Brief Definitive Report

DISTINCT PATTERNS OF HEAVY CHAIN VARIABLE REGION SUBGROUP USE BY HUMAN MONOCLONAL AUTOANTIBODIES OF DIFFERENT SPECIFICITY

By GREGG J. SILVERMAN,* FERNANDO GOÑI,‡ JOSÉ FERNANDEZ,‡ POJEN P. CHEN,* BLAS FRANGIONE,‡ AND DENNIS A. CARSON*

*From the *Department of Basic and Clinical Research, Research Institute of Scripps Clinic, La Jolla, California 92037; and the ‡Department of Medicine and Pathology, New York University Medical Center, New York, New York 10016*

In some of the earliest experiments proving the existence of crossreactive idiotypes (CRIs), Kunkel et al. (1) prepared adult rabbit antiidiotypic antibodies that recognized sets of cold agglutinins (CAs) or rheumatoid factors (RFs) from unrelated individuals. The Wa group represents the largest of the RF-associated CRIs. These RFs use L chains of the minor VkIIIb variable region sub-subgroup. The complete sequence of the 12 Wa CRI^+ RF L chains that have been reported are extremely homologous, and are considered to be the products of a conserved germline Vk gene, termed *Humkv325* (reviewed in reference 2). The products of this gene are identified with antibodies directed against synthetic peptides homologous to the second (anti-PSL2) and third (anti-PSL3) complementarity determining regions (CDRs) of the L chain of the Wa^+ IgM-RF Sie. Also, by correlation of immunoreactivity with Southern blotting and DNA sequencing, the murine monoclonal anti-CRI antibody, 17.109, has been shown to identify a discontinuous idiotype present on intact Igs or isolated L chains derived from genes identical or nearly identical to the germline configuration of *Humkv325* (3).

CRIs associated with the L and H chains of CAs have also been described (reviewed in reference 4). Many CAs bind to the I antigen, a developmentally regulated carbohydrate moiety on the erythrocyte membrane (5). The CAs with anti-I specificity generally use VkIII L chains, which may also derive from the *Humkv325* gene and/or closely related genes (2, 6). As both RFs and CAs frequently use the same Humkv325-derived L chains, we reasoned that the two types of autoantibodies should display quite distinct patterns of H chain use. To test this hypothesis, we used synthetic peptides to generate primary sequence-dependent antibodies specific for the three major human VH protein subgroups (7). Initial studies of RFs from unrelated individuals revealed that Humkv325-derived antibodies often express anti-IgG activity when paired with a subset of VHI H chains (7). In marked contrast, the present results

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show that anti-I CAs with Humkv325-linked idiotypic markers all use H chains of the minor VHII subgroup. Furthermore, four other antierythrocyte antibodies with VkIII L chains display the same VH subgroup restriction.

Materials and Methods

Human Monoclonal CAs and RFs. CAs were isolated from the sera of patients with chronic cold agglutinin disease. The paraproteins Soc, Pos, Kau, and Tri were kindly provided by Dr. J. Oppenheim (New York University, New York, NY). Bat, Bon, Hig, Lea, and Per were donated by W. F. Rosse (Duke University, Durham, NC), and AJ was provided by R. C. Williams, Jr. (University of New Mexico, Albuquerque, NM). CA activity was confirmed using adult human O^+ erythrocytes at 4°C, as described (5). All CAs were found to bind I antigen and not i, or other red cell epitopes (5), and were devoid of cryoglobulin or RF activity by latex fixation.

The monoclonal RF cryoglobulins and CAs were purified from serum as described previously (5, 8). CA activity was undetectable in RF samples at dilutions >1:16.

Antipeptide Antisera. Synthetic peptides were kindly provided by R. Houghten (Scripps Clinic, La Jolla, CA). Sites were selected from the NH_2-terminal end of the constant regions of κ and μ chains about a β bend, representing the sequences VFIFPPSDEQLKSGTASVVC and SASAPTLFPLVSC (one-letter amino acid code), respectively. Other previously described peptides correspond to portions of first framework regions that are diagnostic for the four κ subgroups (9) and three major H chain subgroups (7). The PHI and PHIII peptides used to generate the VHI and III subgroup reagents represent consensus sequences, AEVKKPGASVKVSC and GGLVQPGGSLRLSC, respectively. The PHII peptide, PGLVKPSETLSLTC, which was used to generate the subgroup II antibody, was taken directly from the homologous portion of the translated mRNA sequence of the B cell line V71.2. This H chain was initially thought to derive from the *VHII* gene family, but more recently has been assigned to the newly described *VHIV* gene family (10). The PSL2 and PSL3 peptides correspond, respectively, to the second and third CDR, with adjacent framework regions, of the L chain, of the IgM-RF Sie (2). All synthetic peptides were conjugated to keyhole limpet hemocyanin for immunization of 6-wk-old female NZW rabbits, as described (7, 9).

Subgroup and Idiotypic Analysis. The specificity of the peptide-induced antisera was demonstrated by immunoblot analysis of proteins of known sequence, combined with peptide inhibition studies, as previously described (7, 9). Briefly, the H and L chains of CAs and RFs were electrophoretically separated on 10% polyacrylamide gels containing 0.1% SDS under reducing conditions (7). After transfer of the proteins to nitrocellulose membranes, nonspecific binding sites were blocked with 5% powdered milk in borate buffered saline, pH 8.2 (BBS). Replicate blots with separated H and L chains were reacted with individual peptide-induced antisera, or with control rabbit anti-Ig. After washing and incubation with ^{125}I-protein A (New England Nuclear, Boston, MA), reactive bands were detected by autoradiography, after exposure of the blots to XAR film at −70°C for 24–72 h.

Results and Discussion

Immunoblotting was used to compare the L and H chain variable region subgroups, and the idiotypes, of purified CAs with anti-I activity, and of monoclonal RFs. Displayed in Fig. 1, *left*, is a representative immunoblot that includes five CAs (Per, Bat, Bon, Hig, Lea), and three RFs (Bor, Les, Riv). All eight L chains are recognized by the VkIII-specific antibody. In a parallel experiment, none were recognized by the VkI, VkII, VkIV, or λ-specific reagents (not shown). Within these eight VkIII L chains, seven are reactive with the PSL2-induced anti-CRI, while two (BAT-CA and BOR-RF) also bind the Humkv325-specific marker, anti-PSL3. The immunoblotting results of 10 CAs, and of 30 RFs, are compiled in Table I (present data and reference 7). Of the 10 VkIII CAs, four (BAT, KAU, POS, TRI) probably derive from the *Humkv325* gene, as they bear both the anti-PSL2 and PSL3 CRIs.

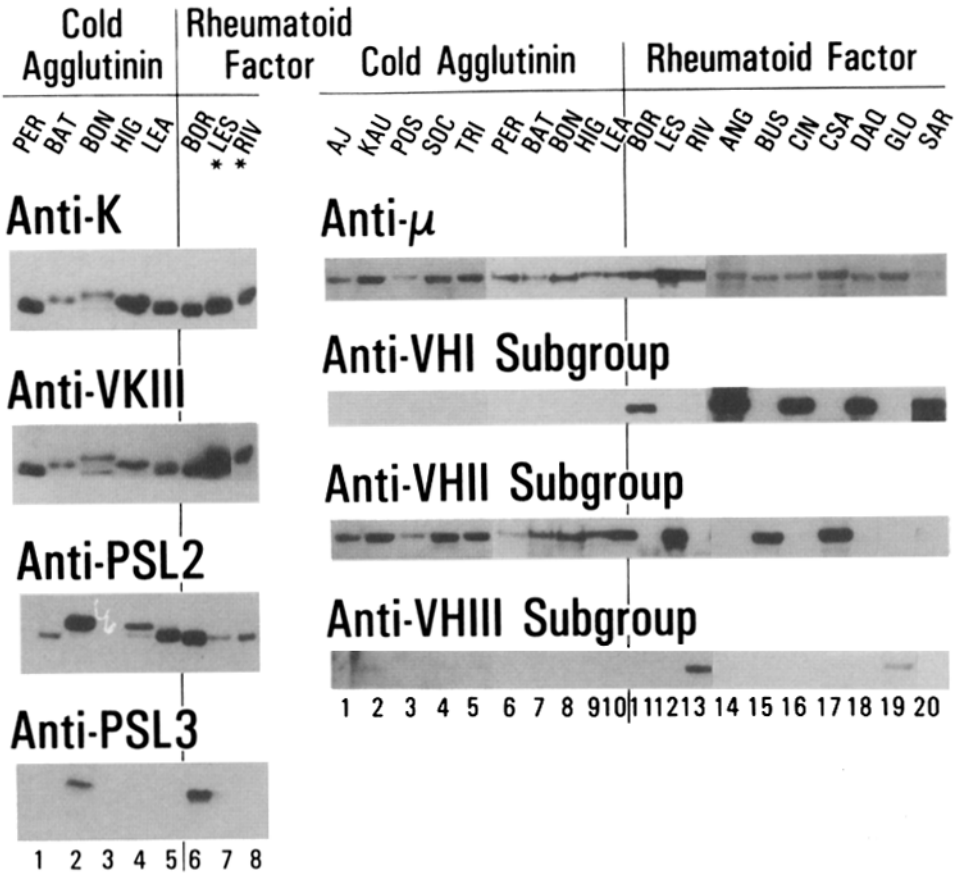

FIGURE 1. Immunoblot analysis of monoclonal IgM-CAs and IgM-RFs. (*Left*) After SDS-PAGE separation and transfer to nitrocellulose, replicate blots of the L chains of five IgM-CAs and three IgM-RFs were reacted with the indicated antipeptide antibodies. (*) VkIIIa L chains by amino acid sequence comparison, and/or reactivity with the 6B6.6 CRI (11). (*Right*) The reactivity of H chains of 10 IgM-CAs and 10 IgM-RFs are displayed.

Additionally, by serologic and amino acid analysis, two CA (AJ, SOC) also derive from the *Humkv325* gene. All 30 RFs in Table I are identified as probable products of the *Humkv325* gene, based upon either reactivity with the anti-PSL3 and/or 17.109 antibodies, or amino acid sequence analysis.

Fig. 1, *right*, shows another representative immunoblot with 10 VkIII CAs and eight Humkv325-derived RFs, which were probed with the anti-H chain antibodies. Two VkIIIa-RFs (Les and Riv) are included for comparison. As compiled in Table I, all CAs and 29:30 Humkv325-derived RFs were reactive with only one of the three VH subgroup-specific antisera (IgM-RF Gol was nonreactive). Most importantly, all CAs were recognized by the antibodies against subgroup II H chain. In contrast, the great proportion (23:30, 77%) of Humkv325-RFs use VHI H chains, while a minority have H chains from the VHII (4:30, 13%), or VHIII subgroups (2:30, 7%). By χ square analysis, this association of subgroup II H chains with CAs, compared with RFs, is highly significant ($p < 0.001$).

TABLE I

Immunoblotting Results of 10 CAs and 30 RFs

Autoantibody	L chain reactivity				H chain reactivity		
	Anti-VkIII subgroup	Anti-PSL2	Anti-PSL3	17.109 CRI	Anti-VHI subgroup	Anti-VHII subgroup	Anti-VHIII subgroup
IgM-VkIII CAs							
AJ*	+	+	−	−	−	+	−
BAT	+	+	+	+	−	+	−
BON	+	+	−	−	−	+	−
HIG	+	+	−	−	−	+	−
KAU*	+	+	+	−	−	+	−
LEA	+	+	−	−	−	+	−
PER	+	+	−	−	−	+	−
POS*	+	+	+	−	−	+	−
SOC*	+	+	−	−	−	+	−
TRI*	+	+	+	−	−	+	−
					0	10 (100%)	0
Humkv325-derived RFs							
AND	+	+	−	+	+	−	−
ANG*	+	+	+	−	+	−	−
ARL	+	+	−	+	+	−	−
BEL	+	+	+	−	+	−	−
BLO	+	+	−	+	+	−	−
BOR*	+	+	+	+	+	−	−
BUS	+	+	+	ND	−	+	−
CIN*	+	+	+	ND	+	−	−
CSA*	+	+	+	ND	−	+	−
CUR*	+	+	+	+	−	+	−
DAQ	+	+	+	ND	+	−	−
DRI	+	+	+	+	+	−	−
FLO*	+	+	+	+	+	−	−
FRA	+	+	+	+	+	−	−
GAL	+	−	+	ND	+	−	−
GAR*	+	+	+	+	+	−	−
GLO*	+	+	+	+	−	−	+
GOL*	+	+	+	−	−	−	−
GOT*	+	+	+	+	−	+	−
JAN	+	+	+	+	+	−	−
KAS*	+	+	+	+	+	−	−
KOK	+	+	+	+	+	−	−
MCD	+	+	+	+	+	−	−
NEU*	+	+	+	−	+	−	−
PAL(m)	+	+	+	+	+	−	−
PAY*	+	+	+	ND	−	−	+
SAR	+	+	+	ND	+	−	−
SCH‡	+	+	+	+	+	−	−
SIE*	+	+	+	+	+	−	−
WOL*	+	+	−	−	+	−	−
					23 (77%)	4 (13%)	2 (7%)

* These L chains have been demonstrated to be Humkv325 derived based on comparison of the complete amino acid sequences and serologic reactivity, and the NH_2-terminal sequence of AJ has been reported to residue 30 (2,6,8,15, and F. Goñi, submitted for publication).

‡ SCH is an IgA, all others are IgM.

Although the VkIII subgroup represents only 13% of κ chains in sera (1), these L chains are used in >60% of IgM-RFs (11), and apparently by most IgM CAs directed against the I antigen. As shown here, the majority (77%) of the Humkv325-derived RFs use H chains of the VHI subgroup, while all VkIII CAs use subgroup II H chains. Other experiments have shown that VkIIIb mAbs that bind to low-density lipoprotein, or intermediate filaments, use H chains of the VHIII subgroup (G. Silverman and F. Goni, unpublished data). Finally, none of these autoantibodies display a pattern of H chain use comparable with VkIII paraproteins of unknown specificity (VHI, 39%; VHII, 17%; and VHIII, 43%) (7); nor does the utilization of the VH subgroups by the two autoantibodies reflect current estimates of VH gene complexity (12).

These experiments are the first to demonstrate a relationship between H chain variable region subgroup utilization and autoantibody specificity. The results provide support to the hypothesis that the binding specificity of many IgM VkIIIb autoantibodies is influenced strongly by the H chain partner.

Although 4:30 IgM-RFs use human subgroup II H chains, these may derive from a different VH gene subset than CAs. In this regard, the genes encoding the VHII protein subgroup have recently been subdivided into at least two distinct VH gene families, designated VHII and VHIV (10), and a related single member gene family, VH6, has also been reported (12). Members of these VH gene families have related nonidentical framework regions, but diverse CDRs, and we are currently working to develop serologic reagents that can discriminate them.

It is possible that the prominent patterns of V gene use by the human IgM autoantibodies that are associated with certain lymphoproliferative disorders are a reflection of an ordered pattern of gene expression during B cell development. Recently, Schroeder et al. (13) have demonstrated restricted VH gene use by B cells in 120-d human fetal liver. One of these genes (30P1) is nearly identical to those used by anti-DNA antibodies that express the 16:6 lupus idiotype (14). Perhaps early B cells that utilize VkIII L chains are preferentially expanded, because they produce a functional antigen receptor when paired with several different germline-encoded HC partners. According to this hypothesis, the interaction of immature B cells with autoantigens in the spleen, peyer's patches, or other lymphoid organs may promote the early expansion of the B cell repertoire.

Summary

Using a panel of antibodies specific for H and L chain variable region subgroups, a panel of human monoclonal cold agglutinin (CA) and rheumatoid factor (RF) autoantibodies were analyzed. The vast majority of the two types of autoantibodies utilized VkIII L chains, many of which probably derive from the *Humkv325* gene. However, while most RFs (77%) utilized VHI H chains, all the CAs used VHII subgroup H chains. These results are consistent with a model of autoantibody generation, wherein binding specificity is H chain defined in a set of antibodies that use a multipotential L chain.

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