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Immunology

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

(immunoglobulins/variable domains/diversity segment/complementarity-determining region)

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ABSTRACT **We have determined the amino acid sequence of the Fv [variable heavy (V_H) and variable light (V_L)] region of a human monoclonal IgM-κ with antibody activity against 3,4-pyruvylated galactose, isolated from the plasma of patient WEA with Waldenström macroglobulinemia. The V_H region has 114 residues, belongs to subgroup III, and has a very short third complementarity-determining region (CDR3), probably due to a small D segment/or an unusual D–J rearrangement (D, diversity; J, joining). The V_L region has 108 residues and belongs to subgroup $V_{\kappa I}$. Compared to other members of the human V_{HIII} and $V_{\kappa I}$ families, WEA Fv does not appear to have significant differences within the framework residues but has unique CDRs that might be responsible for the particular antibody activity. Another IgM-κ (GAL), which has an as-yet-undetermined antibody activity, shares a striking homology in V_κ with WEA, including an identical CDR1.**

It is known by immunochemical (1) and x-ray-crystallographic (2, 3) studies that the Fv region of the immunoglobulins [the variable region of the heavy chain (V_H) and the variable region of the light chain (V_L)] is responsible for the antibody activity and that the specificity resides in the molecular complementarity between structural determinants of the antigen molecule and amino acid residues in the active site.

Although several human monoclonal immunoglobulins have been found to have antibody activity (4–7), the sequences of only a few of them have been determined for the entire Fv region (5, 8). This lack of information is due to the difficulty in finding and obtaining human monoclonal immunoglobulins with antibody activity in sufficient amounts for detailed structural studies and the obstacles associated with screening a wide range of antigens.

The V_L region is encoded by two separate gene segments: *V* (variable) and *J* (joining) (9–11). The V_H region, in addition to its *V* and *J* gene segments, is encoded by a third gene segment called *D* (diversity) (12–18), which in mice (12, 13) was found to encode a portion of the third complementarity-determining region (CDR3). In humans and rabbits, its DNA sequence (18) was also shown to have a striking homology to a portion of the CDR2 (19, 20). Evidence for such minigenes was obtained by independent assortment (21). The multiplicity of these elements in the germ-line or unrearranged DNA allows diversity by recombination, gene conversion, and variations in the joining sites (13–18, 22–25).

In mice it was demonstrated that for a particular antigen a V_H DNA subset or segment is preferentially selected and joined to a particular *D* minigene, which can be recombined to any of the J_H minigenes (13, 23). For L chains, the selection is of one V_L DNA segment that can be joined to any J_L (26).

The availability, in sufficient quantities, of a human monoclonal IgM-κ (WEA) with antibody activity specific for 3,4-pyruvylated galactose present in certain *Klebsiella* polysaccharides (6) encouraged us to determine the primary structure of its Fv region. We now report the complete amino acid sequence of the V regions of H and L chains from protein WEA.

MATERIALS AND METHODS

Isolation of WEA-IgM. IgM-κ WEA, isolated as euglobulin from the plasma of a patient with Waldenström macroglobulinemia, was chromatographed on a Sephadex G-200 (Pharmacia) column equilibrated with 0.1 M sodium phosphate/0.15 M NaCl, pH 7.00, and reprecipitated as euglobulin. Purity was assessed by Ouchterlony double diffusion in agarose, using antisera to μ, γ, α, κ, and λ chains and by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. Presence of J chain was determined by Ouchterlony analysis with anti-J chain (courtesy of J. Mestecky).

Complete Reduction and Alkylation. Protein WEA was completely reduced in 6 M guanidine/0.6 M Tris·HCl/0.001 M EDTA/20 mM dithiothreitol, pH 8.2, for 1 hr at 37°C and alkylated by the addition of iodo[^{14}C]acetic acid (0.7 mCi/mmol, New England Nuclear; 1 Ci = 3.7×10^{10} Bq) to a final concentration of 40 mM for 1 hr at 37°C. H and L chains were separated on a Sephadex G-100 column equilibrated in 1 M acetic acid/5 M guanidine.

Fv Fragment. This fragment was prepared as described by Lin and Putnam (27). IgM-κ was dissolved at 10 mg/ml in 0.02 M sodium acetate/0.15 M NaCl pH 4.00 buffer. Pepsin was added (1:50 enzyme-to-protein weight ratio) and the protein was digested for 24 hr at 4°C, then a new aliquot (1:100) of pepsin was added and digestion was continued for an additional 24 hr. The solution was slowly titrated to neutrality with 1 M NaOH, dialyzed against 0.1 M Tris·HCl/0.15 M NaCl, pH 8.00, and chromatographed on a Sephadex G-100 column equilibrated in the same buffer. The Fv fraction was identified on 12.5% polyacrylamide gels containing 0.1% sodium dodecyl sulfate (28). V_H and V_L regions were separated by HPLC using a linear gradient with a starting buffer of 0.05% trifluoroacetic acid and a final buffer of 0.05% trifluoroacetic acid/acetonitrile, 1:2 (vol/vol).

Fab Fragment. IgM-κ WEA was dissolved at 15 mg/ml in 0.05 M Tris·HCl/11.5 mM $CaCl_2$ pH 8.1 buffer and digested with trypsin (1:100 enzyme-to-protein weight ratio) at 60°C for 45 min (29). Fcμ5 and Fab fragments were separated on a

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Abbreviations: H, heavy; L, light; V, variable; C, constant; J, joining; D, diversity; CDR, complementarity-determining region; FR, framework region.

Sephadex G-200 column and analyzed on 12.5% polyacrylamide gels containing 0.1% sodium dodecyl sulfate.

Cyanogen Bromide (CNBr) Cleavage. WEA Fab fragment was dissolved at 30–40 mg/ml in 70% trifluoroacetic acid, a 5-fold excess (wt/wt) of CNBr (Pierce) was added, and the reaction was allowed to proceed for 16–20 hr at room temperature. CNBr fragments were separated on a Sephadex G-75 column equilibrated with 5% HCOOH/5 M guanidine.

Carboxypeptidase A Digestion. Fragments were dissolved at 0.5% in 0.1 M ammoniun acetate, pH 8.00, and incubated at 37°C with carboxypeptidase A (Sigma) (1:300 enzyme-to-fragment weight ratio) (30). Samples were collected at 2, 5, 10, 30, and 60 min and overnight, and the amino acid(s) released were determined on a Durrum 500 amino acid analyzer. Glutamine, asparagine, and tryptophan were run as standards.

Other Digestions. Tryptic, chymotryptic, and Pronase digestions were performed as described (31) with various times and protein-to-enzyme ratios, depending upon the fragment to be digested. Separation of peptides was accomplished by HPLC (Waters Associates) using a reverse-phase support medium (μBondapak C_{18}). The peptides were eluted at a flow rate of 1 ml/min with a gradient generated by mixing 0.05% trifluoroacetic acid with 0.05% trifluoroacetic acid/acetonitrile, 1:2 (vol/vol). All runs were performed at room temperature. Effluents were monitored by absorbance at 210 nm, and radioactivity was determined by scintillation counting (Beckman LS-250) where appropriate (32). Pronase peptides were subjected to high-voltage electrophoresis at pH 6.5 and stained with 0.1% ninhydrin and chlorine to reveal the presence of pyrrolidone carboxylic acid (33). Presence of tryptophan was determined by specific staining on paper (34).

Amino Acid Analysis and Sequence. Amino acid analysis of peptides hydrolyzed with 6 M HCl/0.1% phenol (31) was performed on a Durrum 500 analyzer. Manual sequencing was accomplished as described (35), and automated Edman degradation was achieved by using a Beckman 890C sequencer and a 0.1 M Quadrol program. Polybrene was used when needed (36). Phenylthiohydantoin derivatives were identified by HPLC with a μBondapak C_{18} column and by amino acid analysis after back-hydrolysis of the derivatives (32).

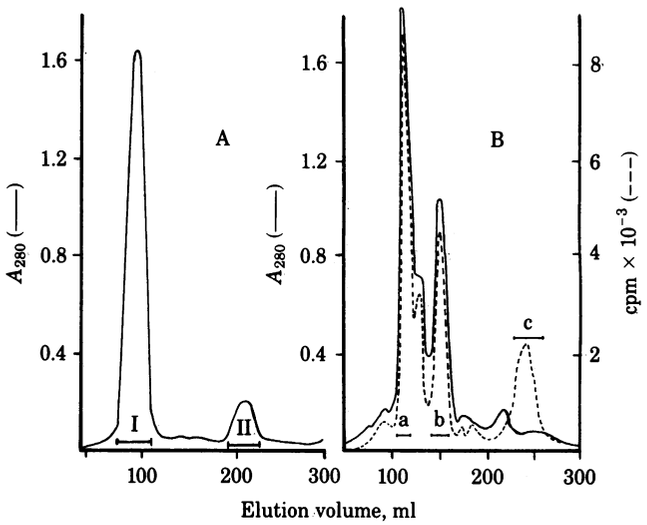

FIG. 1. Profiles from a Sephadex G-75 column (90 × 3 cm) equilibrated in 5% (wt/wt) HCOOH/5 M guanidine. (*A*) Cyanogen bromide digest of WEA Fab. (*B*) Peak I from *A* after complete reduction and ^{14}C-alkylation.

RESULTS

Amino Acid Sequence of WEA V_H. Attempts to remove pyrrolidone carboxylic acid from the amino terminus of WEA H chains were unsuccessful due to the insolubility of the H chain in the deblocking buffer (37). Fab produced by trypsin digestion at 60°C was subsequently cleaved with CNBr and two frac-

Table 1. Amino acid compositions of WEA Fd (V_H-C_μ1) fragments* (residues per mol)

Amino acid	CNBr Ic	CNBr II	CNBr Ib	CNBr Ic-CH1	CNBr Ic-CH1 PR1	PR1A	PR1B	PR2	CNBr Ic T1	T2	T3	CNBr II T1	T2	T3
Cys†	0.59	—	1.52	—	—	—	—	—	—	0.55	—	—	—	—
Asp	3.27	4.95	9.16	1.02	—	—	—	1.04	1.03	—	1.87	1.92	1.04	—
Thr	0.96	1.82	7.54	—	—	—	—	—	—	—	0.99	—	—	—
Ser	5.40	6.41	16.86	1.70	—	—	—	1.74	1.57	2.50	1.05	1.02	0.87	—
Glu	2.93	3.13	5.19	3.16	2.10	2.00	0.99	1.13	3.08	—	—	—	—	1.20
Pro	1.26	1.05	5.92	1.02	—	—	—	0.99	0.93	—	—	—	—	—
Gly	6.29	6.25	7.67	5.02	—	—	—	5.05	5.30	1.11	—	—	—	—
Ala	2.01	2.27	8.40	—	—	—	—	—	—	1.10	1.10	—	—	—
Val	3.04	2.02	8.04	3.00	0.94	1.00	1.01	1.97	2.99	—	—	—	—	—
Ile	—	2.64	1.86	—	—	—	—	—	—	—	—	—	—	—
Leu	4.01	4.16	9.99	3.09	0.96	—	—	1.86	2.98	1.03	—	—	1.02	0.94
Tyr	—	2.74	3.56	—	—	—	—	—	—	—	—	—	1.06	—
Phe	1.95	1.96	3.66	—	—	—	—	—	—	0.95	1.00	—	—	—
Lys	—	2.71	3.66	—	—	—	—	—	—	—	—	1.10	—	—
Arg	1.10	3.28	3.52	—	—	—	—	—	1.09	—	—	—	—	—
Hse	(+)	(+)	(+)	—	—	—	—	—	—	—	(+)	—	—	0.86
Trp‡	—	2.00	≥2	—	—	—	—	—	—	—	—	—	—	—
NH_2 terminus	(−)	Asn	Ser	(−)	(−)	(−)	(−)	Val	(−)	Leu	Thr	Asn	Asn	Leu
Residues	1–34	35–83	84–204	1–18	1–4	1–3	1–2	5–18	1–19	20–27	28–34	73–76	77–80	81–83

T, tryptic; CH, chymotryptic; PR, Pronase; Hse, homoserine; (+), present; (−), nonreactive.
*See Fig. 1.
†Detected as carboxymethylcysteine.
‡Determined by amino acid sequence.

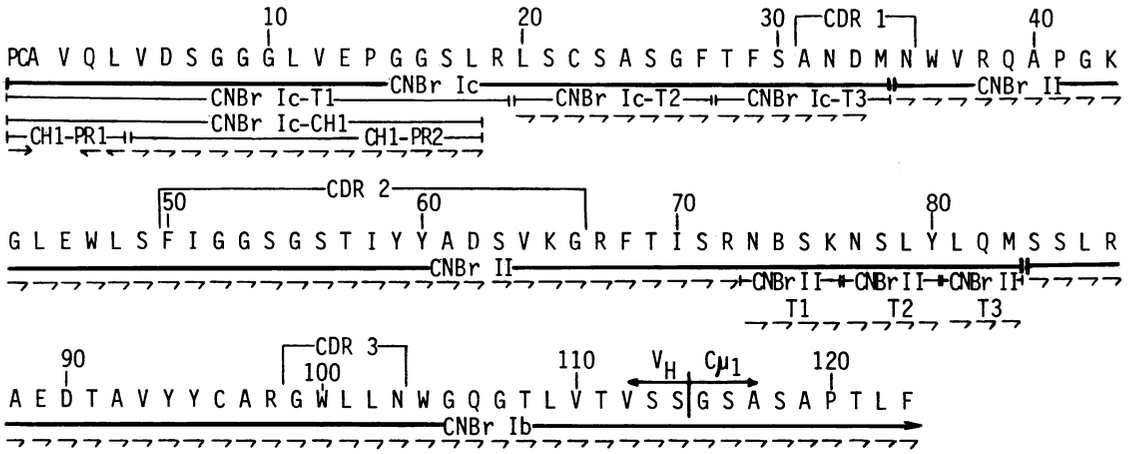

FIG. 2. Amino acid sequence of WEA V_H and amino-terminal positions of the $C_\mu 1$ domain of the μ chain constant region. —, Positions at which the sequence was determined; ←, positions at which the residue was identified by carboxypeptidase digestion and amino acid analysis; →, pyrrolidone carboxylic acid determined by specific stain on paper. Amino acids are identified by the single-letter code: A, Ala; B, Asp or Asn; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; I, Ile; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; and PCA, pyrrolidone carboxylic acid.

tions, CNBr I and CNBr II, were obtained after chromatography on a Sephadex G-75 column (Fig. 1*A*). The CNBr I fragment was reduced, alkylated, and chromatographed on the same Sephadex G-75 column (Fig. 1*B*). Three peaks were obtained. One of them, CNBr Ia, was subjected to automated amino acid sequence analysis and proved to be the L chain, starting at position 5. The amino acid analyses of CNBr fragments Ib and Ic (Fig. 1*B*) and CNBr II (Fig. 1*A*) are shown in Table 1.

CNBr Ic, which yielded no products in the first step of the Edman degradation, was digested with trypsin and the resultant peptides were purified by HPLC. Three peptides were obtained: CNBr Ic-T1, -T2, and -T3. Their amino acid compositions are shown in Table 1. CNBr Ic was also digested with chymotrypsin and the peptides were purified by HPLC. Peptide CNBr Ic-CH1 had a nonreactive amino terminus and its composition (Table 1) is identical to that of CNBr Ic-T1 (minus an arginine residue).

Peptide CH1 was further digested with Pronase and four peptides were obtained: PR1, PR1A, PR1B, and PR2; their amino acid compositions are shown in Table 1. Amino-terminal and carboxyl-terminal analysis of peptide PR1 and the amino acid sequence of peptide PR2 established the sequence of residues 1–18 (Fig. 2). Peptides CNBr Ic-T2 and -T3, products of chymotryptic activity during trypsin digestion, yielded by automated sequence analysis positions 20–27 and 28–34, respectively (Fig. 2). The presence of arginine at position 19 was inferred from the amino acid compositional difference between CNBr Ic-T1 and CNBr Ic-CH1 (Table 1).

Automated amino acid analysis of CNBr II yielded 38 residues that accounted for positions 35–72 (Fig. 2). CNBr II was further digested with trypsin and three peptides were obtained: CNBr II-T1, -T2, and -T3. Their amino acid compositions are listed in Table 1 and their sequences were determined by manual and automated Edman degradation and placed by homology at positions 73–76, 77–80, and 81–83, respectively (Fig. 2). The split between positions 80 and 81 is also due to chymotryptic activity.

CNBr Ib subjected to automated amino acid sequence gave unambiguous results for 40 steps and accounted for positions 84–123 (Fig. 2).

Amino Acid Sequence of WEA V_L. The completely reduced and ^{14}C-alkylated light chain was subjected to automated amino acid analysis and yielded the first 66 residues (Fig. 3); they are characteristic of a $V_{\kappa I}$ (8).

The L chain was then digested with trypsin and peptides were purified on an HPLC system. The sequence of T II (Fig. 3) was determined and gave 29 residues, accounting for positions 62–90. Peptide T II was further digested with chymotrypsin and

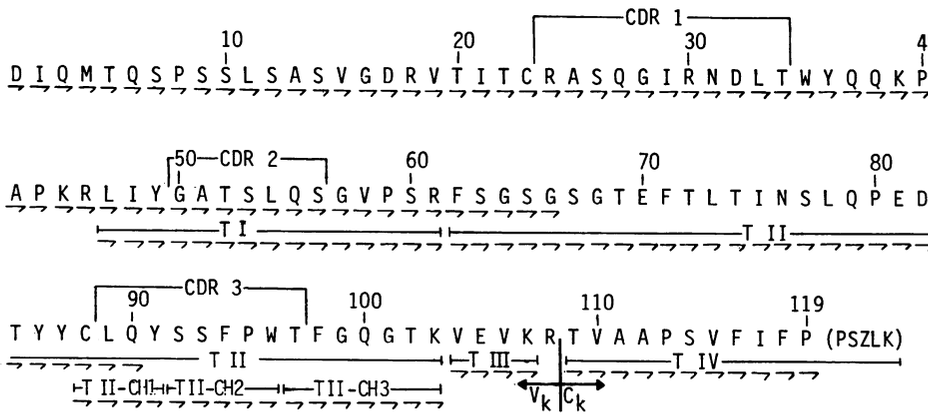

FIG. 3. Amino acid sequence of WEA V_L. —, Positions at which the sequence was determined. The identities of residues 1–66 were obtained by automated amino acid sequence from the isolated, completely reduced, and ^{14}C-alkylated L chain. Amino acids are identified by the single-letter code as in Fig. 2.

Table 2. Amino acid compositions of WEA L chain and its fragments (residues per mol)

Amino acid	L	V_L	T I	T II	T III	T IV	T II-CH1	T II-CH2	T II-CH3
Cys*	4.10	1.05	—	0.62	—	—	0.53	—	—
Asp	16.60	5.86	—	2.33	—	1.05	—	—	—
Thr	18.78	11.63	0.97	5.65	—	1.01	—	—	2.07
Ser	28.37	14.98	2.56	5.82	—	1.90	—	1.87	—
Glu	25.21	12.82	1.18	5.22	1.10	1.82	1.05	—	1.05
Pro	11.17	6.40	0.96	2.23	—	2.67	—	1.00	—
Gly	15.03	10.70	2.01	5.03	—	—	—	—	1.98
Ala	13.10	6.73	1.16	1.23	—	2.24	—	—	—
Val	15.36	6.64	1.02	—	1.74	2.14	—	—	—
Met	1.07	0.86	—	—	—	—	—	—	—
Ile	6.47	4.58	0.92	1.10	—	0.92	—	—	—
Leu	16.22	6.36	1.91	3.18	—	1.05	0.99	—	—
Tyr	8.94	5.08	1.06	2.99	—	—	0.96	—	—
Phe	8.94	5.51	—	4.81	—	1.86	—	1.12	0.98
His	1.85	—	—	—	—	—	—	—	—
Lys	12.60	4.66	—	1.17	1.15	1.23	—	—	0.92
Arg	9.10	5.65	1.26	—	—	—	—	—	—
Trp†	ND	ND	—	(+)	—	—	—	(+)	—

The V_L fragment was obtained by cold pepsin digestion (see text); it accounts for positions 1–116.
* Detected as cysteic acid in the intact molecule and as carboxymethylcysteine in the fragments.
† ND, not determined; (+), determined by stain on paper (34) and amino acid sequence.

the peptides were purified by HPLC. The sequences of peptides T II-CH1, -CH2, and -CH3 (amino acid compositions shown in Table 2) were determined and gave positions 88–91, 92–96, and 97–103, respectively. The tryptophan content of peptide T II-CH2 was determined by specific staining on paper (34). The sequences of T III and T IV were determined and by homology gave positions 104–107 (end of J_κ segment) and 109–119 (beginning of C_κ), respectively. The amino acid composition of V_L (Table 2) indicated the presence of six arginine residues. After trypsin digestion of the V_L fragment, free arginine was obtained and because of the homology with other light chains of the same subgroup, it was placed at position 108.

DISCUSSION

Analysis of V_L. WEA V_L is composed of 108 amino acid residues. The L chain was shown to be κ, both immunologically and by amino acid sequence (Fig. 3); it belongs to the $V_{\kappa I}$ subgroup (positions 1–95) (8). From positions 96 to 108 it appears to be $J_{\kappa I}$ (11) with one substitution at position 106 (valine for isoleucine). An identical substitution is also present in three other proteins previously reported [BJ48, LAY, and EU (8)], perhaps representing polymorphism in the *J* gene (11).

Compared to the human $V_{\kappa I}$ family, WEA V_L exhibits within the framework residues a homology of 95%, having two unusual substitutions at positions 42 and 46 (Fig. 4*A*). The three CDRs are unique for protein WEA L chain, (although CDR1 is shared by another protein GAL as discussed below), with identity to the rest of $V_{\kappa I}$ ranging between 44% and 58%. This low homology is mainly due to what appears to be very rare substitutions at positions 28, 30, 32, 34, 50, 52, 55, 89, 92, 94, and 96. Tryptophan at position 96 is the first amino acid of the $J_{\kappa I}$ segment as deduced from the DNA sequence (11), and, although it is located at the recombination point between $V_{\kappa I}$ and $J_{\kappa I}$, it is rarely expressed.

Analysis of V_H. WEA V_H consists of 114 residues and by its amino acid sequence belongs to the V_{HIII} subgroup. When compared to Kabat numbering (8), it has one amino acid inserted at position 52, three at position 82, and three deleted between 98 and 102. At positions 104–114, the sequence indicates that it can belong to J_H1, J_H4, or J_H5 (17).

WEA V_H region has an amino terminus blocked by pyrrolidone carboxylic acid, and within the framework regions (FRs) has 90% identity with the human V_{HIII} family (Fig. 4*B*), having three unusual substitutions at positions 13, 23, and 82A unique for this protein and three additional substitutions at positions

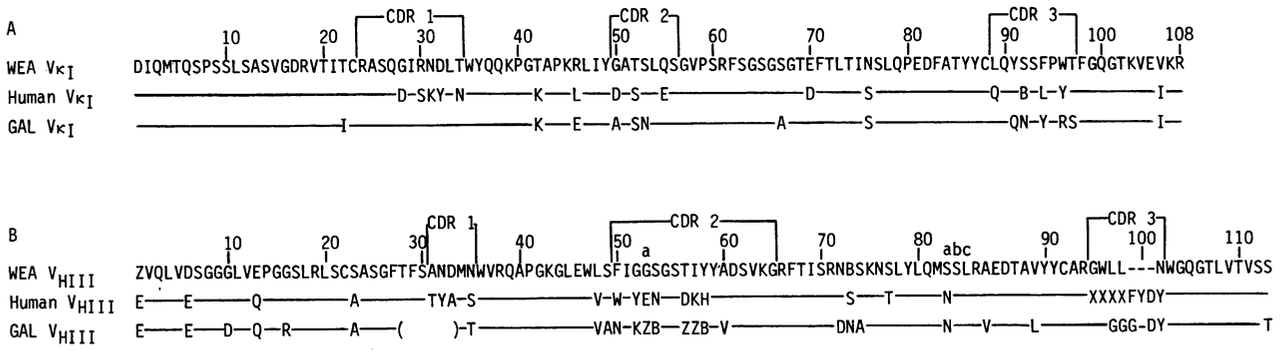

FIG. 4. (*A*) Comparison of the amino acid sequence of V_κ WEA, GAL (38), and human $V_{\kappa I}$. For human $V_{\kappa I}$ the most common residues for each position in the $V_{\kappa I}$ subgroup are indicated (8). A line indicates identity. (*B*) Comparison of the V_H region sequences of WEA, GAL (39), and human V_{HIII}. For human V_{HIII} the most common residues for each position in the V_{HIII} subgroup are indicated (8). ( ), No sequence available; -, residue missing; X, no typical amino acid in the position. Numbering is according to Kabat *et al.* (8).

6, 48, and 77 present in less than 2% of the known human V_{HIII} subgroup sequences.

The three V_H CDRs are unique for this protein because the first two are only 50% identical to the rest of the known V_{HIII} CDRs and the CDR3 is unusually short, extending from position 99 to position 103 (Fig. 2). Compared to the V_{HIII} family, it lacks positions 99, 100, and 101 (Fig. 4*B*) (8). Whether this is due to a short D segment or an unusual recombination to the J_H segment cannot be determined. CDR3 starts after arginine at position 98 (Fig. 2), which represents the 3′ end of the V_H DNA segment, as was shown on a cloned human V_H gene (16). The D segment appears to code for positions 99–102, and position 103 can be created by recombination with J_H1, J_H4, or J_H5 (17). The three J segments have the same amino acid sequence for positions 104–114, although J_H1 or J_H4 appears to be more suitable because a recombination inside the sixth or fourth codons (TAC and CAC), respectively, will use the last two bases A-C before the TGG triplet (tryptophan-104) (17) to build by a joining event an AAC coding triplet for asparagine at position 103. Whether or not this is a normal human VDJ rearrangement selected against 3,4-pyruvylated galactose cannot be answered until more information is available from other human immunoglobulins with the same specificity.

Comparison to Protein GAL. Protein GAL is an IgM-κ (38, 39) that, like protein WEA, belongs to V_{HIII} and $V_{\kappa I}$ subgroups and shares the Km[3] allotype in the C_κ region (40). Antibody activity against 3,4-pyruvylated galactose or agar* was not detected in protein GAL.

The striking homology between the two $V_{\kappa I}$ regions of nearly 90% identity (Fig. 4*A*) is more evident in the first 40 residues, including CDR1, where there is only one difference at position 22. To date there are no other $V_{\kappa I}$ regions known to have a similar CDR1. Up to position 95, there are six differences in the CDRs and five in the FRs, of which three (42, 46, and 52) are unique for WEA and three (22, 67, and 91) are unique for GAL. WEA and GAL V_L regions appear to come from the same or very closely related genes, rarely expressed, with some of the differences accounted for by somatic mutation. This assumption is based on the finding that the human V_κ repertoire is restricted to only 15–20 coding genes that were hypothesized to undergo extensive somatic mutation after selection (41) and that the two proteins are similar to each other while different from the rest of the reported $V_{\kappa I}$ proteins. However, the possibility of gene conversion in the CDR1 zone cannot be excluded (25). Comparison of the amino acid sequence of the V_H regions of proteins WEA and GAL (Fig. 4*B*) indicates striking differences in the CDRs as well as the FRs. However, as with WEA, the CDR3 of protein GAL starts with the sequence Gly-Trp and has a deletion at position 100.

Comparison to other monoclonal proteins with similar or identical specificity should provide new insights into the nature of the active sites.

*Assays by J. Liao and E. A. Kabat (personal communication). There was no precipitation band with *Klebsiella* capsular polysaccharide K33 or with a 20°C extract of agar (6).

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1. Hochman, J., Inbar, D. & Givol, D. (1973) *Biochemistry* **12**, 1130–1135.
2. Poljak, R. J., Amzel, L. M., Chen, B. L., Phizackerly, R. P. & Saul, F. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3440–3444.
3. Segal, D. M., Padlan, E. A., Cohen, G. H., Rudikoff, S., Potter, M. & Davies, D. R. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4298–4302.
4. Kunkel, H. G., Winchester, R. J., Joslin, F. G. & Capra, J. D. (1974) *J. Exp. Med.* **139**, 128–136.
5. Andrews, D. W. & Capra, J. D. (1981) *Biochemistry* **20**, 5816–5822.
6. Kabat, E. A., Liao, G., Bretting, H., Franklin, E. C., Geltner, D., Frangione, B., Koshland, M. E., Shyong, J. & Osserman, E. F. (1980) *J. Exp. Med.* **152**, 979–995.
7. Avrameas, S., Guilbert, B. & Dighiero, G. (1981) *Ann. Immunol. (Inst. Pasteur)* **132C**, 231–236.
8. Kabat, E. A., Wu, T. T. & Bilofsky, H. (1979) *Sequences of Immunoglobulin Chains* (NIH, Bethesda, MD), publ. no. 80–2008.
9. Seidman, J. G. & Leder, P. (1978) *Nature (London)* **276**, 790–795.
10. Bentley, D. L. & Rabbitts, T. H. (1980) *Nature (London)* **288**, 730–733.
11. Hieter, P. A., Maizel, J. V. & Leder, P. (1982) *J. Biol. Chem.* **257**, 1516–1522.
12. Schilling, J., Clevinger, B., Davie, J. M. & Hood, L. (1980) *Nature (London)* **283**, 35–40.
13. Early, P. W., Huang, H., Davis, M. M., Calame, K. & Hood, L. (1980) *Cell* **19**, 981–992.
14. Davis, M. M., Calame, K., Early, P. N., Livant, D. L., Joho, R., Weissman, I. L. & Hood, L. (1980) *Nature (London)* **283**, 733–739.
15. Sakano, H., Kurosawa, Y., Weigert, M. & Tonegawa, S. (1981) *Nature (London)* **290**, 562–565.
16. Rabbitts, T. H., Bentley, D. L., Domnick, W., Forster, A., Matthyssens, G. E. A. R. & Milstein, C. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 867–878.
17. Ravetch, J. V., Siebenlist, U., Korsmeyer, S., Waldmann, T. & Leder, P. (1981) *Cell* **27**, 583–591.
18. Siebenlist, U., Ravetch, J. V., Korsmeyer, S., Waldmann, T. & Leder, P. (1981) *Nature (London)* **294**, 631–635.
19. Wu, T. T. & Kabat, E. A. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5031–5032.
20. Bernstein, K. E., Reddy, E. P., Alexander, C. B. & Mage, R. G. (1982) *Nature (London)* **300**, 74–76.
21. Kabat, E. A., Wu, T. T. & Bilofsky, H. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2429–2433.
22. Sakano, H., Huppi, K., Heinrich, G. & Tonegawa, S. (1979) *Nature (London)* **280**, 288–294.
23. Rao, N. D., Rudikoff, S., Krutzsch, H. & Potter, M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2890–2894.
24. Gough, N. M. & Bernard, O. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 509–513.
25. Baltimore, D. (1981) *Cell* **24**, 592–594.
26. Tonegawa, S., Sakano, H., Maki, R., Traunecker, A., Heinrich, G., Roeder, W. & Kurosawa, Y. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 839–858.
27. Lin, L. C. & Putnam, F. W. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2649–2653.
28. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
29. Plaut, D. & Tomasi, T., Jr. (1970) *Proc. Natl. Acad. Sci. USA* **65**, 318–322.
30. Ambler, R. P. (1967) *Methods Enzymol.* **11**, 436–445.
31. Michaelsen, T. E., Frangione, B. & Franklin, E. C. (1977) *J. Biol. Chem.* **252**, 883–889.
32. Frangione, B., Rosenwasser, E., Penefsky, H. & Pullman, M. E. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7403–7407.
33. Reindel, F. & Hoppe, W. (1954) *Chem. Ber.* **87**, 1103–1107.
34. Reddi, K. K. & Kodicek, E. (1953) *Biochem. J.* **53**, 286–294.
35. Gray, W. R. (1972) *Methods Enzymol.* **25**, 333–343.
36. Klapper, D. F., Wilde, C. E., II, & Capra, J. D. (1978) *Anal. Biochem.* **85**, 126–131.
37. Podel, D. N. & Abraham, G. (1978) *Biochem. Biophys. Res. Commun.* **81**, 176–185.
38. Laure, C. J., Watanabe, S. & Hilschmann, N. (1973) *Hoppe-Seyler's Z. Physiol. Chem.* **354**, 1503–1504.
39. Watanabe, S., Barnikol, H. U., Horn, J., Bertram, J. & Hilschmann, N. (1973) *Hoppe-Seyler's Z. Physiol. Chem.* **354**, 1505–1509.
40. World Health Organization (1976) *Eur. J. Immunol.* **6**, 599–601.
41. Bentley, D. L. & Rabbitts, T. H. (1981) *Cell* **24**, 613–623.