

# 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  $V_{\kappa}$  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  $V_{\text{HI}}$  subgroup, which bear the G6 idiotypic marker. However, among the 14 17.109-reactive RFs, two have heavy chains of the  $V_{\text{HII}}$  subgroup, and another two contain heavy chains of the  $V_{\text{HIII}}$  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  $V_{\kappa}$  gene can combine with several  $V_{\text{H}}$  genes from different  $V_{\text{H}}$  gene subgroups to generate RF activity.

### Introduction

Kunkel and co-workers reported that at least 60% of human monoclonal IgM rheumatoid factor (RF)<sup>1</sup> 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  $V_{\text{HII}}$  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  $V_{\kappa}$  gene that has been cloned, sequenced, and designated either  $V_{\kappa 325}$  or  $V_{\kappa \text{RF}}$  (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  $V_{\text{HI}}$  subgroup, and have  $J_{\text{H4}}$  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  $V_{\text{HI}}$  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  $V_{\text{HI}}$  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  $V_{\text{HI}}$  variable region subgroup. However, four 17.109 CRI-reactive RFs use heavy chains of the  $V_{\text{HII}}$  or  $V_{\text{HIII}}$  subgroups. These results show that a single human

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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.

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$V_L$  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  $\mu$ g peptide-KLH conjugate in Freund's complete adjuvant. Subsequent injections used 200  $\mu$ g 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  $-20^\circ\text{C}$  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 anti-peptide activity and for idiotype assays. Microtiter plates were pre-coated for 4 h with synthetic peptides (5  $\mu$ g/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 pre-coated with affinity purified goat anti-human Ig at 10  $\mu$ g/ml in BBS or with human IgG Fc fragments (Jackson ImmunoResearch Laboratories, Westgrove, PA) at 30  $\mu$ g/ml in BBS, then quenched as above. Paraproteins were then added at 10  $\mu$ g/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  $\mu$ g Ig/ml and incubated overnight at  $4^\circ\text{C}$ . 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')<sub>2</sub> anti-human kappa (Tago Inc., Burlingame, CA). Individual proteins are reported as CRI-reactive if the OD<sub>405</sub> was more than three SDs greater than background values.

**Immunoblot analysis.** The binding specificity of anti-peptide 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

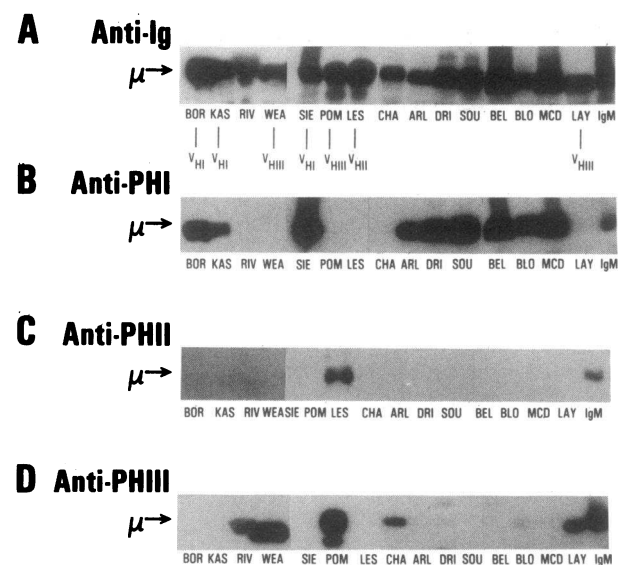
probed either with anti-peptide sera specific for  $V_H$  or  $V_L$  subgroups (18) diluted to 1:200 in BBS, or with goat anti-human Ig (Cappel Worthington Laboratories, Malvern, PA) for 16 h at  $4^\circ\text{C}$ . Alternatively, papers were incubated with antiidiotype 17.109 at 10  $\mu$ g/ml in BBS, and then washed and incubated with rabbit anti-mouse IgG (Cappel Worthington Laboratories) at 10  $\mu$ g/ml in BBS. After washing the papers again, reactive bands were detected by the binding of <sup>125</sup>I-protein A (sp act  $\sim 1$  mCi/mg, at  $2 \times 10^5$  cpm/ml, ICN Radiochemicals, Inc., Irvine, CA). After another washing step, the papers were dried and exposed to Kodak XAR-5 film at  $-70^\circ\text{C}$  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  $\mu$ g/ml of the three peptides, PHI, PHII, and PHIII, overnight at  $4^\circ\text{C}$ . The processed antisera were incubated with nitrocellulose that had been blotted previously with a panel of paraproteins and treated as above.

## Results

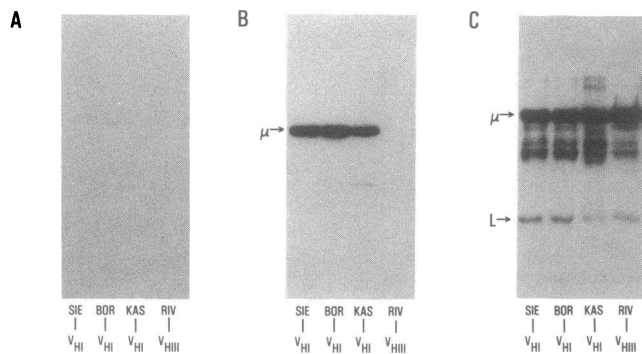
**Preparation and characterization of anti-peptide antisera.** Previous experiments have shown that peptides corresponding to a consensus sequence of the first framework region of light chains could be used to make antisera highly specific for human kappa light chain variable region subgroups (18). Therefore, 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 anti-peptide 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.





**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  $\mu\text{g/ml}$  (A), or with the control peptides PHII and PHIII, both at 100  $\mu\text{g/ml}$  (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 ant subgroup antisera and the monoclonality of each  $\mu$  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  $V_{\kappa 325}$ -RF paraproteins (7, 23, 24). Also included is Pom, a non- $V_{\kappa 325}$  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  $V_{\kappa 325}$ -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- $\kappa$ -RFs had  $V_{HI}$  heavy chains (two of which are also believed to use  $V_{\kappa 325}$  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  $V_{\kappa 325}$  gene. Sequence analyses of four  $V_{\kappa 325}$ -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  $V_{\kappa 325}$ -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  $V_{\kappa 325}$  gene, as indicated by amino acid sequence analysis (15, 23, 24). Both of these  $V_{\kappa 325}$ -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  $V_{\kappa 325}$  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  $V_{\kappa 325}$ -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,  $V_{\kappa 325}$ -light chains have been detected in antibodies with anti-LDLL, 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  $V_{\kappa 325}$  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		Wa CRI
	Kappa subgroup	CRI* (17.109)	Subgroup <sup>‡</sup>	CRI (G6)	
Arl	III	+++	I	++	NT <sup>§</sup>
Bel	III	-	I	+	NT
Blo	III	++	I	++	+
Bor	III	+++	I	+++	+
Cha	I	-	III	-	-
Cor	-(III) <sup>  </sup>	-	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 <sup>†</sup>	-	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 anti-idiotypes G6 and 17.109 was determined by ELISA. Results as expressed as: -, negative; +, OD<sub>410</sub> 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, idiotypic not assigned. || The light chain was assigned a kappa subgroup based on amino acid determination (39). † NA, not applicable. The light chain is a lambda.

V<sub>κ325</sub>-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<sub>H</sub> 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 character-

ized. 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<sub>κ325</sub> (9-11). The present data demonstrate that the heavy chain-associated CRI, G6, is a marker for the products of a subset of V<sub>H</sub> genes.

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

	V <sub>H</sub> Subgroup				G6 CRI <sup>‡</sup>		
	I (%)	II (%)	III (%)	Total tested	+ (%)	- (%)	Total tested
<b>V<sub>κ</sub> subgroup</b>							
<b>RF paraproteins</b>							
V <sub>κIII</sub>	15 (65)	4 (17)	4 (17)	23	12 (67)	6 (33)	18
Non-V <sub>κIII</sub> <sup>§</sup>	0 (0)	0 (0)	3 (100)	3	0 (0)	2 (100)	2
<b>Non-RF paraproteins</b>							
V <sub>κIII</sub>	3 (75)	0 (0)	1 (25)	4	0 (0)	4 (100)	4
Non-V <sub>κIII</sub>	6 (32)	4 (21)	9 (47)	19	2 (11)	17 (89)	19
<b>17.109 CRI<sup>  </sup></b>							
<b>RF paraproteins</b>							
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
<b>Non-RF paraproteins</b>							
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. ‡ Assay results of 25 RF and 23 non-RF paraproteins.

§ Non-V<sub>κIII</sub> light chains include V<sub>κI</sub>, V<sub>κII</sub>, V<sub>κIV</sub>, and lambda. || Assay re-

Most IgM-RFs with light chains derived from the V<sub>κ325</sub> light chain gene use V<sub>HII</sub> heavy chains, most of which bear the G6 idiotope. However, a significant minority of the RF autoantibodies have V<sub>HII</sub> and V<sub>HIII</sub> heavy chains, which indicates that multiple V<sub>H</sub> chains can be used for RF autoantibody synthesis.

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### References

- Kunkel, H. G., V. Agnello, F. G. Joslin, R. J. Winchester, and J. D. Capra. 1973. Cross-idiotypic specificity among monoclonal IgM proteins with anti-gammaglobulin activity. *J. Exp. Med.* 137:331-342.
- Kunkel, H. G. 1984. Cross-reacting idiotypes in the human system. In *The Biology of Idiotypes*. M. I. Greene and A. Nisonoff, editors. Plenum Publishing Corp., New York. 237-246.
- Agnello, V., and J. L. Barnes. 1986. Human rheumatoid factor crossidiotypes. I. Wa and Bla are heat-labile conformational antigens requiring both heavy and light chains. *J. Exp. Med.* 164:1809-1814.
- Kunkel, H. G., R. J. Winchester, F. G. Joslin, and J. D. Capra. 1974. Similarities in the light chains of anti-gamma-globulins showing cross-idiotypic specificities. *J. Exp. Med.* 139:128-136.
- Chen, P. P., F. Goni, S. Fong, F. Jirik, J. H. Vaughan, B. Frangione, and D. A. Carson. 1985. The majority of human monoclonal

IgM rheumatoid factors express a "primary structure-dependent" cross-reactive idiotype. *J. Immunol.* 134:3281-3285.

6. Newkirk, M., P. P. Chen, D. A. Carson, B. Posnett, and J. D. Capra. 1986. Amino acid sequence of a light chain variable region of a human rheumatoid factor of the Wa idiotype group, in part predicted by its reactivity with antipeptide antibodies. *Mol. Immunol.* 23:239-244.

7. Ledford, D. K., F. Goni, M. Pizzolato, E. C. Franklin, A. Solomon, and B. Frangione. 1983. Preferential association of kappa-IIIb light chains with monoclonal human IgM-kappa autoantibodies. *J. Immunol.* 131:1322-1325.

8. Carson, D. A., and S. Fong. 1983. A common idiotype on human rheumatoid factors identified by a hybridoma antibody. *Mol. Immunol.* 20:1081-1087.

9. Radoux, V., P. P. Chen, J. A. Sorge, and D. A. Carson. 1986. A conserved human germline Vk gene directly encodes rheumatoid factor light chains. *J. Exp. Med.* 164:2119-2124.

10. Chen, P. P., K. Albrandt, T. J. Kipps, V. Radoux, F.-T. Liu, and D. A. Carson. 1987. Isolation and characterization of human VkIII germline genes: implications for the molecular basis of human VkIII light chain diversity. *J. Immunol.* 139:1727-1733.

11. Kipps, T. J., S. Fong, E. Tomhave, P. P. Chen, R. D. Goldfien, and D. A. Carson. 1987. High frequency expression of a conserved kappa variable region gene in chronic lymphocytic leukemia. *Proc. Natl. Acad. Sci. USA.* 84:2916-2920.

12. Crowley, J. J., R. D. Goldfien, R. E. Schrohenloher, H. L. Spiegelberg, G. J. Silverman, R. A. Mageed, R. Jefferis, W. J. Koopman, D. A. Carson, and S. Fong. 1988. Incidence of three cross reactive idiotypes on human rheumatoid factor paraproteins. *J. Immunol.* 140:3411-3418.

13. Silverman, G. J., D. A. Carson, K. Patrick, J. H. Vaughan, and S. Fong. 1987. Expression of a germline human kappa chain-associated cross-reactive idiotype after in vitro and in vivo infection with Epstein-Barr virus. *Clin. Immunol. Immunopathol.* 43:403-411.

14. Fong, S., P. P. Chen, T. A. Gilbertson, J. R. Weber, R. I. Fox, and D. A. Carson. 1986. Expression of three cross reactive idiotypes on rheumatoid factor autoantibodies from patients with autoimmune diseases and seropositive adults. *J. Immunol.* 137:122-128.

15. Andrews, D. W., and J. D. Capra. 1981. Complete amino acid sequence of variable domains from two monoclonal human anti-

- gamma globulins of the Wa cross-idiotypic group: suggestion that the J segments are involved in the structural correlate of the idiotype. *Proc. Natl. Acad. Sci. USA.* 78:3799-3803.
16. Newkirk, M. M., R. A. Mageed, R. Jefferis, P. P. Chen, and J. D. Capra. 1987. Complete amino acid sequences of the variable regions of two human IgM rheumatoid factors, Bor and Kas of the Wa idiotypic family reveal restricted use of heavy and light chain variable and joining region gene segments. *J. Exp. Med.* 166:550-564.
  17. Chen, P. P., F. Goni, R. A. Houghten, S. Fong, R. Goldfien, J. H. Vaughan, B. Frangione, and D. A. Carson. 1985. Characterization of human rheumatoid factors with seven antiidiotypes induced by synthetic hypervariable region peptides. *J. Exp. Med.* 162:487-500.
  18. Silverman, G. J., D. A. Carson, A. Solomon, and S. Fong. 1986. Human kappa light chain subgroup analysis with synthetic peptide-induced antisera. *J. Immunol. Methods.* 95:249-257.
  19. Mageed, R. A., M. Dearlove, D. M. Goodall, and R. Jefferis. 1986. Immunogenic and antigenic epitopes of immunoglobulins. XVII. Monoclonal anti-idiotypes to the heavy chain of human rheumatoid factors. *Rheumatol. Int.* 6:179-183.
  20. Chen, P. P., R. A. Houghten, S. Fong, G. H. Rhodes, T. A. Gilbertson, J. H. Vaughan, R. A. Lerner, and D. A. Carson. 1984. Anti-hypervariable region antibody induced by a defined peptide: an approach for studying the structural correlates of idiotypes. *Proc. Natl. Acad. Sci. USA.* 81:1784-1788.
  21. Liu, F.-T., M. Zinnecker, T. Hamaoka, and D. H. Katz. 1979. New procedures for preparation and isolation of conjugates of proteins and a synthetic copolymer of D-amino acids and immunochemical characterization of such conjugates. *Biochemistry.* 18:690-697.
  22. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350-4354.
  23. Goni, F., P. P. Chen, B. Pons-Estel, D. A. Carson, and B. Frangione. 1985. Sequence similarities and cross-idiotypic specificity of L chains among human monoclonal IgM-K with anti-gammaglobulin activity. *J. Immunol.* 135:4073-4079.
  24. Chen, P. P., K. Albrandt, N. K. Orida, V. Radoux, E. Y. Chen, R. Schrantz, F.-T. Liu, and D. A. Carson. 1986. Genetic basis for the cross-reactive idiotypes on the light chains of human IgM anti-IgG autoantibodies. *Proc. Natl. Acad. Sci. USA.* 83:8318-8322.
  25. Powell, R., and V. Agnello. 1983. Characterization of complement-fixing activity and cross-idiotypes of rheumatoid factors in idiopathic mixed cryoglobulinemia. *Clin. Immunol. Immunopathol.* 29:146-151.
  26. Welch, M. J., S. Fong, J. H. Vaughan, and D. A. Carson. 1983. Increased frequency of rheumatoid factor precursor B lymphocytes after immunization of normal adults with tetanus toxoid. *Clin. Exp. Immunol.* 51:299-305.
  27. Coulie, P., and J. Van Snick. 1983. Rheumatoid factors and secondary immune responses in the mouse. II. Incidence, kinetics and induction mechanisms. *Eur. J. Immunol.* 13:895-899.
  28. Nemazee, D. A., and V. L. Sato. 1983. Induction of rheumatoid antibodies in the mouse: regulated production of autoantibody in the secondary humoral response. *J. Exp. Med.* 158:529-545.
  29. Van Snick, J., and P. Coulie. 1983. Rheumatoid factors and secondary immune responses in the mouse. I. Frequent occurrence of hybridomas secreting IgM anti-IgG autoantibodies after immunization with protein antigens. *Eur. J. Immunol.* 13:890-895.
  30. Carson, D. A., P. P. Chen, R. I. Fox, T. J. Kipps, F. Jirik, R. D. Goldfien, G. Silverman, V. Radoux, and S. Fong. 1987. Rheumatoid factor and immune networks. *Annu. Rev. Immunol.* 5:109-126.
  31. Van Snick, J. L., and P. Coulie. 1982. Monoclonal anti-IgG autoantibodies derived from lipopolysaccharide-activated spleen cells of 129/Sv mice. *J. Exp. Med.* 155:219-230.
  32. Shlomchik, M. J., A. Marshak-Rothstein, C. B. Wolfowicz, T. L. Rothstein, and M. G. Weigert. 1987. The role of clonal selection and somatic mutation in autoimmunity. *Nature (Lond.).* 328:805-811.
  33. Shlomchik, M., D. Nemazee, J. Van Snick, and M. Weigert. 1987. Variable region sequences of murine IgM anti-IgG monoclonal autoantibodies (rheumatoid factors). II. Comparison of hybridomas derived by lipopolysaccharide stimulation and secondary protein immunization. *J. Exp. Med.* 165:970-987.
  34. Chen, P. P., D. L. Robbins, F. R. Jirik, T. J. Kipps, and D. A. Carson. 1987. Isolation and characterization of a light chain variable region gene for human rheumatoid factors. *J. Exp. Med.* 166:1900-1905.
  35. Newkirk, M., and D. J. Capra. 1987. Cross-idiotypic specificity among human rheumatoid factors. In *Idiotypes and Disease, Monographs in Allergy*. Vol. 22. M. Zanetti, C. A. Bona, and F. Celeda, editors. Karger AG, Basel, Switzerland. 1-11.
  36. Pons-Estel, B., F. Goni, A. Solomon, and B. Frangione. 1984. Sequence similarities among kappa chains of monoclonal human IgMk autoantibodies. *J. Exp. Med.* 160:893-904.
  37. Goni, F., P. Chen, M. Pizzolato, V. Agnello, D. Carson, and B. Frangione. 1987. Primary structure-dependent cross-reactive idiotypes present in human IgMk monoclonal autoantibodies. *Arthritis Rheum.* 30:S103. (Abstr.)
  38. Agnello, V., F. Goni, J. L. Barnes, M.-T. de la Vega, and B. Frangione. 1987. Human rheumatoid factor crossidiotypes. II. Primary structure-dependent crossreactive idiotype, PSL2-CRI, present on Wa monoclonal rheumatoid factors is present on Bla and other IgMk monoclonal autoantibodies. *J. Exp. Med.* 165:263-267.
  39. Schrohenloher, R. E., and R. B. Hester. 1976. Reassembly of immunoglobulin M heavy and light chains in vitro. *Scand. J. Immunol.* 5:637-646.
  40. Novotny, J., M. Handshumacher, and E. Haber. 1986. Location of antigenic epitopes on antibody molecules. *J. Mol. Biol.* 189:715-721.
  41. Kodaira, M., T. Kinashi, I. Umemura, F. Matsuda, T. Noma, Y. Ono, and T. Honjo. 1986. Organization and evolution of variable region genes of the human immunoglobulin heavy chain. *J. Mol. Biol.* 190:529-541.
  42. Clarke, S. H., K. Huppi, D. Ruzinsky, L. Staudt, W. Gerhard, and M. Weigert. 1985. Inter- and intraclonal diversity in the antibody response to influenza hemagglutinin. *J. Exp. Med.* 161:687-704.
  43. Kabat, E. A., T. T. Wu, H. Bilofsky, M. Reid-Miller, and H. Perry. 1987. Sequences of Proteins of Immunologic Interest. U. S. Dept. of Health and Human Services.
  44. Klapper, D. G., and J. D. Capra. 1976. The amino acid sequence of the variable regions of the light chains from two idiotypically cross reactive IgM anti-gamma globulins. *Ann. Immunol. Inst. Pasteur (Paris).* 127C:261-271.
  45. Goni, F., and B. Frangione. 1983. Amino acid sequence of the Fv region of a human monoclonal IgM (protein WEA) with antibody activity against 3,4-pyruvylated galactose in Klebsiella polysaccharides K30 and K33. *Proc. Natl. Acad. Sci. USA.* 80:4837-4841.
  46. Capra, J. D., and M. M. Newkirk. 1987. Human monoclonal IgG rheumatoid factor (S.F.) appears to use the same V region genes as an IgM rheumatoid factor (RIV). *Fed. Proc.* 46:485. (Abstr.)
  47. Schrohenloher, R. E., and W. J. Koopman. 1986. An idiotype common to rheumatoid factors from patients with rheumatoid arthritis identified by a monoclonal antibody. *Arthritis Rheum.* 29:S28. (Abstr.)
  48. Kaplan, A. P., and H. Metzger. 1969. Partial sequences of six macroglobulin light chains. Absence of sequence correlates to functional activity. *Biochemistry.* 8:3944-3951.
  49. Jirik, F. R., J. Sorge, S. Fong, J. G. Heitzmann, J. G. Curd, P. P. Chen, R. Goldfien, and D. A. Carson. 1986. Cloning and sequence determination of a human rheumatoid factor light-chain gene. *Proc. Natl. Acad. Sci. USA.* 83:2195-2199.