Title
Analysis of the structural correlates for antibody polyreactivity by multiple reassortments of chimeric human immunoglobulin heavy and light chain V segments.

Journal
The Journal of experimental medicine, 180(3)

ISSN
0022-1007

Authors
Ichiyoshi, Y
Casali, P

Publication Date
1994-09-01

DOI
10.1084/jem.180.3.885

Peer reviewed

Analysis of the Structural Correlates for Antibody Polyreactivity by Multiple Reassortments of Chimeric Human Immunoglobulin Heavy and Light Chain V Segments

By Yuji Ichiyoshi and Paolo Casali

From the Department of Pathology and Kaplan Comprehensive Cancer Center, New York University School of Medicine, New York 10016

Summary

Polyreactive antibodies (Abs) constitute a major proportion of the early Ab repertoire and are an important component of the natural defense mechanisms against infections. They are primarily immunoglobulin M (IgM) and bind a variety of structurally dissimilar self and exogenous antigens (Ags) with moderate affinity. We analyzed the contribution of Ig polyvalency and of heavy (H) and light (L) chain variable (V) regions to polyreactivity in recombinatorial experiments involving the V_H-diversity(D)-J_H and V_κ-J_κ gene segments of a human polyreactive IgM, monoclonal antibody 55 (mAb55), and those of a human monoreactive anti-insulin IgG, mAb13, in an in vitro Cγl and C_κ human expression system. These mAbs are virtually identical in their V_H and V_κ gene segment sequences. First, we expressed the V_H-D-J_H and V_κ-J_κ genes of the IgM mAb55 as V segments of an IgG molecule. The bivalent recombinant IgG Ab bound multiple Ags with an efficiency only slightly lower than that of the original decavalent IgM mAb55, suggesting that class switch to IgG does not affect the Ig polyreactivity. Second, we coexpressed the mAb55-derived H or κ chain with the mAb13-derived κ or H chain, respectively. The hybrid IgG Ab bearing the mAb55-derived H chain V segment paired with the mAb13-derived κ V segment, but not that bearing the mAb13-derived H chain V segment paired with the mAb55-derived κ V segment, bound multiple Ags, suggesting that the Ig H chain plays a major role in the Ig polyreactivity. Third, we shuffled the framework 1 (FR1) -FR3 and complementarity determining region 3 (CDR3) regions of the H and κ chain V segments of the mAB55-derived IgG molecule with the corresponding regions of the monoreactive IgG mAb13. The mAb55-derived IgG molecule lost polyreactivity when the H chain CDR3, but not the FR1-FR3 region, was replaced by the corresponding region of mAb13, suggesting that within the H chain, the CDR3 provides the major structural correlate for multiple Ag-binding. This was formally proved by the multiple Ag-binding of the originally monoreactive mAb13-derived IgG molecule grafted with the mAb55-derived H chain CDR3. The polyreactivity of this chimeric IgG was maximized by grafting of the mAb55-derived κ chain FR1-FR3, but not that of the κ chain CDR3. The mAb55-derived κ chain FR1-FR3 (an unmutated V_κ 325 segment), however, failed to yield a polyreactive Ab when grafted onto an IgG that was in all other parts identical with mAb13. Rather, this chimeric molecule showed full specificity for insulin. Thus, the polyreactivity of the human mAb55 can be fully preserved after Ig class switch, and depends primarily on the contribution of structures that are generated through somatic rearrangement events (H chain CDR3), and structures that represent the expression of an unmutated gene (Vκ 325 segment).

Sera of healthy humans and animals contain natural antibodies (Abs) that react with a variety of self and exogenous Ags. Most natural mAbs generated from humans and mice are polyreactive, i.e., they bind multiple, even very heterogeneous, Ags such as polysaccharides, nucleic acids, haptens, and proteins, including structural cellular and tissue components and soluble hormones, generally with moderate intrinsic affinity (for a review see reference 1). Because of their broad reactivity with exogenous Ags, natural Abs may play a major role both in defense against invading bacteria or viruses before specific Abs are generated, and in clearance of cellular debris and certain toxic substances. Because of their reactivity with various self Ags, including DNA, IgG Fc fragment, and phospholipids, natural Abs may serve as templates for

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/94/09/0885/11 $2.00
Volume 180 September 1994 885-895

some of the high-affinity autoantibodies emerging in patients with autoimmune disease.

The multiple Ag-binding activity and primordial Ig class, mainly IgM, of natural Abs contrast with the exquisite specificity and mature Ig class, mainly IgG, of the Ag-induced Abs. Despite their moderate intrinsic affinity, polyreactive Abs generally display a high avidity for Ag due to the decavalency of the predominant IgM class. Consequently, their effectiveness in binding Ag would be expected to decrease dramatically, because of a decrease in overall avidity, after Ig class switch and substitution of a γ for a μ H chain (2). The characteristic spectra of Ag-binding activities of polyreactive Abs presumably reflect fundamental differences in the structure of their Ag-binding sites, as compared to those of Ag-induced (monoreactive) specific Abs, although the limited molecular data available have failed to yield definitive clues to such differences (3–12). It has been suggested that polyreactive Abs rely on the utilization of selected V_H genes, possibly in unmutated configuration, for the recognition of multiple Ags (1, 11, 12). However, recent data have shown that these Abs use various arrays of different V_H and V_L gene segments, as well as different D, J_H, and/or J_L gene segments, suggesting that the somatically generated H chain CDR3 may provide the crucial structural correlate for multiple Ag-binding (6, 13, 14).

To analyze the contribution of different Ig V regions to polyreactivity and to assess the impact of class switch to IgG on the ability of an originally polyreactive IgM Ab to bind multiple Ags, we performed recombinatorial experiments involving a human polyreactive IgM, mAb55 (3) and a human monoreactive anti-insulin IgG, mAb13 (15). These two mAbs were chosen because they use the same V_H and V_L gene segments in a highly similar configuration and with highly conserved FR structures (7, 16). Analysis of the chimeric molecules generated by replacing different portions of the polyreactive mAb55 H and L chain V segments with the corresponding portions of the monoreactive mAb13 and by expressing the "wild-type" or recombinant H and L chain V gene segments juxtaposed with the same $C_\gamma 1$ and C_κ chains, respectively, demonstrating that Ab polyreactivity was not affected by class switch from IgM to IgG and was dependent on the structure of the H chain CDR3 and that of the unmutated V_κ 325 framework 1 (FR1)[1] -FR3 segment.

Materials and Methods

Cloning and Sequencing of the V_H-D-J_H and V_κ-J_κ Genes of mAb55 and mAb13. The polyreactive IgM κ, mAb55, and the monoreactive anti-insulin IgG κ, mAb13, were generated by EBV transformation and somatic cell hybridization techniques using peripheral B cells from a healthy subject and a patient with insulin-dependent diabetes mellitus (IDDM), respectively (3, 15). IgM κ mAb55 was shown to be at least pentameric by size fractionation analysis on a Superose 12 column (Pharmacia LKB Biotechnology, Piscataway, NJ).

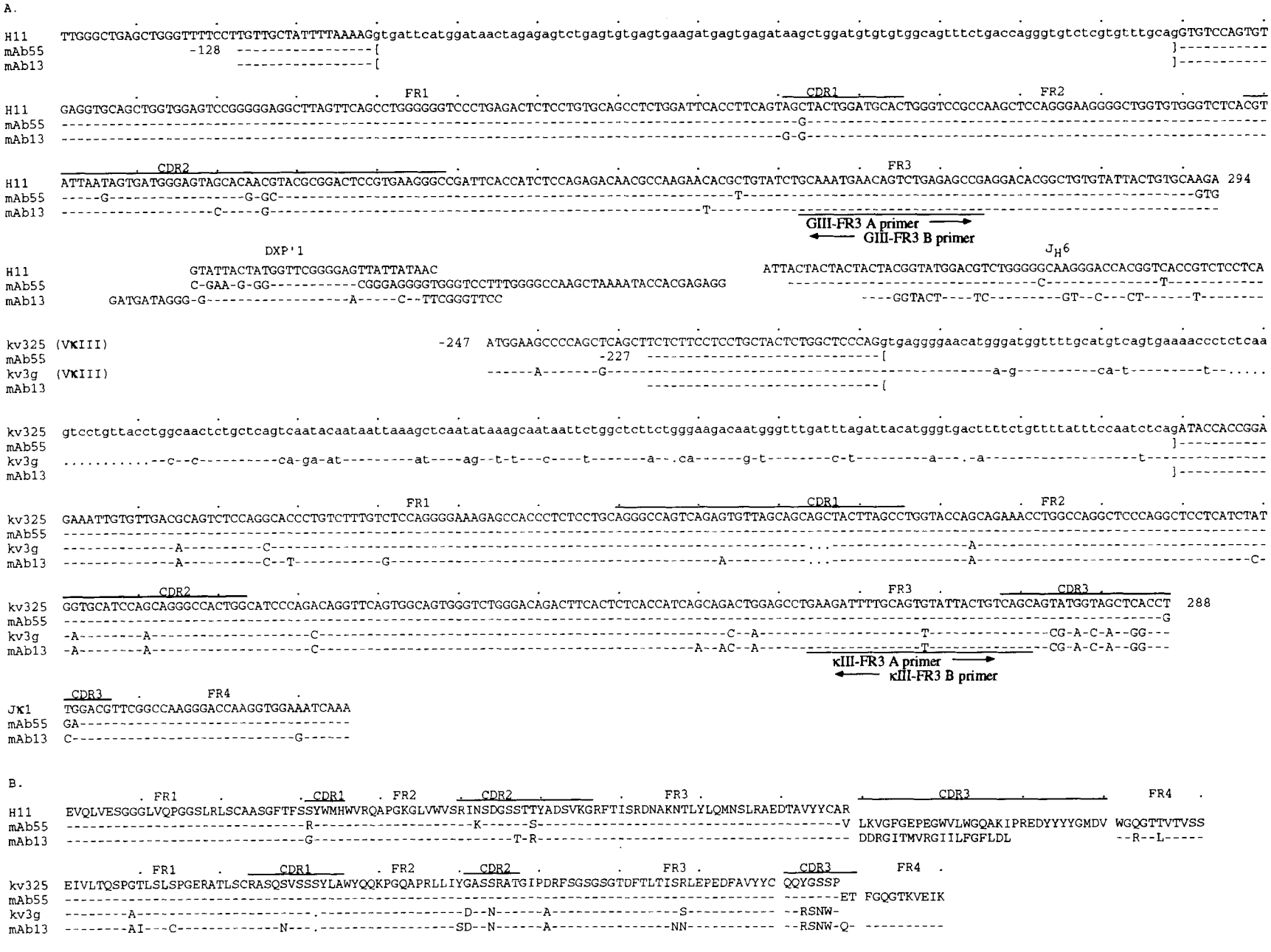
The sequences of the mAb55 V_H-D-J_H, mAb13 V_H-D-J_H, and mAb13 V_κ-J_κ gene segments have been reported (7, 16); that of the mAb55 V_κ-J_κ gene segment was derived for the purpose of these studies using described procedures (7–10). These sequences are depicted in Fig. 1 *A*, and are summarized in Table 1. The two mAb V_H gene sequences were 96.4% identical throughout the coding area, differing from that of the germline H11 gene (17) in five (mAb13) and nine (mAb55) nucleotides. Both mAbs utilized the DXP'1 gene (18) but in different reading frames, and complemented by different unencoded nucleotides and different truncated J_H genes (19). The sequences of the mAb55 and mAb13 V_κIII genes were 93.8% identical throughout the coding area. The mAb55 V_κ gene sequence was identical to that of the germline kv325 gene (20) except for a silent T → G change at position 288; that of the mAb13 V_κ gene differed in six nucleotides from the sequence of the germline kv3g gene (21). Both mAbs used a $J_\kappa 1$ gene (22).

Expression Vectors. The pcDNAIG and pSXRDIG plasmid vectors (23) were used for the in vitro expression of the human Ig V_H-D-J_H and V_κ-J_κ gene segments, respectively. pcDNAIG is a mammalian expression vector derived from pcDNAI/Neo (Invitrogen Corp., San Diego, CA) (Fig. 2 *A*). It encodes a human CMV promoter, a murine leader sequence, a whole human IgG1 H chain, and a Rous sarcoma virus (RSV) LTR-driven neomycin resistance gene. pSXRDIG is a mammalian expression vector derived from pUC18 (Fig. 2 *B*). It contains a SV40 promoter, a murine leader sequence, a whole human Ig κ L chain, and a DHFR gene for selection by methotrexate.

Introduction of mAb55 and mAb13 V_H-D-J_H Gene Segments into pcDNAIG Vector. The unique HindIII and XhoI sites, 5′ and 3′, respectively, of the leader-V_H-D-J_H gene segment in pcDNAIG were utilized for the introduction of the rearranged mAb55 or mAb13 V_H-D-J_H gene segment (Fig. 2 *A*). The mouse H chain leader sequence of pcDNAIG and the V_H-D-J_H gene segment of mAb55 (or mAb13) were amplified in separate PCRs (PCR 1 and PCR 2), and joined by "recombinant" PCR to yield the recombinant "leader-mAb55 (or mAb13) V_HD-J_H" gene segment (Fig. 3 *A*), using the sense H-leader (5′ gggaagcttctcaccatgggatgg 3′) and the antisense H-Ov1 (5′ GCTGCACCTCacactggacacctg-cagagaaag 3′) primers; and the sense H-Ov2 (5′ ttctctgcaggtgtc-cagtgtGAGGTGCAGCTG 3′) and the antisense H-FR4 (5′ gggctcgagactcaccTGAGGAGACGGTAACCG 3′ for mAb55 $J_H 6$ or 5′gggctcgagactcaccTGAGGAGACAGTGACCA 3′ for mAb13 $J_H 2$) primers (uppercase letters denote the sequences derived from the Ig V gene sequence of mAb55 or mAb13; lowercase letters denote the sequences derived from the expression vectors; sequences of restriction sites are underlined). The sequences of the primers used at the ends to be joined (H-Ov1 and H-Ov2) were complementary and allowed for partial annealing of the PCR 1 and PCR 2 products and performance of a recombinant PCR by addition of excess

[1] *Abbreviations used in this paper:* Av_{rel}, relative avidity (g/μl); ds, double stranded; FR, framework region; IDDM, insulin-dependent (type I) diabetes mellitus; ss, single stranded.

Figure 1. Nucleotide (*A*) and deduced amino acid (*B*) sequences of the V_H, D-J_H, V_κ, and J_κ gene segments of mAb55 and mAb13. The top sequence in each cluster is that of the germline gene to which the remaining sequences of the cluster were compared. Dashes indicate identities. Solid lines on the top of each cluster depict CDRs. Dots represent deletions. Lowercase letters denote untranslated sequences. The sequences encompassed by the GIII-FR3 and κIII-FR3 primers are underlined. The present sequences are available from EMBL/GenBank/DDBJ under accession numbers L-08084, L-32748, D-16833, and D-16834.


A.
H11
mAb55
mAb13
FR1
CDR1
FR2
CDR2
FR3
GIII-FR3 A primer
GIII-FR3 B primer
DXP'1
J_H6
kv325 (VκIII)
mAb55
kv3g (VκIII)
mAb13
κIII-FR3 A primer
κIII-FR3 B primer
CDR3
FR4
Jκ1
B.
H11
mAb55
mAb13
kv325
mAb55
kv3g
mAb13

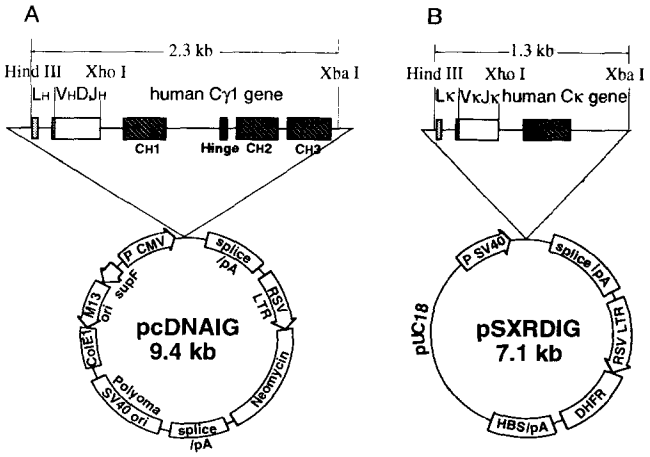


Figure 2. Structure of the pcDNAIG (*A*) and pSXRDIG (*B*) expression vectors. The segment between HindIII and XbaI sites, comprising the Ig H or κ chain gene is detailed. Boxes indicate exons: (*Shaded boxes*) mouse leader exons; (*open boxes*) human Ig V chain gene exons (*hatched boxes*) human Ig C chain gene exons. Solid lines indicate introns and noncoding sequences. (*Co1E1*) Co1E1 origin bases; (*DHFR*) dihydrofolate reductase gene for methotrexate selection; (*HBS pA*) HBV surface antigen Poly(A) site; (L_H) mouse H chain leader sequence; (L_κ) mouse κ leader sequence; (*M13 ori*) M13 origin bases; (*P CMV*) human CMV promoter; (*P SV40*) SV40 promoter; (*RSV LTR*) RSV LTR sequence; (*supF*) supF suppressor tRNA gene; (*SV40 Polyoma ori*) SV40 origin bases and polyoma origin bases; (SV40 splice/poly A site; (*Neomycin*) neomycin resistance gene.

H-leader and H-FR4 primers (Fig. 3 *A*). The recombinant fragment was sequenced, to ensure that no unintended mutations were introduced during PCR amplification (8, 10, 16, 24), digested with HindIII and XhoI, ligated into pcDNAIG previously digested with HindIII and XhoI and freed of its original V_H-D-J_H gene segment. The recombinant pcDNAIG plasmids were amplified by transformation of competent MC1061/P3 cells (Invitrogen Corp.), and selection with ampicillin and tetracycline. Plasmid DNA was isolated using a plasmid kit (Qiagen Inc., Chatsworth, CA).

Introduction of mAb55 and mAb13 V_κ-J_κ Gene Segments into pSXRDIG Vector. The unique HindIII and XhoI sites, 5′ and 3′, respectively, of the leader-V_κ-J_κ gene segment in pSXRDIG, were utilized for the insertion of the mAb55 or mAb13 V_κ-J_κ gene segment (Fig. 2 *B*). The murine κ-leader sequences of pSXRDIG and the mAb55 (or mAb13) V_κ-J_κ gene segment were amplified in separate PCRs and joined by recombinant PCR (Fig. 3 *A*). The sequences of the primers used for these PCRs were as follows: sense κ-leader (5′ gggaagcttatcaagatgaagtca 3′), antisense κ-Ov1 (5′ CAACACAATTTCgcatctggaacctgcagtcagaga 3′); sense κ-Ov2 (5′ gcaggttccagatgcGAAATTGTGTTGACGCAGTCT 3′), antisense κ-FR4 (5′gggctcgagacttacgTTTGATCTCCACCTTGG 3′). The recombinant fragment, leader-mAb55 V_κ-J_κ or leader-mAb13 V_κ-J_κ, was inserted into pSXRDIG after digestion with HindIII and XhoI. Recombinant pSXRDIG plasmids were amplified in competent DH5α cells by selection with ampicillin.

Construction of Recombinant (rec)V_H13-55 and recV_H55-13 Gene Segments. The method used to construct the recV_H13-55 gene segment is schematized in Fig. 3 *B*. The sequence encoding the leader through the FR3 area of mAb13 H chain V segment was PCR amplified using the sense H-leader and antisense GIII-FR3 B (5′ CGGCTCTCAGACTGTTCATTGC 3′) primers (Fig. 1 *A*). The sequence encoding the FR3 though FR4 area of mAb55 H chain V segment was PCR amplified using the sense GIII-FR3 A (5′ GCAATGAACAGTCTGAGAGCCG 3′) (the reverse complement of GIII-FR3 B) and the antisense H-FR4 primers. The two amplified fragments were purified and joined by recombinant PCR. The same primers, except for the H-FR4 primer, and recombinant PCR were used for the construction of the recV_H55-13 gene segment. The recV_H13-55 and recV_H55-13 gene segments were introduced into pcDNAIG as described above.

Construction of recV_κ13-55 and recV_κ55-13 Gene Segments. The

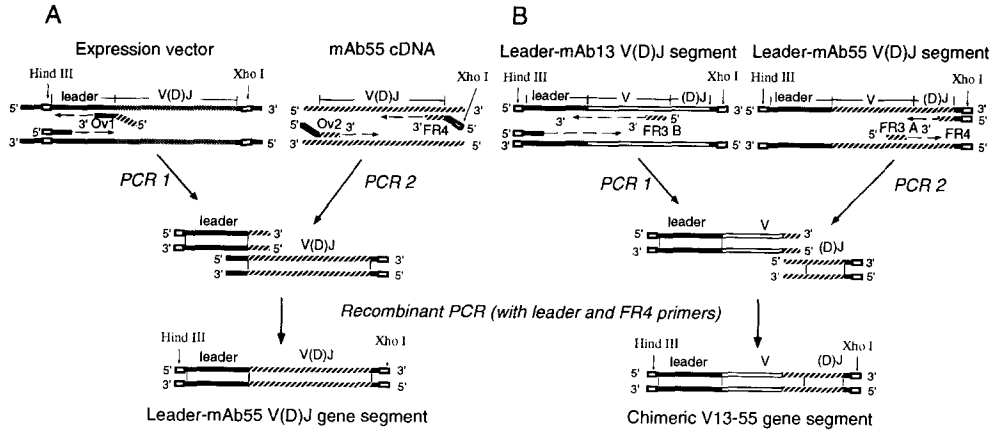


Figure 3. (*A*) Construction of the recombinant "mouse leader-human mAb55V-(D)-J" gene segment (leader-mAb 55V_H-D-J_H or leader-mAb 55 V_κ-J_κ). Solid (■), shaded (▩), and hatched (▨) lines depict the sequences of the expression vector (pcDNAIG or pSXRDIG), that of the V-(D)-J gene segment to be substituted, and that of the V-(D)-J gene segment of mAb55, respectively. (*Broken arrows*) Nucleotide chain elongation by DNA polymerase. (Open boxes) Restriction sites. The mouse leader Ig V gene segment in the expression vector and the V_H-D-J_H or V_κ-J_κ gene segment of mAb55 were amplified in separate PCR (*PCR 1* and *PCR 2*), and joined by recombinant PCR. Primers used at the end to be joined (primers *Ov1* and *Ov2*) were made complementary to one another by including nucleotides at the 5′ end that are complementary to the 3′ portion of the other primer (see Materials and Methods). Primers "leader" and "FR4" were designed to yield final recombinant products bearing HindIII and XhoI sites used for the introduction into the expression vectors. (*B*) Construction of the chimeric V13-55 (recV_H13-55 or recV_κ13-55) gene segment. Gene segments amplified from the recombinant leader-mAb 13-V-(D)-J gene sequence by the leader and FR3 B primers (PCR 1), and amplified from the recombinant leader-mAb 55V-(D)-J gene sequence by the FR3 A and FR4 primers (*PCR 2*) were joined by recombinant PCR. See Materials and Methods for the sequences of the sense FR3 A (GIII FR3 A and κIII FR3 A) and the antisense FR3 B (GIII FR3 B and κIII FR3 B) primers.

method used to construct the recV$_\kappa$13-55 and recV$_\kappa$55-13 gene segments was similar to that used for construction of the recV$_H$ segments (Fig. 3 *B*). The sequence encoding the leader though the FR3 area of mAb13 and mAb55 κ V segment was PCR amplified using the sense κ-leader and antisense κIII-FR3 B (5′ GCTGACAG-TAATACACTGCAAAATCTTC 3′) primers (Fig. 1 *A*). The sequence encoding the FR3 though FR4 area of mAb55 or mAb13 κ V segment was amplified using the sense κIII-FR3 A (5′ GAA-GATTTTGCAGTGTATTACTGTCAGC 3′) (reverse complement of κIII-FR3 B) and antisense κ-FR4 primers. For each pair of segments, the two amplified DNAs were purified, joined by recombinant PCR, and introduced into pSXRDIG.

Cell Culture and Transfection. Mammalian F3B6 cells were used in all transfection experiments. F3B6 is the Ig nonsecretor human-mouse heterohybridoma used as fusion partner for the generation of mAb13 and mAb55 (3, 15). F3B6 cells were cultured in RPMI-1640 (BioWhittaker, Walkersville, MD) with 10% FCS, 1% L-glutamine, and antibiotics (FCS-RPMI), washed, and then resuspended in FCS-RPMI at 10^7/ml. The cell suspension (750 μl) containing pcDNAIG (4 μg) and pSXRDIG (4 μg) vector DNA was transferred into an ice-cold electroporation cuvette with a 0.4-cm gap (Invitrogen Corp.). After application of an electric pulse of 750 V/cm with a capacitance of 1,000 μF using the Electroporator™ (Invitrogen Corp.) and a power supply (model 4000; GIBCO BRL, Gaithersburg, MD), the cuvette was kept on ice for 10 min. Cells were then transferred to a flask containing 10 ml of prewarmed FCS-RPMI. After a 48-h culture, culture medium was changed to selective medium containing 0.4 mg/ml of G418 (Geneticin; GIBCO BRL), and cells were distributed into 96-well flat-bottom plates. Neomycin only was used as selecting agent, because in transfectants expressing only pcDNAIG (H chain), the accumulation of unsecretable H chain molecules leads to cell death. Clumps of transformant cells were detected within 7–10 days. After 2 wk, culture fluids were tested by ELISAs using plates coated with goat F(ab′)$_2$ fragment specific to human Ig μ + γ + α H chains. Double γ1 and κ chain producer cells were identified by developing separate ELISA plates with peroxidase-conjugated affinity-purified goat anti-human Ig γ and κ chain probes (Cappel, Organon Teknika Corp., Durham, NC) (3–5). In each transfection, 5 –12 clones secreting IgG κ were generated. The three most efficient secretors were expanded and frozen.

Ab Purification, Binding Assays, and Measurement of Relative Avidity (Av_{rel}). IgG mAbs were purified from culture supernatant by 50% ammonium sulfate precipitation followed by absorption of the solubilized IgG onto a GammaBind G-Sepharose column (Pharmacia LKB Biotechnology), and elution with 100 mM glycine-HCl buffer (pH 2.7). Eluates were brought to pH 7.5 by addition of neutralizing buffer (pH 9.0), dialyzed against PBS, and stored in aliquots at 4°C. mAb binding to human recombinant insulin (Eli Lilly Research Laboratories, Indianapolis, IN), single stranded (ss)DNA, human recombinant IL-1β (BASF Biotech Corp., Worcester, MA), polyclonal human IgG Fc fragment, tetanus toxoid, and BSA was measured using appropriate ELISAs (3–5). IgG bound to solid-phase IgG Fc fragment were measured using a peroxidase-conjugated, affinity-purified goat anti-human Ig κ chain probe (Cappel, Organon Teknika Corp.). Binding of mAbs to soluble insulin was measured by competitive ELISA (3–10, 15, 16). Briefly, increasing amounts (0.25–100 μg) of soluble insulin were mixed with a constant amount of Ab in 100 μl of PBS containing 0.05% Tween 20 (PBS-Tween) and 1% BSA. After an 18-h incubation at room temperature, the mixtures were transferred into ELISA plates precoated with insulin. After a 1-h incubation and subsequent washing with PBS-Tween, the amount of Ab bound to the solid-phase insulin was measured. For each Ab, the binding measured after incubation under identical conditions but in the absence of soluble insulin (always >1.00 OD at 492 nm; negative control <0.04) represented 100% binding activity. The concentration (g/μl) of soluble insulin yielding 50% inhibition of Ab binding to solid-phase insulin was used to express the Av_{rel}.

Results

Structure and Specificity of the Human Polyreactive mAb55 and Monoreactive mAb13. To define the relative contribution of the whole Ig H and L chain V segments and/or their discrete regions of polyreactivity, we generated a variety of chimeric Ig molecules by replacing different portions of polyreactive mAb55 H and κ chain V segments with the corresponding portions of monoreactive mAb13. mAb55 is a "prototypic" natural human Ab in that it was generated from a healthy subject, and is a polyreactive IgM with moderate affinity for various ligands (3). The anti-insulin mAb13 is a prototypic Ag-induced "mature" human autoantibody in that it stemmed by affinity maturation from the insulin-specific autoantibody response in a patient with IDDM, and is a monoreactive and highly specific IgG (21, 22). Deduced amino acid sequences of mAb55 and mAb13 V_H-D-J_H genes were 94.9% identical throughout the V_H area, differing in four amino acids in the CDR1 and CDR2 and one amino acid in the FR3, but were highly divergent in length and composition of the somatically generated CDR3 (Fig. 1 *B*). The two mAb H chain FR4 sequences differed only in two amino acids. The two mAb V$_\kappa$III segment sequences were 87.6% identical, differing in four amino acids (including one deletion) in the CDR1 and CDR2 and seven in the FRs (Fig. 1 *B*). The mAb55 Vκ segment represented the expression of an unmutated Vκ325 gene. The two mAb κ chain CDR3s were identical in length, but differed in five of the nine amino acids. The κ chain FR4 sequences were identical.

Thus, mAb55 and mAb13 were highly similar or identical in their V_H, H chain FR4, V_κ and κ chain FR4 segments, but substantially different in their H chain CDR3s, and, to a lesser extent, κ chain CDR3s. The mAb55 and mAb13 dose-saturable bindings to human insulin, human IL-1β, ssDNA, human IgG Fc fragment, tetanus toxoid, and BSA are depicted in Fig. 4, *A* and *C*. The mAb55 Av_{rel} for human recombinant insulin, used as a measure of the mAb ability to bind Ag in the fluid phase, was approximately two orders of magnitude lower than that of mAb13 (Table 1).

Effect of Class Switch on mAb55 Polyreactivity. Decavalency has been claimed to be an important factor in IgM polyreactivity. To verify whether the pentameric structure of the mAb55 is in fact a major determinant of the ability of this Ab to bind multiple Ags, we juxtaposed the mAb55-derived V_H-D-J_H and V_κ-J_κ gene segments with human Cγ1 and C$_\kappa$ genes, respectively, by recombination into the Cγ1 pcD-NAIG and C$_\kappa$ pSXRDIG vectors, and expressed them by simultaneous transfection of F3B6 cells. 10 stable transformant clones secreting human IgG1 κ were generated, three of which were expanded to produce recombinant mAb55 IgG1 κ molecules (designated recIgG55). The purified monomeric recIgG55 Ab

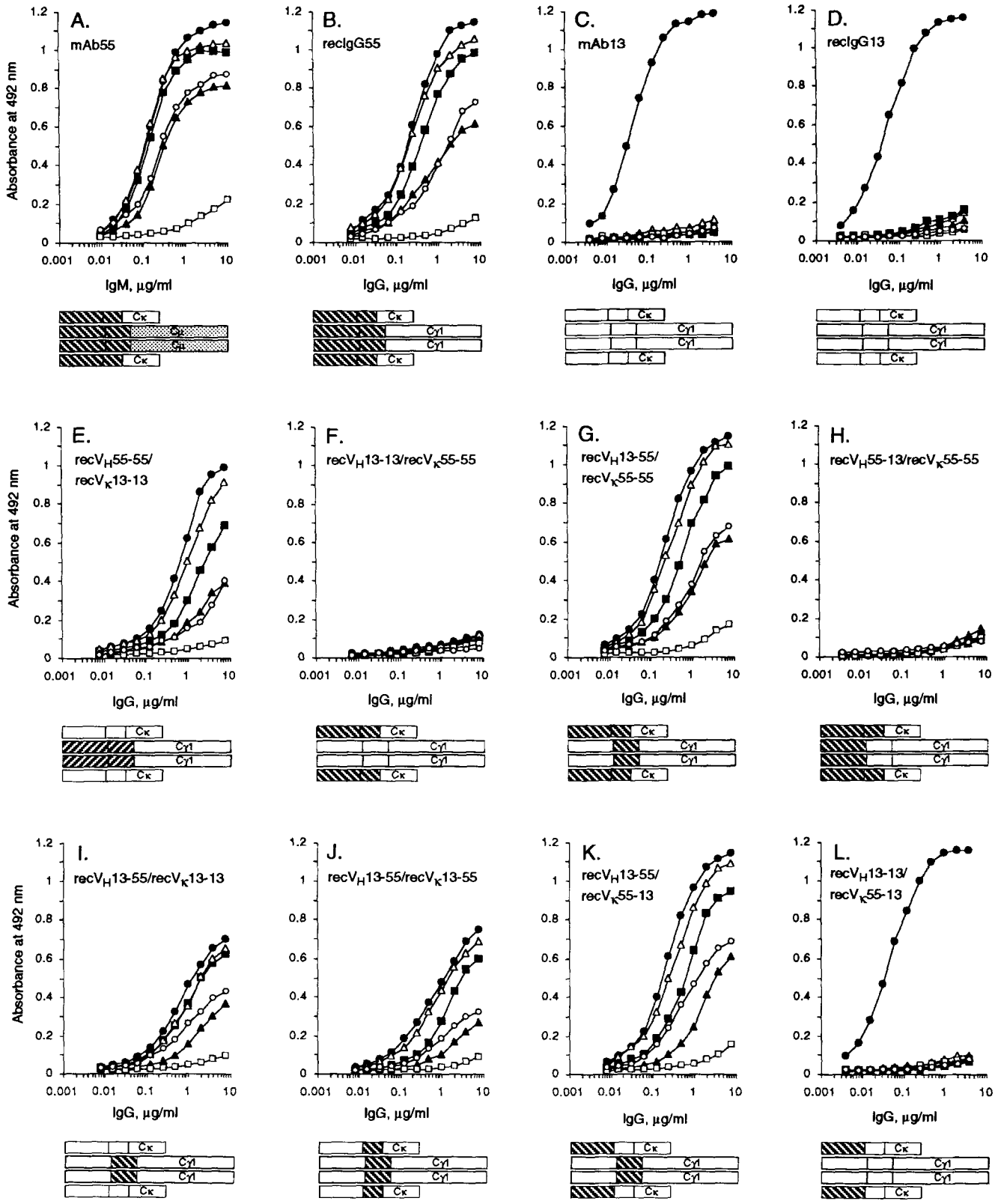


Figure 4. Binding of the original IgM κ mAb55 (*A*), recIgG55 (*B*), original IgG κ mAb13 (*C*), recIgG13 (*D*), $recV_H55\text{-}55/recV_\kappa13\text{-}13$ (*E*), $recV_H13\text{-}13/recV_\kappa55\text{-}55$ (*F*), $recV_H13\text{-}55/recV_\kappa55\text{-}55$ (*G*), $recV_H55\text{-}13/recV_\kappa55\text{-}55$ (*H*), $recV_H13\text{-}55/recV_\kappa13\text{-}13$ (*I*), $recV_H13\text{-}55/recV_\kappa13\text{-}55$ (*J*), $recV_H13\text{-}55/recV_\kappa55\text{-}13$ (*K*), and $recV_H13\text{-}13/recV_\kappa55\text{-}13$ (*L*) antibodies to human recombinant insulin (●), ssDNA (△), tetanus toxoid (■), human recombinant IL-1β (▲), human IgG Fc fragment (○), and BSA (□). Bars below each panel schematize the composition of each original or recombinant antibody. (*Open boxes*) Human Cγ and Cκ segments; and (*shaded boxes*) human Cμ segments. (*Hatched boxes*) The V segments derived from the polyreactive mAb55; (*open boxes*) those derived from the monoreactive anti-insulin IgG mAb13. The Ag-binding activity of each antibody is expressed as optical absorbance at 492 nm.

Table 1. *Structure and Properties of Human mAb55 and mAb13*

Clone	Source	Chains H	Chains L	Reactivity	Av_{rel} for insulin	V_H segment V_H gene	V_H segment D gene	V_H segment J_H gene	V_κ segment V_κ gene	V_κ segment J_κ gene
					g/μl					
mAb55	Healthy subject	μ	κ	Polyreactive	5.0×10^{-7}	H11*	DXP'1‡	J_H6§	kv325‖ (V_κIII)	$J_\kappa1$¶
mAb13	IDDM patient	γ1	κ	Monoreactive (anti-insulin)	4.9×10^{-9}	H11	DXP'1	J_H2	kv3g** (V_κIII)	$J_\kappa1$

* The sequence of the germline H11 gene has been reported by Rechavi et al. (17).
‡ D genes have been reported by Ichihara et al. (18).
§ J_H genes have been reported by Ravetch et al. (19).
‖ The sequence of germline kv325 gene has been reported by Kipps et al. (20).
¶ J_κ genes have been reported by Heiter et al. (22).
** The sequence of germline kv3g gene has been reported by Pech et al. (21).

bound in a dose-saturable fashion and with an efficiency only slightly lower than that of the original pentameric IgM mAb55 to the six self and exogenous Ags tested (Fig. 4, *A* and *B*). This slightly lower multiple Ag-binding efficiency was reflected in a slightly lower Av_{rel} for insulin of the recIgG55 when compared with its IgM counterpart (8.5×10^{-7} vs. 5.0×10^{-7} g/μl). These experiments show that class switch from IgM to IgG does not significantly affect the ability of mAb55 to bind multiple Ags, and that polyreactivity is a function inherent to the V portion of the Ig molecule.

Contribution of the mAb55 V_H-D-J_H Segment to Polyreactivity. To evaluate the relative contribution of the whole V_H-D-J_H and V_κ-J_κ segments to the polyreactivity of the recIgG55 Ab, we first inserted the V_H-D-J_H and V_κ-J_κ gene segments derived from mAb13 into the Cγ1 pcDNAIG and C_κ pSXRDIG vectors, respectively, and simultaneously expressed the recombinant Ig chains by transfection of F3B6 cells. Pairing of the recombinant H and κ chains yielded a fully functional molecule, designated recIgG13, which bound insulin in a dose-saturable fashion and with an efficiency comparable to that of the original mAb13 (Av_{rel} for insulin was 7.5×10^{-9} g/μl, compared with 4.9×10^{-9} g/μl for mAb13) but none of the other Ags tested (Fig. 4, *C* and *D*).

Having shown that the expression of mAb55 and mAb13 V gene segments juxtaposed with the same Cγ1 and C_κ genes in a recombinant system did not essentially alter the distinct original Ag-binding features of the two mAbs, we constructed: (*a*) the hybrid IgG molecule $recV_H$55-55/$recV_\kappa$13-13 bearing the mAb55-derived H chain V segment and the mAb13-derived κ chain V segment, and (*b*) the hybrid IgG molecule $recV_H$13-13/$recV_\kappa$55-5 bearing the mAb13-derived H chain V segment and the mAb55-derived κ chain V segment. Although somewhat less efficiently (Av_{rel} for insulin, 1.2×10^{-6} g/μl), the $recV_H$55-55/V_κ13-13 hybrid Ab bound multiple Ags in a fashion similar to that of the recIgG55 ($recV_H$55-55/$recV_\kappa$55-55) and the original polyreactive IgM mAb55 molecules (Fig. 4, *E*, *B*, and *A*). In contrast, the $recV_H$13-13/$recV_\kappa$55-55 hybrid Ab did not bind any of the Ags tested (Fig. 4 *F*). The failure of this Ab to bind insulin may have been due to a "damping" effect by the $recV_\kappa$55-55 segment on the inherent insulin specificity of the $recV_H$13-13 segment, since the $recV_H$13-13 segment showed full expression of insulin specificity (Ichiyoshi, Y., M. Zhou, and P. Casali, manuscript submitted for publication) when paired with the V_L segment of mAb10 (14) which is very different in sequence from the V_L segment of mAb13 or mAb55. This phenomenon is reminiscent of the finding by Radic et al. (25) who showed that the H chain of murine anti-DNA 3H9 Ab contains most or all of the required determinants for double stranded (ds)DNA binding, but its pairing with certain L chains can result in abrogation of this binding. Thus, the mAb55 V_H-D-J_H segment is necessary and sufficient for multiple Ag-binding.

Role of the H Chain CDR3 in mAb55 Polyreactivity. To determine whether mAb55 polyreactivity rests in the V_H segment itself (FR1 through FR3 sequence) or the somatically generated H chain CDR3, we evaluated the functional role of the discrete V_H segment and CDR3, using: (*a*) the $recV_H$13-55 gene segment, constructed by juxtaposing the mAb13-derived V_H sequence with the mAb55-derived D-J_H sequence; and (*b*) the $recV_H$55-13 gene segment, constructed by juxtaposing the mAb55-derived V_H sequence with the mAb13-derived D-J_H sequence. In these gene recombinations, the last residue of the V_H FR3, which is a Val in mAb55 and an Arg in mAb13, was regarded as part of the CDR3, because FR residues adjacent to CDR might contribute to the conformation of the CDR loop itself (26). The two chimeric gene segments were inserted into the Cγl pcDNAIG vectors, and separately expressed by transfection of F3B6 cells along with the V_κ-J_κ gene segment derived from the polyreactive mAb55 ($recV_\kappa$55-55) to yield the $recV_H$13-55/$recV_\kappa$55-55 and $recV_H$55-13/$recV_\kappa$55-55 Ab mol-

ecules. Consistent with the role of the H chain CDR3 in providing the structural correlate for polyreactivity, the recV$_H$13-55/recV$_\kappa$55-55, but not the recV$_H$55-13/recV$_\kappa$55-55, IgG Ab bound multiple Ags (Fig. 4, *G* and *H*). In fact, the extent to which the recV$_H$13-55/recV$_\kappa$55-55 molecule bound to the Ags tested was comparable (AV_{rel} for insulin, 9.4×10^{-7} g/μl) to that of the recIgG55 and that of the original IgM mAb55 (Fig. 4, *B* and *A*). Thus, the CDR3, not the V$_H$ segment, is the crucial element providing the structural correlate for the polyreactivity displayed by the mAb55 H chain. This contention was further strengthened by the demonstration that grafting of the mAb55-derived H chain CDR3 onto a molecule which was in all other parts identical to mAb13 enabled the molecule to bind multiple Ags. The Ab resulting from the pairing of the recV$_H$13-55 gene segment with the recV$_\kappa$13-13 gene segment bound to all Ags tested, although less efficiently, in a fashion similar to that of the recV$_H$55-55/recV$_\kappa$13-13 hybrid molecule, which was encoded by the whole arm mAb55-derived V$_H$-D-J$_H$ gene segment (Fig. 4, *I* and *E*).

Role of the V$_\kappa$ Segment in mAb55 Polyreactivity. Analysis of the recV$_H$13-55/recV$_\kappa$13-13 molecule emphasized the role of the H chain CDR3 in Ag-binding. It also indicated that the mAb55 κ chain contributed to polyreactivity. This was initially suggested by the slightly suboptimal binding to multiple Ags displayed by the recV$_H$55-55 segment paired with the recV$_\kappa$13-13 segment (Fig. 4 *E*). To determine which portion of the mAb55 V$_\kappa$ chain was synergistic with the H chain in binding Ags, we substituted the V$_\kappa$ FR1-FR3 or κ chain CDR3-FR4 sequence of the recV$_H$13-55/V$_\kappa$13-13 molecule with the respective corresponding sequences derived from mAb55. To this end, we constructed: (*a*) the recV$_\kappa$13-55 gene segment, by juxtaposing the mAb13-derived V$_\kappa$ sequence, encoding the FR1-FR3, with the mAb55-derived V$_\kappa$-J$_\kappa$ sequence, encoding the CDR3-FR4; and (*b*) the recV$_\kappa$55-13 gene segment, by juxtaposing the mAb55-derived V$_\kappa$ sequence, encoding the FR1-FR3, with the mAb13-derived V$_\kappa$-J$_\kappa$ sequence, encoding the CDR3-FR4. The two chimeric gene segments were inserted into the C$_\kappa$ pSXRDIG vectors, and separately expressed by transfection of F3B6 cells as paired with the recV$_H$13-55 gene segment, to yield recV$_H$13-55/recV$_\kappa$13-55 and recV$_H$13-55/recV$_\kappa$55-13 molecules. Grafting of the mAb55-derived V$_\kappa$FR1-FR3 sequence, but not the κ chain CDR3-FR4 sequence, onto the L chain of the recV$_H$13-55/recV$_\kappa$13-13 molecule significantly enhanced the multiple Ag-binding activity of this Ab (Fig. 4, *K* and *J*). In fact, the recV$_H$13-55/recV$_\kappa$55-13 molecule bound multiple Ags with an efficiency (Av_{rel} for insulin, 8.4×10^{-7} g/μl) comparable to that of the integral recIgG55 Ab (Fig. 4 *B*). The germline configuration of the mAb55-derived V$_\kappa$325 FR1-FR3 segment is crucial in Ag-binding by the autologous H chain CDR3, but may not be sufficient to independently provide a structural correlate for polyreactivity, as grafting of the mAb55-derived V$_\kappa$ gene segment onto a molecule encoded in all other parts by mAb13-derived gene segments failed to generate a polyreactive Ab. Instead, this Ab, recV$_H$13-13/recV$_\kappa$55-13, bound specifically to insulin but to none of the other Ags (Fig. 4 *L*). Its exquisite specificity for insulin (Av_{rel}, 6.0×10^{-9} g/μl) was comparable to that measured for recIgG13 and that of the original mAb13 (Fig. 4, *D* and *C*).

Discussion

In the present studies, we investigated the contributions of valency and of primary structure of V segments to the multiple Ag-binding of a human polyreactive IgM Ab, mAb55. The recombinant Ig V$_H$ and V$_L$ gene expression system we adopted was designed to produce a complete human IgG1 κ molecule. Accordingly, the IgM mAb55-derived V$_H$-D-J$_H$ and V$_\kappa$-J$_\kappa$ gene segments were expressed and secreted as a recombinant bivalent monomer (designated recIgG55), effectively "switching" the polyreactive IgM to IgG. On a weight-to-weight basis, the multiple Ag-binding of the recIgG55 molecule closely mimicked that of the wild-type IgM mAb55, showing that IgM to IgG class switch does not necessarily affect the characteristic feature of a polyreactive Ab, and that recognition of multiple Ags is a function that is absolutely restricted to the Ig V domains. This finding suggests that the role of natural polyreactive IgM extends far beyond that of providing the structural elements of the idiotypic regulatory network in early ontogeny, and that of enhancing opsonization and/or complement activation after binding invading microorganisms in the early phases of infection. Natural polyreactive IgM-producing cells may include the precursors of cells producing high-affinity polyreactive IgG or IgA autoantibodies or Abs to exogenous microbial Ags, after undergoing an Ag-dependent process of clonal activation and expansion entailing class switch and, possibly, somatic mutation and Ag selection (8–10).

The experiments involving the hybrid IgG molecules generated by coexpressing the mAb55-derived H or κ chain V segment with the mAb13-derived κ or H chain V segment, respectively, clearly demonstrated that the H chain plays a major role in mAb55 polyreactivity. This is consistent with previous observations that the specificity of monoreactive-specific Abs and autoantibodies is mainly determined by the H chain V segment (26, 27), although in some cases the L chain seems to play a critical role in defining epitope specificity (28, 29). Individual Ig H chains can bind Ag independently of the contribution of any L chain, as originally shown by Utsumi and Karush (30) in an isolated rabbit H chain to *p*-azophenyl-β-lactoside, and by Jaton et al. (31) in an isolated rabbit H chain to the 2,4-dinitrophenyl group. These early findings have recently been extended by experiments showing efficient Ag-binding of cloned murein V$_H$ domains, "single domain antibodies," to lysozyme or KLH (32), and by the observation that a broad Ag-binding repertoire is provided by naturally occurring Ig H chain dimers in the camel (33). Finally, x-ray crystallography has demonstrated in at least three Ag-Ab systems that the number of Ag-contacting residues in the H chain far exceeds that in the L chain (34, 35).

The experiments, in which the presence or absence of the mAb55-derived H chain CDR3 sequence resulted in acquisi-

tion or abolition, respectively, of polyreactivity, whether in a mAb55 or mAb13-like structural context, indicated that the CDR3 provides a critical structural correlate for the multiple Ag-binding activity of mAb55. The generated chimeric molecules satisfied the criteria set by Chothia and Lesk (26), i.e., they were identical in those canonical FR sequences that are adjacent to the CDRs, and are critical for the full preservation of the CDR loop structure. Thus, our choice of Abs and experimental design for shuffling the FR and CDR sequences should have prevented the structural deleterious effects which might be observed when CDR sequences are grafted onto FR contexts different from those of the original Ab molecule (37, 38). The H chain CDR3 of mAb55 is exceptionally long (31 amino acids), identifying a structure potentially capable of providing multiple contact interfaces, which may partially overlap, for different epitopes (1, 39). Crystallographic analysis has shown that at least in the case of relatively large protein Ags, multiple residues within the H chain CDR3 interface with Ag even in specific (monoreactive) Abs (35, 40, 41). However, as suggested by Chothia and Lesk (26), the important role of the H chain CDR3 in Ab specificity may arise from the central position of this structural element in the binding site rather than from its sheer size (or number of contact residues). In at least some polyreactive Abs, a wide H chain CDR3 loop may be critical for accommodating different Ags, but relatively short H chain CDR3s have been reported in other human polyreactive Abs (5, 7–12). In fact, the H chain CDR3 structures of human polyreactive Abs so far described are highly divergent in length and composition, and do not allow for the identification of any obvious common motif possibly responsible for polyreactivity (5–12). This structural heterogeneity might underlie the functional "uniqueness," i.e., the discrete Ag-binding features, of each polyreactive mAb (5, 8, 9), but at the same time it raises the possibility that, in certain Abs, polyreactivity is mediated primarily by structures other than the H chain CDR3.

An important role of the H chain CDR3 in providing the structural correlate for multiple Ag-binding has been suggested by Harindranath et al. (6) for human RF IgM autoantibodies, by Ikematsu et al. (8) and by Kasaian et al. (10) for other human IgG and IgA autoantibodies, and by Chen et al. (13), who showed that the H chain CDR3 was the most obvious parameter distinguishing polyreactive from monoreactive Ag-induced Abs in a panel of 84 murine mAbs. This hypothesis was substantiated by Martin et al. (14). They generated transfectomas to pair the cross-reactive idiotype (CRI) 17.109^+ κL chain of SMI, a polyreactive IgM RF utilizing a CRI $G6^+$ V_HI segment, with the Ig H chain of SMI or 10 other nonpolyreactive IgMs. All these IgMs had $G6^+$ H chains encoded by the same V_HI gene as SMI, but different D-J_H (CDR3) sequences. None of the 10 recombinant IgM κ Abs utilizing a H chain other than SMI was polyreactive, suggesting that the polyreactivity of SMI was dependent on the structure of the H chain CDR3. Our findings strengthen these observations by directly demonstrating that the grafting of the H chain CDR3 of a polyreactive Ig onto an originally monoreactive Ig results in multiple Ag-binding, and show further that an appropriate L chain V segment is necessary to fully express the polyreactive potential of the Ig H chain CDR3. Other L chains have been shown to not only contribute to Ag specificity of polyreactivity but also to efficiently bind Ags independently of H chains. Dimers of the MOPC 315 L chain bind ϵ-*N*-(2,4 dinitrophenyl)-L-lysine and 4-(α-*N*-alanine)-7-nitrobenz-2-oxa-1, 3-diazide (42, 43); and dimers of the L chain Bence-Jones Mcg protein bind dinitrophenyl compounds, ϵ-dansyllysine, colchichine, 1,10-phenanthroline, methadone, morphine, meperidine, 5-acetyluracil, caffeine, theophylline, menadione (vitamin K_3), triacetin, and other compounds (44, 45). In the case of mAb55, the direct multiple Ag-binding activity of the V_κ325 segment remains to be defined as this V_κ segment failed to generate a polyreactive Ab when paired with the V_H segment of mAb13. The FR1-FR3 sequence of the mAb55-derived κ chain V segment is identical to the FR1-FR3 sequence of the germline kv325 gene, and to that of the V_κ (SMI) segment used by Martin et al. (14) to express the polyreactivity of the SMI H chain. In fact, the mAb55 and SMI κ chains are identical not only in their FR1-FR3 sequence, but also in their FR4 sequence, and in the first seven of nine amino acids of the CDR3. The kv325 and other closely related segments of the V_κIIIb subgroup are frequently utilized to encode natural polyreactive Abs, as well as disease-associated autoantibodies, particularly RFs in autoimmune patients and chronic lymphocytic leukemia patients (20, 24, 46). The FR residues of V_κIIIb segments, which are putatively involved in the binding of the IgG Fc fragment (47), may also serve in stabilizing or defining the multiple Ag-binding site in polyreactive Abs.

Although limited to a single Ab pair, the present findings suggest that both monoreactive and polyreactive Ag-binding sites can be generated utilizing identical H and L chain V gene segments in conjunction with discrete primary structures of the H chain CDR3 (see Fig. 4, *K* and *L*). Previous observations have shown that the pool of V_H genes used by polyreactive natural Abs overlaps, at least partially, with that of V_H genes recruited in high-affinity immune responses to foreign Ags in healthy subjects or to self Ags in autoimmune patients patients (1, 5–10, 16, 24, 48). In contrast, the H chain CDR3 constitutes an important source of diversity in the expressed Ab repertoire, since its structure results from complex somatic rearrangement events, often involving more than one D gene in different orientations, and "unencoded" nucleotide additions (19, 49, 50). Computer analysis by Sanz (50) of more than 500 sequences from a CDR3-specific cDNA library revealed that these human Ig H chain sequences have the potential to generate more than 10^{14} different peptides. In view of the findings of Martin et al. (14), however, only a minority of somatically generated H chain CDR3s would give rise to Abs binding multiple Ags. The frequency of fully polyreactive Abs would be further reduced by the requirement for an appropriate L chain V segment, as shown here, and/or other H or L chain structures. This expected paucity of newly generated polyreactive Abs contrasts with the consistent findings that polyreactive Ab-producing cells account for up to 30% of the adult, and possibly more of the neo-

natal, human B cell repertoire (for a review see reference 51). Polyreactive Ab-producing cell precursors may be positively selected by contact with self Ags in early ontogeny, in a fashion similar to T cells in the thymus, where cells bearing receptors with low affinity for self Ags are recruited along the T cell maturation pathway (52). A somatic selection and amplification of human polyreactive Ab-producing cell clones would be further supported by the findings suggesting that most of the peripheral blood B cells in mice are ligand selected (53). The extension of combined structural and functional studies to other polyreactive Abs may help define the generality of the present findings and elucidate the role of these Abs in development and disease.

We are indebted to Dr. Kirk Fry (Genelabs Technologies, Inc., Redwood City, CA) for his generous gift of the pcDNAIG and pSXRDIG expression vectors. We thank Dr. Hideyuki Ikematsu for valuable suggestions, and the Eli Lilly Corp. for the gift of human recombinant insulin. Ms. Nancy Pacheco provided skillful technical assistance.

This work has been supported by the U.S. Public Health Service grant AR-40908. This is publication 26 from The Jeanette Greenspan Laboratory for Cancer Research.

Address correspondence to Dr. Paolo Casali, Department of Pathology, MSB-599, New York University School of Medicine, 550 First Avenue, New York, NY 10016.

Received for publication 10 January 1994 and in revised form 24 May 1994.

References

1. Riboldi, P., M.T. Kasaian, L. Mantovani, H. Ikematsu, and P. Casali. 1993. Natural antibodies. *In* The Molecular Pathology of Autoimmune Disease. C.A. Bona, K. Siminovitch, M. Zanetti, and A.N. Theofilopoulos, editors. Harwood Academic Publishers GmbH, Chur, Switzerland. 45–64.
2. McHeyzer-Williams, M.G., and G.J.V. Nossal. 1988. Clonal analysis of autoantibody-producing cell precursors in the preimmune B cell repertoire. *J. Immunol.* 141:4118.
3. Ueki, Y., I.S. Goldfarb, N. Harindranath, M. Gore, H. Koprowski, A.L. Notkins, and P. Casali. 1990. Clonal analysis of a human antibody response: quantiation of precursors of antibody-producing cells and generation and characterization of monoclonal IgM, IgG, and IgA to rabies virus. *J. Exp. Med.* 171:19.
4. Kasaian, M.T., H. Ikematsu, and P. Casali. 1992. Identification and analysis of a novel human $CD5^-$ B lymphocyte subset producing natural antibodies. *J. Immunol.* 148:2690.
5. Sanz, I., P. Casali, J.W. Thomas, A.L. Notkins, and J.D. Capra. 1989. Nucleotide sequence of eight human natural autoantibodies V_H region reveal apparent restricted use of V_H families. *J. Immunol.* 142:4054.
6. Harindranath, N., I.S. Goldfarb, H. Ikematsu, S.E. Burastero, R.L. Wilder, A.L. Notkins, and P. Casali. 1991. Complete sequence of the genes encoding the V_H and V_L regions of low and high affinity monoclonal IgM and IgA1 rheumatoid factors produced by $CD5^+$ B cells from a rheumatoid arthritis patient. *Int. Immunol.* 3:865.
7. Ikematsu, H., N. Harindranath, Y. Ueki, A.L. Notkins, and P. Casali. 1993. Clonal analysis of a human antibody response II. Sequence of the V_H gene of human IgM, IgG, and IgA to rabies virus reveal preferential utilization of V_HIII segments and somatic hypermutation. *J. Immunol.* 150:1325.
8. Ikematsu, H., M.T. Kasaian, E.W. Schettino, and P. Casali. 1993. Structural analysis of the V_H-D-J_H segments of human polyreactive IgG mAb: evidence for somatic selection. *J. Immunol.* 151:3604.
9. Harindranath, N., H. Ikematsu, A.L. Notkins, and P. Casali. 1993. Structure of the V_H and V_L regions of polyreactive and monoreactive human natural antibodies to HIV-1 and *E. coli* β-galactosidase. *Int. Immunol.* 5:1523.
10. Kasaian, M.T., H. Ikematsu, J.E. Balow, and P. Casali. 1994. Structure of the V_H and V_L segments of monoreactive and polyreactive IgA autoantibodies to DNA in patients with SLE. *J. Immunol.* 152:3137.
11. Chen, P.P., M.-F. Liu, S. Sinha, and D.A. Carson. 1988. A 16/6 idiotype-positive anti-DNA antibody is encoded by a conserved V_H gene with no somatic mutation. *Arthritis Rheum.* 31:1429.
12. Siminovitch, K.A., V. Mesiner, P.C. Kwong, Q.-L. Song, and P.P. Chen. 1989. A natural autoantibody is encoded by germline heavy and lambda light chain variable region genes without somatic mutation. *J. Clin. Invest.* 84:1675.
13. Chen, C., M.P. Stenzel-Poore, and M.B. Rittenberg. 1991. Natural auto- and polyreactive antibodies differing from antigen-induced antibodies in the H chain CDR3. *J. Immunol.* 147:2359.
14. Martin, T., S.F. Duffy, D.A. Carson, and T.J. Kipps. 1992. Evidence for somatic selection of natural autoantibodies. *J. Exp. Med.* 175:983.
15. Casali, P., M. Nakamura, F. Ginsberg-Fellner, and A.L. Notkins. 1990. Frequency of B cells committed to the production of antibodies to insulin in newly diagnosed patients with insulin-dependent diabetes mellitus and generation of high affinity human monoclonal IgG to insulin. *J. Immunol.* 144:3741.
16. Ikematsu, H., Y. Ichiyoshi, E.W. Schettino, M. Nakamura, and P. Casali. 1994. V_H and Vκ segment structure of anti-insulin IgG autoantibodies in patients with insulin-dependent diabetes mellitus. Evidence for somatic selection. *J. Immunol.* 152:1438.
17. Rechavi, G., B. Bienz, D. Ram, Y. Ben-Neriah, J.B. Cohen, R. Zakut, and D. Givol. 1982. Organization and evolution of immunoglobulin V_H gene subgroups. *Proc. Natl. Acad. Sci. USA.* 79:4405.
18. Ichihara, Y., H. Matsuoka, and Y. Kurosawa. 1988. Organization of human immunoglobulin heavy chain diversity gene

loci. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:4141.
19. Ravetch, J.V., U. Siebenlist, S.J. Korsmeyer, T. Waldmann, and P. Leder. 1981. Structure of the human immunoglobulin μ locus: characterization of embryonic and rearranged J and D genes. *Cell.* 27:583.
20. Kipps, T.J., E. Tomhave, P.P. Chen, and D.A. Carson. 1988. Autoantibody-associated κ light chain variable region gene expressed in chronic lymphocytic leukemia with little or no somatic mutation: implication for etiology and immunotherapy. *J. Exp. Med.* 167:840.
21. Pech, M., and H. Zachau. 1984. Immunoglobulin genes of different subgroups are interdigitated within the Vκ locus. *Nucleic Acids Res.* 12:9229.
22. Heiter, P.A., J.V. Maizel, Jr., and P. Leder. 1982. Evolution of human immunoglobulin κ J region gene. *J. Biol. Chem.* 257:1516.
23. Larrick J.W., E.F. Wallace, M.J. Coloma, U. Bruderer, A.B. Lang, and K. Fry. 1992. Therapeutic human antibodies derived from PCR amplification of B-cell variable regions. *Immunol. Rev.* 130:69.
24. Mantovani, L., R.L. Wilder, and P. Casali. 1993. Human rheumatoid B-1a ($CD5^+B$) cells make somatically hypermutated high affinity IgM rheumatoid factors. *J. Immunol.* 151:473.
25. Radic, M.Z., M.A. Mascelli, J. Erikson, H. Shan, and M.G. Weigert. 1991. Ig H and L chain contributions to autoimmune specificities. *J. Immunol.* 146:176.
26. Chothia, C., and A.M. Lesk. 1987. Canonical structures for the hypervariable regions of immunoglobulins. *J. Mol. Biol.* 196:901.
27. Kabat, E.A., and T.E. Wu. 1991. Identical V region amino acid sequences and segments of sequences in antibodies of different specificities: relative contributions of V_H and V_L genes, minigenes, and complementarity-determining regions to binding of antibody-combining sites. *J. Immunol.* 147:1709.
28. Sanz, I., and J.D. Capra. 1987. Vκ and Jκ gene segments of A/J Ars-A antibodies: somatic recombination generates the essential arginine at the junction of the variable and joining regions. *Proc. Natl. Acad. Sci. USA.* 84:1085.
29. Protolano, S., G.D. Chazenbalk, J.S. Hutchison, S.M. Mclachlan, and B. Rapoport. 1993. Lack of promiscuity in autoantigen-specific H and L chain combinations as revealed by human H and L chain "Roulette." *J. Immunol.* 150:880.
30. Utsumi, S., and F. Karush. 1964. The subunits of purified rabbit antibody. *Biochemistry.* 3:1329.
31. Jaton, J.-C., N.R. Klinman, D. Givol, and M. Sela. 1968. Recovery of antibody activity upon reoxidation of completely reduced polyalanyl heavy chain and its Fd fragment derived from anti-2,4-dinitrophenyl antibody. *Biochemistry.* 7:4185.
32. Ward, E.S., D. Gussow, A.D. Griffiths, P.T. Jones, and G. Winter. 1989. Binding activities of a repertoire of single immunoglobulin variable domains secreted from *Escherichia coli*. *Nature (Lond.).* 341:544.
33. Hamers-Casterman, C., T. Atarhouch, S. Muyldermans, G. Robinson, C. Hamers, E. Bajyana Songa, N. Bendahman, and R. Hamers. 1993. Naturally occurring antibodies devoid of light chains. *Nature (Lond.).* 363:446.
34. Segal, D.M., E.A. Padlan, G.N. Cohen, S. Radikoff, M. Potter, and D.R. Davies. 1974. The three-dimensional structure of a phosphorylcholine-binding mouse immunoglobulin Fab and the nature of the antigen binding site. *Proc. Natl. Acad. Sci. USA.* 71:4298.
35. Amit, A.G., R.A. Mariuzza, S.E.V. Phillips, and R.J. Poljak. 1986. Three-dimensional structure of an antigen–antibody complex at 2.8 Å resolution. *Science (Wash. DC).* 233:747.
36. Stanfield, R.L., T.M. Fieser, R.A. Lerner, and I.A. Wilson. 1990. Crystal structure of an antibody to a peptide and its complex with peptide antigen at 2.8 Å. *Science (Wash. DC).* 248:712.
37. Reichmann, L., M. Clark, H. Waldmann, and G. Winter. 1988. Reshaping human antibodies for therapy. *Nature (Lond.).* 332:323.
38. Kao, C.-Y.Y., and J. Sharon. 1993. Chimeric antibodies with anti-dextran-derived complementary-determining regions and anti-*p*-azophenylarsonate-derived framework regions. *J. Immunol.* 151:1968.
39. Richards, F.F., W.H. Koningsberg, R.W. Rosenstein, and J.M. Varga. 1975. On the specificity of antibodies. *Science (Wash DC).* 187:130.
40. Sheriff, S., E.W. Silverton, E.A. Padlan, G.H. Cohen, S.J. Smith-Gill, B.C. Finzel and D.R. Davies. 1987. Three-dimensional structure of an antibody-antigen complex. *Proc. Natl. Acad. Sci. USA.* 84:8075.
41. Padlan, E.A., E.W. Silverton, S. Sheriff, G.H. Cohen, S.J. Smith-Gill, and D.R. Davies. 1989. Structure of an antibody–antigen complex: crystal structure of the HyHEL-10 Fab-lysozyme complex. *Proc. Natl. Acad. Sci. USA.* 86:5938.
42. Zidovetski, R., A. Licht, and I. Pecht. 1979. Effect of interchain disulfide bond on hapten binding properties of light chain dimer of protein 315. *Proc. Natl. Acad. Sci. USA.* 76:5848.
43. Zidovetski, R., A. Licht, and I. Pecht. 1981. Positive cooperativitly in the hapten binding by the V_L dimer of protein 315. *Mol. Immunol.* 18:491.
44. Edmundson, A.B., E.R. Ely, R.L. Girling, E.E. Abola, M. Schiffer, F.A. Westholm, M.D. Fausch, and H.F. Deutsch. 1974. Binding of 2,4-dinitrophenyl compounds and other small molecules to a crystalline λ-type Bence-Jones dimer. *Biochemistry.* 13:3816.
45. Tribbick, G., A.E. Edmundson, T.J. Mason, and M.H. Geysen. 1989. Similar binding properties of peptide ligands for a human immunoglobulin and its light chain dimer. *Mol. Immunol.* 26:625.
46. Radoux, V., P.P. Chen, J.A. Sorge, and D.A. Carson. 1986. A conserved human germline Vκ gene directly encodes rheumatoid factor light chains. *J. Exp. Med.* 164:2119.
47. Hay, F.C., A.J. Soltys, G. Tribbick, and H.M. Geysen. 1991. Framework peptides from κIIIb rheumatoid factor light chains with binding activity for aggregated IgG. *Eur. J. Immunol.* 21:1837.
48. Pascual, V., and J.D. Capra. 1991. Human immunoglobulin heavy-chain variable region genes: organization, polymorphism, and expression. *Adv. Immunol.* 49:1.
49. Yamada, M., R. Wasserman, B.A. Richard, S. Shane, A.J. Caton, and G. Rovera. 1991. Preferential utilization of specific immunoglobulin heavy chain diversity and joining segments in adult human peripheral blood B lymphocytes. *J. Exp. Med.* 173:395.
50. Sanz, I. 1991. Multiple mechanisms participate in the generation of diversity of human H chain CDR3 regions. *J. Immunol.* 147:1720.
51. Casali, P., M.T. Kasaian, and G. Haughton. 1993. B-1 (CD5 B) cells. *In* Autoimmunity: Physiology and Disease. A. Coutinho and M.D. Kazatchkine, editors. Wiley-Liss, Inc., New York. 57–88.
52. Janeway, C.A. 1994. Thymic selection: two pathways to life and two to death. *Immunity.* 1:3.
53. Gu, H., D. Tarlinton, W. Müller, K. Rajewsky, and I. Förster. 1991. Most peripheral B cells in mice are ligand selected. *J. Exp. Med.* 173:1357.