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Three-Dimensional Structure of the Fab from a Human IgM Cold Agglutinin

Ana Cauerhff,* Bradford C. Braden, ‡ Julio Garcia Carvalho, ‡ Ricardo Aparicio, ‡ Igor Polikarpov, ‡ Juliana Leoni,* and Fernando A. Goldbaum §

Cold agglutinins (CAs) are IgM autoantibodies characterized by their ability to agglutinate in vitro RBC at low temperatures (4–22°C) (1, 2). These autoantibodies cause hemolytic anemia in patients with CA disease (CAD). CAs appear in the context of monoclonal gammopathies secondary to B cell dyscrasias ranging from benign to malignant lymphoproliferation (3–5). They can also be detected in normal patients at low titers (6), though these titers increase with different infectious processes (7–9).

Many diverse Ags are recognized by CAs, most frequently those belonging to the I/i system. These are oligosaccharides composed of repeated units of N-acetyllactosamine, expressed in a linear form (i) on fetal RBC or in a branched form (I) on adult RBC.

Early studies revealed that the vast majority of Abs with CA activity reacted with a polyclonal antisera (10) generated by an isolated CA molecule. This phenomenon is called “idiotypic cross-reactivity” because these types of autoantibodies share an antigenic structure (or idiotope). The rat mAb 9G4 (11) served to identify the cross-reacting idiotope associated with the expression of a particular variable domain of the heavy chain (VH) region. Amino acid and nucleotide sequence analyses have confirmed this VH as derived from the VH4-34 (VH4-21) germline gene (12, 13). The presence of a VH chain derived from VH4-34 is necessary both for the CA property and for the idiotope that is recognized by 9G4 mAb. Abs derived from the VH4-34 gene also recognize different autoantigens as is the case of some rheumatoid factors (14), anti-DNA Abs (15), and the anti-D Abs (16).

Anti-I/i CAs show a great variability in VhCDR3, suggesting that this hypervariable region could not be directly involved in the specific recognition or that the mode of binding is different among CAs. Li et al. (17) postulated that the VH-FR1 region is essential in the specific recognition of the anti-I/i system, whereas the VhCDR3 and variable domain of the light chain (VL) dictate the fine specificity and strength of binding. Most of the anti-I CAs VL domains are derived from the VκIII germline gene, although some are encoded by VκI or VκII. In contrast, CAs with anti-i activity make no preferential usage of L chains.

The IgM KAU CA was obtained from the serum of a patient suffering CAD. Its amino acid sequence has been determined by Leoni and coworkers (18). As all CAs that recognize the I/i system, the VH domain is derived from the VH4-34 germline gene, showing a single point mutation in the VHCDR1 (Gl31Asp). The VL domain is derived from the kv305 germline gene (VκIIIb). The Fab from IgM KAU was crystallized, and preliminary x-ray diffraction data was reported (19).

Here we present the three-dimensional structure of the Fab KAU. Its combining site shows an extended cavity, as expected of an anti-carbohydrate Ab. Conservation of key binding site residues among anti-I/i CAs indicates that this is a common feature of this family of autoantibodies. We also describe the first high-resolution structure of the human IgM CA first constant domain of the heavy chain; anti-Id, anti-idiotypic Ab; rmsd, root mean square deviation; PDB, Protein Data Bank.

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chain: constant domain of the light chain (\(\text{C}_{\text{H}}1\text{C}_{\text{L}}\)) domain and compare its features with those of other human and murine isotypes.

**Materials and Methods**

**Molecular replacement and structure refinement**

In a previous work (19) we described the preliminary x-ray diffraction of a single crystal of Fab KAU to a resolution of 2.8 Å. Using synchrotron light at the Laboratorio Nacional de Luz Sincrotron (LNLS, Campinas, Brazil) we obtained a data set comprising 33,333 unique reflections (96.8% complete between 13.0 and 2.8 Å resolution, 99.0% complete in the last resolution shell between 2.9 and 2.8 Å).

This crystal belongs to \(P_{3}121\) space group with cell dimension of \(a = 114.23\,\text{Å}, c = 172.78\,\text{Å}; \alpha = \beta = 90^\circ, \gamma = 120^\circ\). Estimation of solvent content in this crystal, using the Matthews coefficient (20), indicated that there are two Fabs in the asymmetric unit (\(V_m = 3.38\,\text{Å}^3\text{Da}^{-1}\) and solvent content \(= 63\%\)). The crystal structure of KAU Fab was initially determined at 2.8 Å by the molecular replacement method using the program AmoRe (21). The search model consisted of the Fab of Ab 3db (22) (Protein Data Bank (PDB) code 1dqb). Several of the best rotation functions and translation searches were used for the translation search. The seven best translation function solutions were subjected to rigid body refinement using reflections in the resolution range 13.8–3.5 Å. The two most significant solutions have correlation coefficients of 0.219 and 0.210, whereas all other solutions were below 0.145. Combining these two solutions resulted in an \(R\) factor of 0.497 and correlation coefficient of 0.344. In all of these calculations, the search model elbow angle was not changed.

Several cycles of model building with the program O (23) followed by refinement of atomic coordinates with Refmac (24) reduced the \(R_{\text{cryst}}\) factor to 0.268, the \(R\) factor to 0.216, root mean square deviation (r.m.s.d) bond lengths to 0.016 Å, and r.m.s.d bond angles to 3.3°. Appropriate amino acid substitutions were made in the model structure using the program KAU primary structure (18). Omit maps were calculated after the final cycle of refinement to check the integrity of the model.

At this point, data to 2.28 Å from another trigonal crystal form became available (space group \(P_{3}21\), cell dimensions \(a = b = 110.9\,\text{Å}, c = 170.8; \alpha = \beta = 90^\circ, \gamma = 120^\circ\); \(V_m = 3.50\,\text{Å}^3\text{Da}^{-1}\), and solvent content 65%). This data set was obtained from a single crystal grown as described (19). Briefly, Fab KAU was concentrated to 5.5 mg/ml and crystallized using 17% PEG 8000 and 0.1 M HEPES, pH 7.5, as mother liquor, and the temperature was kept at 4°C. This crystal was subsequently cryocooled using 12% PEG 400 as cryoprotectant, and a data set was collected at –123°C using Synchrotron light at the Laboratorio Nacional de Luz Sincrotron. At this step, molecular replacement using the 2.8 Å structure as a probe was performed with this new data set. Refinement was initiated with a rigid body procedure using the program CNS (25) followed by ten cycles of manual model adjustment and addition of solvent water molecules using the program TURBO (26) and simulated annealing refinement using the program CNS, resulting in an \(R\) factor of 0.18 and \(R_{\text{free}}\) of 0.22. This model consists of two Fab molecules with a total of 6594 protein atoms; 614 solvent molecules have been included in the model structure (see Table I).

**Quality of the model**

Results of the refinement of the KAU Fab crystal structure are summarized in Table I. In one of the Fabs present in the unit cell \(C_{\text{H}}1\) residues 135–140 and 199–201 have no associated electron density and have been removed from the model. Likewise, \(C_{\text{L}}1\) residues 135–142 and 199–201 in the second Fab have been removed due to lack of electron density. All residues in the \(V_{\text{H}}, C_{\text{H}}1,\) and \(V_{\text{L}}\) domains of both molecules are well resolved. Only two nonglycyl residues in each Fab, \(V_{\text{L}}\) Ala52 and \(V_{\text{H}}\) Asp31, are in disallowed regions of a Ramachandran plot (data not shown). Similar occurrences in tight turns have been observed in other Fabs (27–29).

Calculation of a Fo-Fc difference Fourier map using the final model coordinates reveals electron densities at the \(C_{\text{H}}1\) glycosylation site. This density is continuous with the side chain of \(C_{\text{H}}1\) Asn166, indicating covalent bonding to the Fab. However, this density is unresolved, with breaks in the glycosidic linkages, and is only approximately the length of a trisaccharide. Attempts to model and refine an oligosaccharide into this density resulted in significant (>2.5 σ) error peaks in a Fo-Fc Fourier.

Superposition between the two Fabs using all Cα atoms gave a r.m.s.d of 0.63 Å (0.48 Å using only Cα atoms <2 r.m.s differences). Superposition of the two Fvs gave values of 0.34 Å (0.30 Å); meanwhile, the superposition of the \(C_{\text{H}}1-C_{\text{L}}\) modules resulted in a r.m.s.d of 0.50 Å (0.27 Å).

Solvent-excluded surface areas were computed with the probe radius of 1.7 and 1.4 Å using the program GRASP (30). Superposition alignments were made using the programs ALIGN (31) and Macromodel (32). The final coordinates have been deposited in the RCSB Protein Data Bank (RCSB ID: PDB 1DN0).

**Results**

The Fab KAU shows the usual Ig fold of Ab molecules (33). The two Fabs in the asymmetric unit are positioned in opposite directions and have their interfaces at the C domains. The two Fabs can be superimposed by a rotation of ~180°.

The final coordinates of the Fab A-B include 429 residues, 214 in the heavy chain and 215 in the light chain, whereas for the other Fab in the asymmetric unit, 212 amino acids of the heavy chain could be located. The quantitative agreement between the two Fabs gives a measure of the accuracy of the structural analysis (see Materials and Methods). The elbow angle made by the two pseudodyads of the variable and constant domains is 170° for both Fabs. This value falls within the range of those of other Fabs, from 127° to 227° (34). cis-P proline residues occur at position 8, 96, and 142 for the light chain and at position 155 for the heavy chain.

**Variable region**

The quaternary structure of this region is similar to the previously described human and mouse Abs. The Fab KAU \(V_{\text{H}}\) (VeIllBb) and \(V_{\text{H}}\) (VeIIIb) domains form a compact module like other Fvs with an average buried surface area of 1587 Å² (788 Å² for \(V_{\text{L}}\) and 799 Å² for \(V_{\text{H}}\)). The \(V_{\text{L}}-V_{\text{H}}\) interaction is mediated by 220 atomic contacts made mainly by \(V_{\text{H}}\) residues Leu45, Trp47, Tyr106, and Thr109 and \(V_{\text{L}}\) residues Gln63, Thr66, and Tyr92. However, this density is unresolved, with breaks in the glycosidic linkages, and is only approximately the length of a trisaccharide. Attempts to model and refine an oligosaccharide into this density resulted in significant (>2.5 σ) error peaks in a Fo-Fc Fourier.

Superposition between the two Fabs using all Cα atoms gave a r.m.s.d of 0.63 Å (0.48 Å using only Cα atoms <2 r.m.s differences). Superposition of the two Fvs gave values of 0.34 Å (0.30 Å); meanwhile, the superposition of the \(C_{\text{H}}1-C_{\text{L}}\) modules resulted in a r.m.s.d of 0.50 Å (0.27 Å).

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**Table I. Refinement statistics summary of the Fab fragment of the anti-KAU Ab**

<table>
<thead>
<tr>
<th>Program CNS</th>
<th>Resolution range (Å)</th>
<th>17.0–2.28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unique reflections ((F &gt; 0; F_{o} &gt; 1))</td>
<td>45,494</td>
<td></td>
</tr>
<tr>
<td>Final (R_{\text{cryst}})</td>
<td>0.180</td>
<td></td>
</tr>
<tr>
<td>Final (R_{\text{free}})</td>
<td>0.224</td>
<td></td>
</tr>
<tr>
<td>r.m.s.d bond length (Å)</td>
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<td></td>
</tr>
<tr>
<td>r.m.s.d bond angle (°)</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Mean temperature factor (Å²)</td>
<td>28.2</td>
<td></td>
</tr>
</tbody>
</table>

* \(R_{\text{cryst}} = \sum h k l \left| F_{o} \right| - \sum h k l \left| F_{c} \right| / \sum h k l \left| F_{o} \right| *

* \(R_{\text{free}}\) is calculated in the same manner as \(R_{\text{cryst}}\), but from 4599 reflections not used for refinement.
KAU, Val in 409.5.3) and 29 (Val in KAU, Ile in 409.5.3) with the extra bulk of the Val-Ile interaction in 409.5.3, resulting in a higher loop conformation at residue 29 and a subsequent change in the positions of residues 30–31. Moreover, Tyr 71 in 409.5.3 makes a hydrogen bond to the amide of residue 31. This hydrogen bond is missing in the KAU structure as a result of the Phe framework residue. As such, the conformational differences between KAU L1 and 409.5.3 L1 bear a striking similarity to the A and B subclasses of an L1 class 2 loop with the subclasses defined by interactions with residue 71. The resolution at which the 1f7 (3.0Å) and Fab 409.5.3 (2.9 Å) structures were solved, precluded their analysis of canonical structure by Al-Lazikani et al. (38). As such, the characteristics of L1 canonical group 6 are still open to further developments.

The KAU combining site shows an extended cavity (39, 40) and a neighboring pocket (see Fig. 2A). Residues from the hypervariable loops V[H] CDR3, V[L] CDR1, and V[L] CDR3 form the cavity whereas the small pocket is defined essentially by residues from the hypervariable loops V[H] CDR1 and V[H] CDR2.


The cavity floor communicates with the pocket floor by means of Pro98H (V[H] CDR3). The pocket floor is continued by Tyr33H and some main chain atoms of residues Asp31H (V[H] CDR1), Asp101H, and Thr102H (V[H] CDR3). The pocket is completely surrounded by polar and charged residues like Thr102H, Asp101H, (V[H] CDR3), Asp31H (V[H] CDR1), His53H, Asn52H, Ser54H, and Ser56H (V[H] CDR2).

Constant region

The first C[H] domain of the human IgM (Cm1) displays the basic Ig fold as do other constant regions from different isotypes (41). The Cm1 and C[L] domains are covalently joined by a disulfide bond between the residues Cys134H and Cys215L. Both domains face each other, related by a pseudodyad angle of 167.0°.

**FIGURE 2.** Analysis of the KAU combining site. A, Combining site surface colored by electrostatic potential (GRASP). Residues that constitute the extended groove are labeled. B, Conservation of combining site residues among 32 sequenced anti-I/i CAs (as described in references and BLAST database). The figure is color coded based on the percentage of CAs containing the same residue at this particular position. Purple, 66–100%; red, 33–66%; yellow, 0–33%.
The interaction between C$_{\mu}$1 and C$_{\lambda}$k results in a buried surface of 2211 Å$^2$ (1158 Å$^2$ for C$_{\mu}$1 and 1053 Å$^2$ for C$_{\lambda}$k). The Van der Waals contacts, hydrogen bonds, the disulfide bond, and the salt bridge between C$_{\mu}$1 and C$_{\lambda}$k are listed in Table II. The salt bridge occurs between Lys219H (N$_Z$2) and Glu124L (O$_{E2}$) with a distance of 2.5 Å and forming an angle of 104.5°. It is worth noting that Glu124L also participates in an equivalent salt bridge with Lys221H of CH 1 from mouse IgG$_2$A isotype (41) (as shown in Abs 17/9 and 33F12, PDB entries 1HIL, Ref. 42 and 1AXT, Ref. 43, respectively) and mouse IgG$_1$κ (Ab NMC-4, PDB entry 1OAK) (44).

In total, C$_{\mu}$1 and C$_{\lambda}$k establish 223 atomic contacts in Fab KAU A-B (247 contacts in Fab C-D). Light chain residues Phe119L, which makes 40 contacts, and Gln125L, which makes 22 contacts, account for 22% of contacts with C$_{\mu}$1. On C$_{\mu}$1, residues Phe129H, which makes 32 contacts, and Phe176H, which makes 32 contacts, represent 27% of C$_{\lambda}$k. Pro177, and Thr188 of the heavy chain make eight hydrogen bonds between C$_{\mu}$1 and C$_{\lambda}$k. Thir180 of the heavy chain makes 32 contacts, and Phe176H, which makes 32 contacts, with CL (see Table II).

Electron density corresponding to a trisaccharide moiety (see Materials and Methods) can be visualized in the C$_{\lambda}$k interface, allowing us to compare the contact residues at this interface. Eighteen of the twenty residues of C$_{\mu}$1 KAU that contact C$_{\lambda}$k make equivalent contacts on C$_{\lambda}$k RF-AN with C$_{\lambda}$λ. This analysis shows a remarkable conservation in this interface regardless of the isotype of the partner light chain.

The constant domains C$_{\mu}$1 from KAU (human IgM), HIL (human IgG1) (46), D44.1 (murine IgG1) (47) and Bv04–01 (murine IgG2b) (48) were aligned by a least-squares superposition to compare the folding conservation among these isotypes. The α-carbon structure of the IgM KAU C$_{\lambda}$1 domain is very similar to all other C$_{\lambda}$1 domains analyzed (Fig. 3). The rmsd obtained for the Cα positions for the 73 spatially corresponding residues of KAU vs other isotypes are 0.83Å for human IgG1, 0.94Å for murine IgG1, and 1.03Å for IgG2b. The C$_{\lambda}$1 domain superimposes well overall with the exception of two segments, 163–168 and 194–204.

In the region 163–168, the IgM C$_{\lambda}$1 domain deviates substantially from the other isotypes conformation (Fig. 3B), a fact that can be explained, at least in part, by the presence of a covalently attached carbohydrate at residue Asn166 of the IgM. Human IgM conformation at residues 163–168 has a similar orientation to that of murine IgG2b, where this loop is pointing toward the hinge region, in clear contrast with the conformation observed in human IgG1, murine IgG1, (Fig. 3B) IgG2a and IgG3 C$_{\lambda}$1 domains (49).

The backbone conformation of the IgM C$_{\lambda}$1 domain also differs significantly from other isotypes structure in the segment 194–204. This loop is three amino acids longer in human IgM and displays a more extended conformation, even though residues 199–201 were not modeled due to poor electron density.

The cis-Pro155 in Fab KAU aligns with its counterpart cis in the isotypes analyzed, as well as the intrachain S-S bond formed by Cys148-Cys208 in KAU. A very mobile segment formed by residues 135–140 in Fab KAU corresponds to a mobile segment in the rest of the isotypes at similar position.

The C$_{\mu}$1 half-cystine forming the interchain disulfide bond with the light chain is located at the N-terminal end of the domain in

| Table II. Atomic contacts between the constant domains of the light and heavy chains of Fab KAU$^a$ |
|                                | F  | P  | L  | V  | S  | C  | A  | L  | Q  | R  | G  | F  | P  | V  | L  | R  | T  | Q  | L  | K  | Totals |
| F 117                           |   | 2 | 9 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 11 |
| I 118                           |   | 2 | 1 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 3  |
| F 119                           | 16| 10| 9 | 5  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 40 |
| P 120                           |   | 1 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 1  |
| S 122                           | 5 | 3 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 8  |
| E 124                           | 8 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 13 |
| Q 125                           | 3 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 22 |
| S 132                           | 1 | 3 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 4  |
| V 134                           | 5 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 5  |
| L 136                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 8  |
| N 138                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 8  |
| N 139                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 16 |
| Q 161                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 6  |
| E 162                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 11 |
| S 163                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 18 |
| V 164                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 17 |
| T 165                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 4  |
| S 175                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 10 |
| L 176                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 6  |
| S 177                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 13 |
| T 179                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 4  |
| T 181                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 2  |
| C 215                           |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 1  |
| Totals                          | 32| 3 | 11| 13| 2 | 14| 1 | 6 | 16| 3 | 32| 15| 8 | 3 | 17 | 3 | 16 | 2 | 5 | 223 |

$^a$ The heavy chain residues are across the page and the light chain residues are down the page. The numbers represent van der Waals contacts otherwise indicated as follows: * represent a hydrogen bond, ● represents a disulphide bond, and ○ represents a salt bridge.
most of the isotypes. IgG1 is an exception, where the cystine donated by the heavy chain is found at the carboxyl end of CH1 (50). As shown in the Fig. 3A, in human IgM the half-cystine is in the loop between the strands 4-1 (A) and 4-2 (B) of the four-stranded β sheet, whereas in murine IgG1 is located in the C-terminal loop arising from the strand 3-3 (G), pertaining to the three-stranded β sheet. Consequently, the half-cystine in both isotypes do not superimpose, but their counterpart in the light chain (located also in a loop) can accommodate itself to interact to form the interchain disulfide bond.

The structural alignment also shows that the CH1-CL interface is mainly conserved during the isotype switch process from IgM to IgG isotypes. In effect, Fig. 3 shows the close correspondence of the α-carbon trace at the interface. This result is in agreement with the sequence alignment analysis of IgM against IgG1 isotypes made by Padlan and coworkers (51).

Variable region

The usage of the VH4-34 gene is very common among autoantibodies, such as CAs, rheumatoid factors, anti-Rh, and anti-DNA Abs. This restricted gene usage is striking in the case of anti-i/i CAs where all the analyzed Abs belong to this family. Therefore, the structural description of KAU encoded by this gene, is important to understand the molecular basis of anti-i/i autoimmune recognition.

The mAb 9G4 recognizes a public idiotope present in all CAs encoded by VH4-34 (11). As was described before, the area reacting with 9G4 is localized in framework region 1 (FR1; residues 23–25). Accordingly, previous studies (52, 53) showed that binding of anti-idiotypic Ab (anti-Id) 9G4 to the FR1 region of VH4-34-encoded Abs blocked the hemagglutination activity of CAs with i/i activity. The KAU three-dimensional structure shows that the 9G4 cross-reactive idiotope (residues 23–25 of FR1) and the combining site do not overlap. The inhibitory effect of 9G4 anti-Id could be explained by an induced conformational change on FR1 upon binding of the anti-Id or by steric hindrance because of the bulky nature of the anti-Id.

The topography of the combining site (Fig. 2A) shows a pocket and a cavity formed by hydrophobic and aromatic residues, surrounded by polar and charged amino acids such as Asp31H, Asp215H, and His104H. Comparison with the binding site of the soybean lectin bound to the Ag I of RBC (SBA, PDB files 2sba, 1sbd, 1sbe) (54, 55), shows that both have a similar topography and overall residue composition. The soybean agglutinin has a pocket formed by hydrophobic residues (Phe128, Ala87, Ile216, Leu214) and make hydrogen bonds with the I Ag by means of surrounding residues, which typically participate in such interactions with carbohydrate moieties (Asp88, Asp215, Asn130, His104). That pocket resembles the Fab KAU combining site topography, consequently, the way KAU interacts with the I Ag could be similar.

The topography of the KAU combining site is consistent with the binding activity and genetic origin of human CAs (56–59). Functional assays show that RBC agglutination produced by KAU is abrogated by treatment of the cells with β-endogalactosidase.
and inhibited by the linear (i) and branched (I) Ags (A. C., unpublished observation), suggesting that KAU recognizes a large carbohydrate Ag.

To gain insight into the common structural features between anti-I/i autoantibodies, all known sequences of anti-I/i CAs (see Fig. 2B) were analyzed in terms of the Fab KAU CDRs and combining site. KAU H1 loop (residues 26–33 of the VH region) folds with the group 1 canonical structure. All the important residues for this conformation (60) are conserved in all the analyzed anti-I/i CAs, suggesting that the packing and conformation of KAU H1 loop is similar in all human anti-I/i. Most of sequenced anti-I/i CAs have the same residues in the H2 loop (residues 52–56 of the VH region). As in KAU, they should have a canonical group 1 H2 structure.

It was also postulated that the VH4-34-encoded region is mainly responsible for Ag I specificity, while VH3DR3 and VH2 modulate the affinity (17). Fig. 2B shows the KAU combining site with its residues colored according to the degree of conservation of those amino acids among anti-I/i CAs. It is clear that most of VH3DR1 and all VH2DR2 residues are conserved among anti I/i CAs. These two loops form the pocket’s wall. FR2 residues Trp 47H, Glu 50H and FR3 residue Asn 58H, that form the first segment of the external perimeter, and Ser93L and Ser94L (both of V L CDR3), that extend this outer limit of the cavity, are remarkably conserved. Therefore, all of these highly conserved residues constitute most of the external limit of the whole combining site.

The small pocket at the KAU combining site, formed essentially by VH4-34 encoded residues (V H CDR1, FR2, and FR2) could explain the VH4-34 restriction among CAs. Differences in composition at V H CDR3, V L CDR3, and V H CDR1 would explain the diversity in the fine specificity of this family of autoantibodies. Further three-dimensional structural studies of other anti-I/i CAs, free and complexed with its carbohydrate Ags are needed to ascertain the structural basis of this autoimmune recognition.

Acknowledgments

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