresponded to diagnostic amino acid residues in the heavy chain first framework regions. This method has been successfully used to derive antibodies against the four known kappa subgroups (18). The RF proteins were also tested for reactivity with the 17.109 antibody, and with the murine MAb G6 that defines a heavy chain CRI on human RFs (19). The results demonstrate that most 17.109-reactive RFs contain heavy chains that bear the G6 CRI and belong to the V_{HI} variable region subgroup. However, four 17.109 CRI-reactive RFs use heavy chains of the V_{HII} or V_{HIII} subgroups. These results show that a single human

This is publication 4746BCR from the Research Institute of Scripps Clinic, La Jolla, California.

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Received for publication 29 September 1987 and in revised form 30 December 1987.

1. *Abbreviations used in this paper:* BBS, borate-buffered saline; CRI, cross-reactive idiotype; KLH, keyhole limpet hemocyanin; PH, peptide heavy chain; RF, rheumatoid factor.

J. Clin. Invest.
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0021-9738/88/08/0469/07 $2.00
Volume 82, August 1988, 469–475

V_κ gene can associate with V_H genes from different subgroups to generate RF activity.

Methods

Human monoclonal IgM-RFs. The paraproteins and monoclonal IgM-RFs from patients with essential mixed cryoglobulinemia or Waldenstrom's macroglobulinemia were isolated in this laboratory, or were kindly donated by Drs. V. Agnello, M. Newkirk, J. D. Capra, A. Solomon, and H. Metzger.

Peptides. Peptides were synthesized by a solid phase method as previously described (20). The three peptides used in these studies corresponded to portions of the first framework region sequences of human heavy chains. They represent consensus sequences of the three known subgroups; and were designated peptide heavy chain (PH) I, PHII, and PHIII (Table I). The chosen prototype subgroup sequences were distinct; the greatest homology was only 50% between PHII and PHIII. The peptides were coupled to keyhole limpet hemocyanin (KLH) through a terminal cysteine moiety, using *m*-maleimido-benzoyl-*N*-hydroxysuccinamide ester (21).

Subgroup specific reagents and antiidiotypic reagents. For heavy chain subgroup reagents, 4–6-wk-old female New Zealand White rabbits were immunized at 2-wk intervals with the peptide-KLH conjugates (18). Initial injections contained 500 μg peptide-KLH conjugate in Freund's complete adjuvant. Subsequent injections used 200 μg of the conjugates emulsified in incomplete adjuvant. Rabbits were bled 1 wk after the second injection and every 2 wk thereafter. Sera were stored at −20°C until used. Rabbit antibodies specific for the four kappa chain subgroups were made as described previously (18).

Paraproteins were tested for reactivity with two murine monoclonal antiidiotypic antibodies, 17.109 and G6. The 17.109 antibody has been described previously (8), and recognizes an idiotope in the kappa variable region that identifies the products of the $V_{\kappa 325}$ gene (10, 11). The G6 anti-CRI was prepared by immunization with the IgM-RF Kok, and recognizes an idiotope in RF heavy chains (19).

ELISA. An ELISA method was used for initial screening of antipeptide activity and for idiotype assays. Microtiter plates were precoated for 4 h with synthetic peptides (5 μg/ml) in isotonic borate-buffered saline, pH 8.2 (BBS). Nonspecific binding sites were then blocked by incubation with 1% BSA in BBS. Serial dilutions of rabbit antisera in BBS were then added and incubated overnight. After washing the plates with 0.05% Tween 20 in BBS, they were developed with alkaline phosphatase-conjugated goat anti–rabbit IgG (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD).

For idiotype testing, microtiter plates were precoated with affinity purified goat anti–human Ig at 10 μg/ml in BBS or with human IgG Fc fragments (Jackson Immunoresearch Laboratories, Westgrove, PA) at 30 μg/ml in BBS, then quenched as above. Paraproteins were then added at 10 μg/ml in BBS. After washing the globulin fractions of ascitic fluids containing either antibodies G6 or 17.109 or a control murine MAb, one or the other was added at a concentration of 5 μg Ig/ml and incubated overnight at 4°C. The plates were washed and antibody binding was detected with alkaline phosphatase-conjugated goat anti–mouse Ig antibody (Kirkegaard and Perry Laboratories). To ensure that the plates had been equally coated, replicate wells were developed with alkaline phosphatase-conjugated goat $F(ab')_2$ anti–human kappa (Tago Inc., Burlingame, CA). Individual proteins are reported as CRI-reactive if the OD_{405} was more than three SDs greater than backround values.

Immunoblot analysis. The binding specificity of antipeptide antisera and antibody 17.109 were also assessed by an immunoblot method, as previously described (14, 18, 22). Briefly, the heavy and light chains of Igs were separated by electrophoresis in 10% polyacrylamide gels containing 0.1% SDS, after boiling them for 1 min in 4% (vol/vol) 2-mercaptoethanol. After electrotransfer to nitrocellulose paper, nonspecific binding sites were quenched by incubation with BBS containing 5% powdered milk. The blotted papers were then probed either with antipeptide sera specific for V_H or V_κ subgroups (18) diluted to 1:200 in BBS, or with goat anti–human Ig (Cappel Worthington Laboratories, Malvern, PA) for 16 h at 4°C. Alternatively, papers were incubated with antiidiotype 17.109 at 10 μg/ml in BBS, and then washed and incubated with rabbit anti-mouse IgG (Cappel Worthington Laboratories) at 10 μg/ml in BBS. After washing the papers again, reactive bands were detected by the binding of ^{125}I-protein A (sp act ~ 1 mCi/mg, at 2×10^5 cpm/ml, ICN Radiochemicals, Inc., Irvine, CA). After another washing step, the papers were dried and exposed to Kodak XAR-5 film at −70°C for 16–48 h.

Peptide inhibition studies. Peptide inhibition studies were performed by liquid phase absorption. Aliquots of each of the three peptide-induced antisera were diluted 1:200 in BBS, and then were incubated separately with 0.01–100 μg/ml of the three peptides, PHI, PHII, and PHIII, overnight at 4°C. The processed antisera were incubated with nitrocellulose that had been blotted previously with a panel of paraproteins and treated as above.

Results

Preparation and characterization of antipeptide antisera. Previous experiments have shown that peptides corresponding to a consensus sequence of the first framework region of light chains could be used to make antisera highly specific for human kappa light chain variable region subgroups (18). Therefore, a similar strategy was used to make heavy chain subgroup–specific reagents. After immunization with first framework peptides, all rabbits produced high titer (> 1:10,000) antisera when tested against the immunizing peptide by ELISA (data not shown).

The specificity of binding of the antisubgroup antisera was then tested by the immunoblotting of proteins of known sequence. As expected, each antipeptide antiserum bound to Igs belonging to the appropriate V_H subgroup (Fig. 1 and Table I). Fig. 1 shows that anti-PHI reacts with three of three V_{HI}-RFs (Bor, Kas, Sie), anti-PHII reacts with the V_{HII}-RF (Les), and

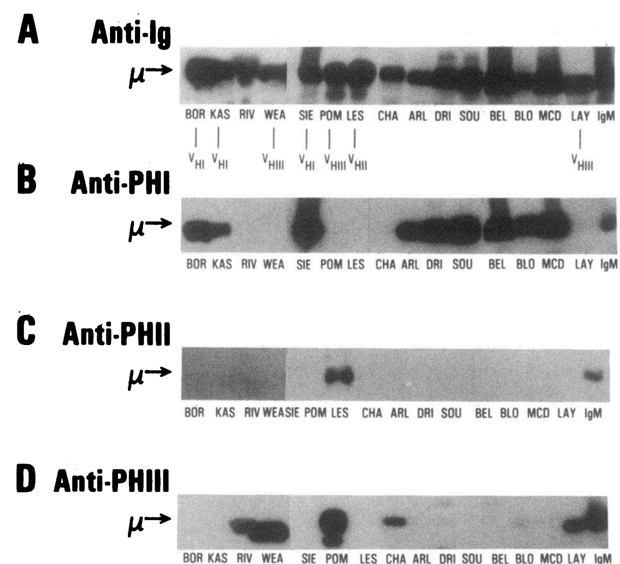

Figure 1. Immunoblot analysis of 14 human monoclonal IgM-RFs and one non-RF IgM. For simplicity, only the heavy chain results are shown, and the known heavy chain subgroups of some paraproteins, as determined by sequence analysis, are indicated below the figure. (*A–D*) Four identical RF sets probed with the indicated antibodies.

*Table I. Specificity of Human Heavy Chain Subgroup Reagents**

	Sequences of first framework peptides																
	Residue number														Subgrouping results		
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Anti-V_{HI}‡	Anti-V_{HII}	Anti-V_{HIII}
V_{HI}																	
PHI§	A	E	V	K	K	P	G	A	S	V	K	V	S	C			
Sie	—	—	—	—	—	—	—	S	—	—	R	—	T	—	+	—	—
Wol	—	—	—	—	—	—	—	S	—	—	R	—	—	—	+	—	—
Bor	—	—	—	—	—	—	—	S	—	—	—	—	T	—	+	—	—
Kas	—	—	—	—	—	—	—	S	—	—	—	—	—	—	+	—	—
V_{HII}																	
PHII (Les)	P	G	L	V	K	P	S	E	T	L	S	L	T	C	—	+	—
V_{HIII}																	
PHIII	G	G	L	V	Q	P	G	G	S	L	R	L	S	C			
Pom	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
Lay	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+
Wea	—	—	—	—	E	—	—	—	—	—	—	—	—	—	—	—	+

* Numbering according to Kabat et al. (43). Except Wea, all other IgMs were reported by Capra and co-workers (15, 16, 44). The non-RF, Wea, was previously reported (45). ‡ Reactivity of the paraprotein with the antiserum by immunoblotting is indicated by (+); lack of reactivity is indicated by (−). § PHI represents a consensus sequence from all V_{HI} proteins reported (43). A comparable peptide with the exact Sie first framework sequence yielded equivalent results (data not shown). ‖ An identical amino acid residue is used in the sequence.

anti-PHIII reacts with two of two V_{HIII}-RFs (Pom and Lay) and the V_{HIII}–non-RF (Wea). The RF Les was used as a prototype V_{HII} subgroup protein (T. Kipps and D. A. Carson, unpublished observation). Fig. 2 shows that anti-PHI also reacts with an additional V_{HI}-RF (Wol). Furthermore, the binding of anti-PHI to the three V_{HI}-heavy chains was completely blocked by the immunizing peptide at 0.1 μg/ml (Fig. 3 *A*). In contrast, preincubation of the antiserum with 100 μg/ml of

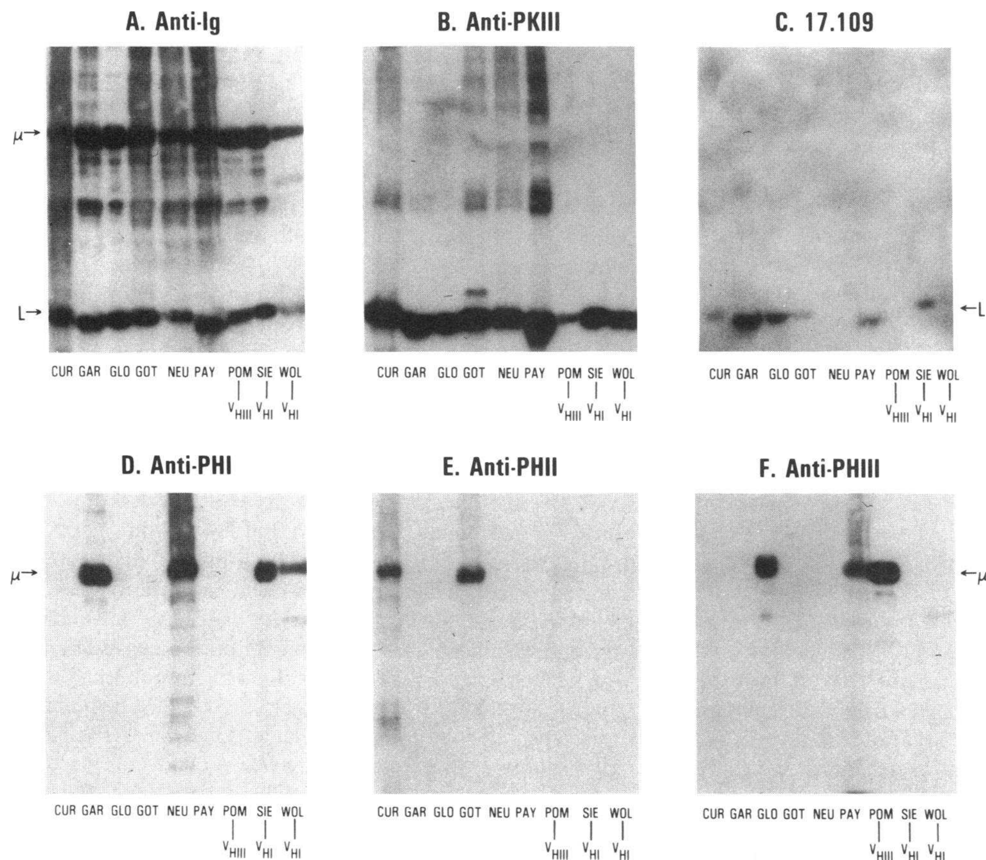

Figure 2. Immunoblot analysis of eight $V_{\kappa 325}$-RFs and the IgM-RF Pom. Separated heavy and light chains were reacted with the indicated antibodies. The anti-PKIII is the peptide-induced $V_{\kappa III}$ specific antibody; 17.109 is an MAb that recognizes a CRI on most $V_{\kappa 325}$-encoded light chains.

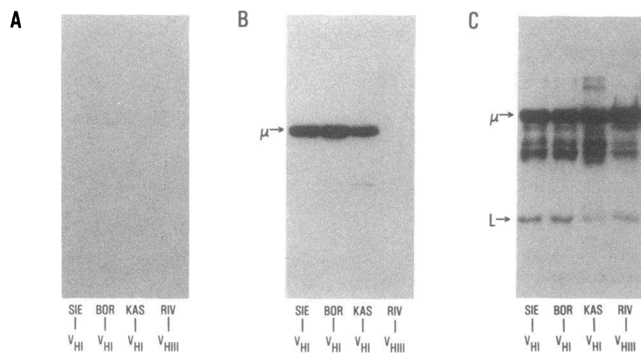

Figure 3. Specific inhibition of the anti-PHI antiserum. The separated light and heavy chains of four IgM-RF paraproteins were reacted with anti-PHI at a 1:200 dilution after preincubation with either immunizing peptide at 0.1 μg/ml (*A*), or with the control peptides PHII and PHIII, both at 100 μg/ml (*B*). (*C*) Incubated with an anti-Ig reagent.

peptide corresponding to the other V_H subgroups caused no inhibition (Fig. 3 *B*). Equivalent results were obtained from analyses of two non-RF V_{HI} paraproteins, and from studies of other paraproteins with the anti-PHII and anti-PHIII sera (data not shown). Together, these data clearly demonstrate the fine specificity of the peptide-induced subgrouping reagents.

Subgroup characterization of human RFs. To characterize the heavy chain subgroups of RF autoantibodies, 27 of these paraproteins were tested by immunoblotting. Fig. 1 displays reactivity of the three antipeptide antisera with 14 RFs and 1 non-RF paraprotein. Here, in addition to three known V_{HI}-RFs, the anti-PHI reacts with six other RFs (Arl, Dri, Sou, Bel, Blo, and Mcd). In contrast, anti-PHIII reacts with only one additional RF (Cha). The fine specificity of the peptide-induced antisubgroup antisera and the monoclonality of each μ heavy chain is demonstrated by the reactivity of each RF with only one of the heavy chain subgroup-specific reagents. All RFs tested were reactive with one of the three V_H antisera.

Fig. 2 displays the immunoblot analysis of eight $V_{\kappa325}$-RF paraproteins (7, 23, 24). Also included is Pom, a non-$V_{\kappa325}$ IgM-RF (1, 4). As illustrated, four of the heavy chains reacted with the V_{HI} specific antibody, two with the antibody against V_{HII}, and three with the antibody against V_{HIII}. Again, the specificity of these reagents in identifying heavy chain subgroups is demonstrated.

Kappa subgroups were assigned to the associated light chains of the RF paraproteins using peptide-induced antisera, which have previously been described. The monoclonality of light chains was confirmed by reactivity with only one of the four reagents (data not shown). Only 1 of 25 kappa light chains was nonreactive (Cor), and its subgroup was assigned based on amino acid sequence. The results of the heavy and light chain subgroup analyses are summarized in Tables II and III.

Reactivity of RFs with antiidiotypic antibodies. Proteins were tested by ELISA and immunoblotting for reactivity with MAb 17.109, which recognizes a light chain CRI, and with G6, which binds to a heavy chain-associated CRI. The RF paraproteins Gar, Neu, Cur, Got, and Pay could not be assayed for G6 binding, as samples of intact protein were not available. The results of these studies are shown in Tables II and III, along with reported data for the Wa CRI (1, 4, 25). As demonstrated, 10 RFs that expressed the 17.109 light chain CRI had V_{HI} heavy chains. Four other $V_{\kappa325}$-17.109 RFs had non-V_{HI} heavy chains, of which two were V_{HII} and two were V_{HIII}. In contrast, only 5 of 11 17.109 negative IgM-κ-RFs had V_{HI} heavy chains (two of which are also believed to use $V_{\kappa325}$ light chains, Neu and Wol). Furthermore, among $V_{\kappa III}$ proteins, 10 of 14 17.109-reactive RFs had V_{HI} heavy chains of which 9 were G6 reactive, while 5 of 9 17.109 negative RFs had V_{HI} heavy chains of which 3 were G6 reactive.

Discussion

The present study was directed at understanding the genetic and structural basis of human RFs. The RF autoantibodies have been demonstrated to represent a physiologic component of the normal secondary immune response in the mouse and the human (26–30). These autoantibodies are produced by 3–20% of murine hybridomas derived from LPS-stimulated splenic B cells (31, 32). To explain this large representation of RF-secreting B cells within the normal immune system, Schlomchik et al. determined the sequences of RFs that arise in LPS-stimulated mice. They found that murine RF activity was generated by a limited set of light chains in association with a diverse group of heavy chains (33).

A majority of human RF cryoglobulins have been shown to express the Wa CRI. Many of these proteins also bear the 17.109 CRI, which has been shown to be a phenotypic marker for the $V_{\kappa325}$ gene. Sequence analyses of four $V_{\kappa325}$-RFs revealed an exclusive use of V_{HI} genes. These results suggested that human RFs, in contrast to murine RFs, were heavy chain subgroup-restricted. To resolve the apparent difference between RFs, we studied a large panel of human RF paraproteins. The results of our experiments showed that, although the great majority of $V_{\kappa325}$-encoded RFs use V_{HI} heavy chains, a minority do use heavy chains from other V_H subgroups. The data are entirely consistent with our previous observation concerning the heterogeneity of human RF heavy chains (17).

It should be noted that, out of the 9 of 23 $V_{\kappa III}$-RFs that did not express the 17.109 CRI, two (Neu and Wol) almost certainly derive from the $V_{\kappa325}$ gene, as indicated by amino acid sequence analysis (15, 23, 24). Both of these $V_{\kappa325}$-encoded RF light chains are associated with V_{HI} heavy chains. However, four other 17.109 CRI-negative RF molecules (Cor, Les, Riv, and Pom) use $V_{\kappa IIIa}$ light chains, which are not encoded by the $V_{\kappa325}$ gene (34, 35), and they are associated with non-V_{HI} heavy chains. Thus, at least two separate $V_{\kappa III}$ light chain genes can be used for RF synthesis in humans (12).

To clarify the normal distribution of $V_{\kappa325}$-17.109 Igs, we performed a separate analysis of non-RF paraproteins. In this series, the light chain associated CRI was detected on 2 of 23 non-RF paraproteins (1 of 9 V_{HI}, 0 of 5 V_{HII}, and 1 of 9 V_{HIII} proteins, see Table III). Recently, $V_{\kappa325}$-light chains have been detected in antibodies with anti-LDL, antiintermediate filament, and anti-DNA histone activity (23, 24, 36–38), and recent studies indicate that these non-RF antibodies use V_{HII} and V_{HIII} heavy chains (Silverman, G. J., et al., unpublished observation). Together, the data indicate that the $V_{\kappa325}$ light chain is important, but that it is not sufficient for RF activity. Apparently, shared properties of certain heavy chains are necessary to endow Fc binding activity to these molecules. There are at least two possible alternatives to explain why V_{HI} heavy chains, especially those bearing the G6 CRI, are used preferentially by

Table II. Subgroup and Idiotypic Analysis of IgM RF Paraproteins

	Light chain		Heavy chain		
	Kappa subgroup	CRI* (17.109)	Subgroup‡	CRI (G6)	Wa CRI
Arl	III	+++	I	++	NT§
Bel	III	–	I	+	NT
Blo	III	++	I	++	+
Bor	III	+++	I	+++	+
Cha	I	–	III	–	–
Cor	–(III)‖	–	II	–	NT
Cur	III	+	II	NT	NT
Dri	III	+++	I	+++	+
Fra	III	+	I	+++	NT
Gar	III	+++	I	NT	NT
Glo	III	+++	III	–	+
Got	III	++	II	NT	NT
Kas	III	+++	I	+++	+
Koh	NA¶	–	III	–	–
Kok	III	++	I	+++	NT
Lay	I	–	III	–	–
Les	III	–	II	–	NT
McD	III	++	I	+++	+
Neu	III	–	I	NT	NT
Palm	III	–	I	++	NT
Pay	III	+	III	NT	NT
Pom	III	–	III	–	–
Riv	III	–	III	–	–
Sie	III	+++	I	+++	+
Sou	III	–	I	–	+
Tal	NT	–	III	–	–
Wol	III	–	I	+++	+

The RFs Arl, Bel, Blo, Cha, Dri, McD, Sou, Tal, and Glo and associated Wa idiotypic reactivity have previously been described by Kunkel, Agnello, and co-workers (1, 4, 15, 25). Glo was initially listed as Gl. The RFs Bor, Kas, Lay, Pom, Riv, and Sie have previously been described by Capra, Newkirk, and co-workers (4, 15, 16, 44, 46). The RF Cor has previously been described by Schrohenloher and co-workers (12, 47). The RFs Cur, Glo, Got, Neu, and Pay and their light chain sequences have previously been described reported by Frangione, Goni, and co-workers (7, 23). The RFs Fra and Kok have previously been described by Jefferis and co-workers (19). The RF Koh has previously been described by Kaplan and Metzger (48). The RFs Les and Palm, and the light chain sequence of the former, have previously been described (12, 17, 34, 49). Palm was initially listed as Pal. * Reactivity with the monoclonal antiidiotypes G6 and 17.109 was determined by ELISA. Results as expressed as: –, negative; +, OD_{410} between 0.100 and 0.400; ++, between 0.401 and 0.700; +++, between 0.701 and 1.000. 17.109 reactivity of Neu, Gar, Cur, and Got is listed as previously described (14). ‡ Reactivity with the subgroup specific antipeptide reagents was evaluated by immunoblotting. Subgroup was assigned for heavy chains or kappa light chains if the protein was reactive with only one of the antipeptide antisera. Certain proteins were nonreactive with all related antisera and were designated "–". § NT, idiotype not assigned. ‖ The light chain was assigned a kappa sugroup based on amino acid determination (39). ¶ NA, not applicable. The light chain is a lambda.

$V_{\kappa 325}$-RFs. First, light-heavy chain pairing may be nonrandom, irrespective of RF activity, but this seems unlikely as preliminary studies of 17.109 affinity-purified Ig from a variety of donors failed to show a V_H subgroup preference. It is more probable that specific sites within the heavy chain variable regions play a direct role in Fc binding. The chain recombination experiments of Schrohenloher and Hester support this conclusion (39).

In the present study insights into the V gene origins of a diverse panel of human RF paraproteins were gained, by the use of antibodies with predetermined specificity against sequences in the first framework regions of the heavy and light chain variable regions. Using antibodies specific for the three heavy chain and four kappa light chain subgroups, 44 of 45 kappa light chains and all of 50 heavy chains were characterized. Thus, the first framework region of human heavy chains appears to contain a primary sequence-dependent epitope that can be used to discriminate variable region subgroups. The strong immunogenicity of the first framework region may be due to the presence of a β bend at residues 13–16 (18, 40). The reactivity of the great majority of Ig molecules with these reagents may be explained by the observation that mutations in the framework regions are more often silent than in adjacent hypervariable regions (41, 42).

In summary, the data indicates that the 17.109, G6, and Wa CRIs identify overlapping subsets of IgM-RFs. Previous experiments have shown that the light chain–associated CRI, 17.109, is the marker of a germline gene, $V_{\kappa 325}$ (9–11). The present data demonstrate that the heavy chain–associated CRI, G6, is a marker for the products of a subset of V_{HI} genes.

*Table III. Summary of Subgroup and CRI Associations of Paraproteins**

	V_H Subgroup				G6 CRI‡		
	I (%)	II (%)	III (%)	Total tested	+ (%)	− (%)	Total tested
V_κ subgroup							
RF paraproteins							
$V_{\kappa III}$	15 (65)	4 (17)	4 (17)	23	12 (67)	6 (33)	18
Non-$V_{\kappa III}$§	0 (0)	0 (0)	3 (100)	3	0 (0)	2 (100)	2
Non-RF paraproteins							
$V_{\kappa III}$	3 (75)	0 (0)	1 (25)	4	0 (0)	4 (100)	4
Non-$V_{\kappa III}$	6 (32)	4 (21)	9 (47)	19	2 (11)	17 (89)	19
17.109 CRI‖							
RF paraproteins							
CRI +	10 (71)	2 (14)	2 (14)	14	9 (90)	1 (10)	10
CRI −	5 (45)	2 (18)	4 (36)	11	3 (30)	7 (70)	10
Non-RF paraproteins							
CRI +	1 (50)	0 (0)	1 (50)	2	0 (0)	2 (100)	2
CRI −	8 (38)	4 (19)	9 (43)	21	2 (10)	19 (90)	21

* Tal omitted. ‡ Assay results of 20 RF and 23 non-RF paraproteins. § Non-$V_{\kappa III}$ light chains include $V_{\kappa I}$, $V_{\kappa II}$, $V_{\kappa IV}$, and lambda. ‖ Assay results of 25 RF and 23 non-RF paraproteins.

Most IgM-RFs with light chains derived from the $V_{\kappa 325}$ light chain gene use V_{HI} heavy chains, most of which bear the G6 idiotope. However, a significant minority of the RF autoantibodies have V_{HII} and V_{HIII} heavy chains, which indicates that multiple V_H chains can be used for RF autoantibody synthesis.

Acknowledgments

The authors thank Dr. R. Houghton for providing the synthetic peptides, and Drs. V. Agnello, M. Newkirk, J. D. Capra, A. Solomon, and H. Metzger for proteins. We appreciate Dr. G. Rhodes and Dr. A. Olson for their helpful discussions, and the Basic Clinical Research word-processing department for secretarial assistance.

Funding for this research was supported in part by grants AI-00866, AR-25443, AG-04100, AR-07144, AM-02594, and RR-00833 awarded by the National Institutes of Health (NIH), and by a grant from the Gingold Sloan Foundation. G. J. Silverman, and F. Goni are postdoctoral fellows of the Arthritis Foundation. G. J. Silverman is a recipient of an NIH Physician Scientist Award. R. A. Mageed is a postdoctoral fellow of the Arthritis and Rheumatism Council (U.K.). S. Fong is a recipient of an NIH Research Career Development Award (AG-00279).

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