Evidence for Somatic Selection of Natural Autoantibodies

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Summary

Natural autoantibodies are primarily immunoglobulin M (IgM) antibodies that bind to a variety of self-antigens, including self-IgG. Accounting for a large proportion of the early B cell repertoire, such polyspecific autoantibodies are speculated to contribute to the homeostasis and/or competence of the primary humoral immune system. Recent studies indicate that the leukemia cells from most patients with chronic lymphocytic leukemia (CLL) also express such IgM autoantibodies. Similarly, the leukemia cells from many CLL patients react with murine monoclonal antibodies (mAbs) specific for crossreactive idiotypes (CRIs) associated with human IgM autoantibodies. In particular, leukemic cells frequently react with G6, a mAb specific for an Ig heavy chain (H chain)-associated CRI, and/or with 17.109, a mAb that defines a κ light chain (L chain)-associated CRI. Generated against IgM rheumatoid factor (RF) paraproteins, G6 and 17.109 each recognize a major CRI that is present in many IgM RF paraproteins. Furthermore, over 90% of the IgM paraproteins found to bear both H and L chain–associated CRIs also are found to have RF activity. Molecular characterization of these CRIs demonstrates that each is a serologic marker for expression of a highly conserved Ig V gene. As such, the frequent production of IgM polyspecific autoantibodies in CLL simply may reflect the frequent use of such highly conserved autoantibody-encoding Ig V genes with little or no somatic mutation. To test this hypothesis, we generated murine transfectomas to pair the 17.109-reactive κ L chain of SMI, a 17.109/G6-reactive CLL population, with the Ig H chain of SMI or other G6-reactive leukemia cells or tonsillar lymphocytes. Cotransfection of vectors encoding the Ig H and L chains of SMI generated transfectomas that produce IgM_κ RF autoantibodies reactive with human IgG1 and IgG4. In contrast to G6/17.109-reactive IgM_κ RF Waldenstrom's paraproteins, the SMI IgM_κ also reacts with several other self-antigens, including myoglobin, actin, and ssDNA. However, cotransfection of the SMI L chain with a vector encoding any one of 10 different G6-reactive Ig H chains generated transfectomas that produce IgM_κ antibodies without detectable polyspecific autoantibody activity. These results indicate that polyspecific antiself-reactivity of G6/17.019-reactive Ig is dependent on the somatically generated Ig third complementarity determining region. Collectively, these studies imply that selection may be responsible for the frequent expression of polyspecific autoantibodies in CLL and early B cell ontogeny.

The leukemia cells from many patients with chronic lymphocytic leukemia (CLL)[1] produce polyspecific IgM autoantibodies. Early studies by Preud'homme and Seligmann (1) indicated that CLL patients have leukemia cells that frequently bear surface membrane IgM that has RF activity, or binding activity for human IgG. In another limited survey, four of thirteen CLL patients were found to have leukemia cells that expressed surface IgM (sIgM) with binding activity for human IgG (2). Subsequent studies demonstrated that over half of all CLL patients have leukemia cells that can be stimulated to secrete autoantibodies in vitro that bind to a variety of self-antigens, most notably human IgG (3–6). Although each of the leukemia cell cultures in these studies was monoclonal, the secreted autoantibodies generally were polyspecific, each Ig binding to two or more distinct self-antigens, e.g., human IgG, ssDNA, dsDNA, histones, cardiolipin, actin, thyroglobulin, and/or cytoskeletal components. Collectively, these studies indicate that the leukemia cells from most CLL patients may express polyreactive autoantibodies.

[1] *Abbreviations used in this paper:* BBS, borate buffered saline; CLL, chronic lymphocytic leukemia; CRI, crossreactive idiotypes.

Similarly, the leukemia cells of many CLL patients react with mAbs specific for crossreactive idiotypes (CRIs) of IgM autoantibodies. Approximately one-fifth of the patients with κ L chain-expressing CLL, for example, were found to have leukemia cells that express a κ L chain-associated CRI defined by a mAb, designated 17.109 (7, 8). Furthermore, a high proportion of CLL patients have leukemia cells that react with G6 (8, 9), a mAb specific for an Ig H chain-associated CRI. Generated against monoclonal IgM RF paraproteins, both 17.109 and G6 recognize CRIs present on many IgM autoantibodies, particularly RFs (10–12). In addition, over 90% of the monoclonal IgM paraproteins that have both H and L chain CRIs are noted to have RF activity (13).

Evaluation of the molecular basis for expression of these CRI reveals that each is a serologic marker for a conserved Ig V gene expressed with little or no somatic mutation (14, 15). For example, the 17.109-reactive (17.109^+) neoplastic B cells from unrelated patients express nearly identical V_κ genes that share more than 99% nucleic acid sequence homology with a germline V_κIII gene isolated from placental DNA, designated *Humkv325* (14–16). Similarly, G6-reactive ($G6^+$) leukemia cells from unrelated patients, as well as $G6^+$ tonsillar lymphocytes from healthy donors (17), express homologous V_H genes of the V_H1 subgroup, designated 51p1 (18, 19). Conceivably, the frequent expression of IgM autoantibodies in CLL may be a direct consequence of the repeated use of such highly-conserved Ig V genes. This model is supported by the work of Sanz et al. (20), suggesting that natural polyreactive autoantibodies are encoded by a restricted repertoire of nonmutated Ig V genes.

However, this model minimizes the contribution of the third CDR (CDR3) to the autoreactive binding activity of the Ig expressed in CLL. This region of the Ig H chain is encoded by the D and J_H gene segments, that undergo recombination and NH_2-terminal nucleic acid base insertion immediately prior to V_H gene rearrangement (21, 22). Accordingly, the sequence of the CDR3 generally is idiosyncratic to each Ig V_H gene rearrangement, as has been noted with all $G6^+$ H chains sequenced to date (17, 18, and unpublished observations). In view of the large potential for diversity in the CDR3 of $G6^+$ H chains (17), the random pairing of a 17.109^+ κ L chain with any $G6^+$ Ig H chain may not be anticipated to form an autoantibody if the CDR3 is critical to autoantibody binding activity.

To examine this, we generated murine transfectomas to pair the 17.109^+ κ L chains of SMI, a $17.109/G6^+$ CLL B cell population, with each of several different $G6^+$ H chains expressed by normal or leukemic B cells. We find that the myeloma cells cotransfected with the original pair of Ig H and L chain genes of SMI secrete polyreactive IgM_κ RF autoantibodies. However, myeloma cells co-transfected with the SMI κ L chain gene and any one of ten different G6 H chain genes produced IgM_κ that failed to have such polyspecific autoreactivity. Collectively, this study indicates that the polyspecific autoreactivity of the "natural" autoantibodies frequently noted in CLL and early B cell ontogeny is a selected specificity.

Materials and Methods

Antibodies. Purified polyclonal human IgG was obtained from Cappel Research Products (Durham, NC). We obtained purified myeloma proteins of each IgG isotype from Dr. Hans Spiegelberg (University of California, San Diego, School of Medicine, La Jolla, CA). Another set of purified myeloma proteins of each IgG isotype was purchased from Calbiochem Corp. (San Diego, CA). G6, a murine IgG1 mAb reactive with an autoantibody H chain-associated CRI (12) was obtained from Drs. Rizgar Mageed and Roy Jefferis (University of Birmingham, Birmingham, England). DA4-4, an IgG1 anti-human μ H chain-producing hybridoma (23), was obtained from the American Type Tissue Culture Collection (Rockville, MD). Anti-human κ or anti-human λ mAb-producing hybridomas were as described (7, 14). The IgM_κ RF paraprotein BOR, isolated from the plasma of a patient with mixed cryoglobulinemia, was donated by Dr. R. Wistar, Jr. (National Naval Medical Center, Bethesda, MD). The IgM Waldenstrom's paraprotein, ME591, is as described (24). MOPC-21, an $IgG1_\kappa$ mouse myeloma protein of unknown specificity, was produced by P3X63Ag8 (25). This cell line was obtained from the American Type Culture Collection. Antibodies were precipitated from ascites or culture supernatants in 50% ammonium sulfate. After at least 1 h at 4°C, the samples were spun for 10 min at 10,000 *g*. The harvested Ig precipitate was suspended and dialyzed extensively in PBS, pH 7.2. Column chromatography of transfectoma Ig was performed with Superose-6™ in PBS at 4°C using an FPLC apparatus (Pharmacia, Uppsala, Sweden).

Vector Constructions. We isolated the rearranged gene complexes encoding the κ L chain and the μ H chain of $G6\text{-}CRI^+/17.109\text{-}CRI^+$ leukemic cells of CLL patient, SMI. For this we partially digested SMI genomic DNA with Sau3a. Large sized fragments were isolated on sucrose density gradients for ligation into λ dash™ with BamHI compatible overhangs (Stratagene Inc., La Jolla, CA), as described (26). For the κ L chain, a 17.8-kb BamHI fragment was isolated that contained the κ L chain promoter, rearranged V_κ gene, L chain enhancer and κ L chain constant region exon. This fragment was ligated in pSV2-gpt, a SV40 DNA plasmid vector with the *Escherichia coli* gpt gene (*Ecogpt*), encoding xanthine-guanine phosphoribosyltransferase (27). Expression of this gene allows for selection of transfected mammalian cells with mycophenolic acid (27, 28), as described (29) (see Fig. 1 *A*). In addition, a recombinant clone was isolated from the SMI genomic library that contained the rearranged H chain of SMI. An EcoRI/HindIII digest of this recombinant insert yielded a 3.4-kb fragment that contained the Ig promoter, the functionally rearranged V_H gene and the H chain enhancer region. This fragment was subcloned into Bluescript™ (Stratagene Inc.).

Unique restriction sites were introduced at sites flanking the H chain variable region gene to allow for the removal and subsequent insertion of different rearranged V_H genes isolated via PCR. For this, ssDNA was synthesized using the R408 helper phage (Stratagene Corp.). Unique restriction enzyme sites then were introduced via site-directed mutagenesis using synthetic oligonucleotides and the Oligonucleotide-Directed-Mutagenesis-System™ version 2 (Amersham Corp., Arlington Heights, IL). A ClaI site was introduced 5′ of the leader sequence and a SalI site was introduced 3′ of the J_H segment. Mutants initially were screened by digestion of plasmids prepared from independent colonies and checked by dsDNA sequencing using oligonucleotide complementary to sequences 100-bp contiguous to the introduced site, as described (14).

This modified 3.4-kb rearranged V_H gene fragment was ligated onto an 7.4-kb BamHI-HindIII fragment containing the human μ constant region exons excised from pN· ×-μTNP (30), that originally was derived from the cloned segment λ C75 (31. This fragment, in turn, was ligated onto an EcoRI/BamHI fragment of pN· ×-μTNP (30), containing the *E. coli* neo^r gene, the SV40 ori, pBR322 ori, and ampicillin resistance gene to create pRTM1 (see Fig. 1 *B*). The plasmid pN· ×-μTNP originally was obtained from Dr. M. J. Shulman (University of Toronto, Toronto, Canada) (30).

Synthetic Oligonucleotides. PCR primers, site-directed mutagenesis oligonucleotides and probes were synthesized on a Pharmacia Gene Assembler (Pharmacia). Probes were 5′ end-labeled with γ-[^{32}P]ATP and polynucleotide kinase, as described (7).

Cloning Rearranged G6-encoding V_H Genes Into pRTM1. Rearranged G6-encoding V_H1 genes isolated from normal tonsillar (17) or neoplastic B cells (18) were cloned and sequenced in Bluescript™ (Stratagene Inc.) as described (15, 17, 18). The rearranged V_H1 genes of SIC, HEN, BRA, and HUR were cloned from the genomic DNA isolated from CD5 B cell lymphoma tissues of patients with G6-reactive CLL (8). SMI, AND, and NEI were isolated from G6-reactive leukemia cells (18). To clone each rearranged V_H1 gene into pRTM1, we performed PCR on each recombinant plasmid using oligonucleotides specific for the V_H1 leader sequence (dGCA TCG ATA ATC ACC ATG GAC), or to a J_H consensus sequence (dGCG TCG ACT CTG AGG AGA CGG TGA) that have the flanking restriction enzyme sites ClaI or SalI, respectively. This generated PCR products of ~660 bp that were digested with ClaI and SalI, to yield V_H1-containing inserts that were purified via agarose gel electrophoresis. These inserts were ligated into pRTM1 that had been digested with SalI and ClaI and then isolated free of the SMI V_H1 insert via 0.8% agarose gel electrophoresis. The ligated plasmids each were used to transform XL-1Blue™ (Stratagene, Inc.). Ampicillin-resistant transformants were screened via blot hybridization with a radiolabeled oligonucleotide (dGCG TAG TTT GCT GTA CC) specific for a sequence within the CDR2 of the V_H1 gene(s) encoding the G6-CRI, as described (18, 32).

Transfectomas. P3X63-Ag8.653, a nonsecreting mouse myeloma cell line (33), was obtained from the American Type Culture Collection. These cells were cultured in RPMI-1640 (Whittaker Bioproducts, Walkersville, MD) containing 20% FCS (HyClone Laboratories, Inc., Logan, UT) and 2-mM L-glutamine (Mediatech Inc., Washington, DC). Cells were washed and then suspended in ice cold RPMI at 1.5 × 10^7/ml. One ml of the cell suspension was then mixed with 2-mg linearized vector DNA and then transferred into a cold plastic cuvette containing two aluminum electrodes (Bio-Rad Laboratories, Richmond, CA). An electric pulse of 1,000 V/cm with a capacitance of 960 mF was applied to the solution using a Gene-Pulser™ apparatus (Bio-Rad Laboratories), as described (34). After the electric pulse, the cells were kept on ice for 10 min and then transferred to a flask of warm culture medium. 2 d after the electroporation, the medium was adjusted to 1 mg/ml of G418 (Geneticin; Gibco Bethesda Research Laboratories, Gaithersburg, MD) and/or 250 mg/ml xanthine (Sigma Chemical Co., St. Louis, MO), 15 mg/ml hypoxanthine (Sigma Chemical Co.), and 1 mg/ml mycophenolic acid (Sigma Chemical Co.), respectively, to select for transfectomas expressing *E. coli* neo^r and/or gpt genes of the transfected plasmids.

ELISA. To measure the concentrations of IgM, IgM_κ, or CRI-bearing Ig, polystyrene microtiter plates were incubated with murine mAbs HB-57 (anti-human IgM), 17.109, or G6, at 5–10 μg/ml of borate buffered saline (BBS, pH 8.2). After incubation for over 1 h, plates were washed and then exposed to 1% bovine gelatin (J. T. Baker Inc., Phillipsburg, NJ) in BBS for 1 h at room temperature to block residual protein binding sites. Culture supernatants, concentrated transfectoma Ig, or purified IgM_κ paraprotein were titrated in 1% gelatin in BBS and then added to the washed plates for a 1 h incubation at room temperature. Plates were then washed with 0.05% Tween in BBS before addition of alkaline phosphatase-conjugated goat antibodies specific for the human Ig μ chain or κ L chain (Southern Biotech., Birmingham, AL) to assay for IgM or IgM_κ, respectively. After another 1-h incubation at room temperature, plates were washed and then developed with aminoethylcarbozole in carbonate buffer (pH 9.8). The optical density at 405 nm of each of the wells was accessed using a ThermoMax™ (Molecular Devices Corp., Menlo Park, CA) ELISA plate reader linked to a MacIntosh^{Plus} (Apple Computer, Cupertino, CA) computer for protein concentration analyses using Δ SOFT™ software (BioMetallic Inc., Princeton, NJ). For standards we used the IgM_κ RF paraprotein, BOR, and an IgM_κ Waldenstrom's paraprotein, ME591, of irrelevant specificity. Binding to other self-antigens was measured similarly, except that the polystyrene plates were coated with bovine actin, bovine thyroglobulin, purified singlestranded calf thymus DNA (Sigma Chemical Co.), polydeoxyinosine (poly dI), or poly-deoxythymidine (poly dT) (Pharmacia Inc., Piscataway, NJ). Plates without antigen but treated with 1% gelatin were used to measure gelatin binding activity. For this, test samples were diluted in 1% BSA faction V (Boehringer Mannheim Biochemicals, Indianapolis, IN), or in 0.05% gelatin without any detectable difference in plate binding activity.

To evaluate the ability of anti-idiotype to inhibit antigen binding, varying amounts of murine mAb were added to individual wells containing SMI IgM_κ at a final concentration of 2 μg/ml. After a 30-min incubation at room temperature, the samples were assessed for their ability to bind antigen-coated plates, as described above. The binding activities of antibody with inhibitor were compared with those of serial dilutions of antibody without inhibitor to obtain the effective antigen-binding antibody concentrations. The percent inhibition of antigen binding was calculated as [1-(effective antigen-binding antibody concentration)/(actual concentration)] × 100.

Results

We generated transfectomas to pair different G6$^+$ IgM H chains with the 17.109$^+$ κ L chain of SMI, a 17.109/G6$^+$ CLL cell population. For this, we generated pRTM1, a human μ chain expression vector (Fig. 1 *B*). This vector contains the functionally rearranged SMI V_H1 gene flanked by unique restriction enzyme sites, ClaI and SalI, that were introduced via site-directed mutagenesis. This vector enabled us to exchange the SMI H chain variable with any one of several different functional V_HDJ_H exons of other G6$^+$ leukemia cells or tonsillar lymphocytes.

From pRTM1, we generated ten additional vector constructs that encode highly homologous Ig H chain variable regions (Fig. 2). Each variable region is encoded by a rearranged V_H1 gene that shares >99% nucleic acid sequence homology to 51p1, a V_H1 gene expressed during early fetal development (17–19, and unpublished observations). Like SMI, H chain constructs SIC, HEN, AND, NEI, BRA, and HUR were derived using the rearranged V_H1 gene expressed by G6$^+$

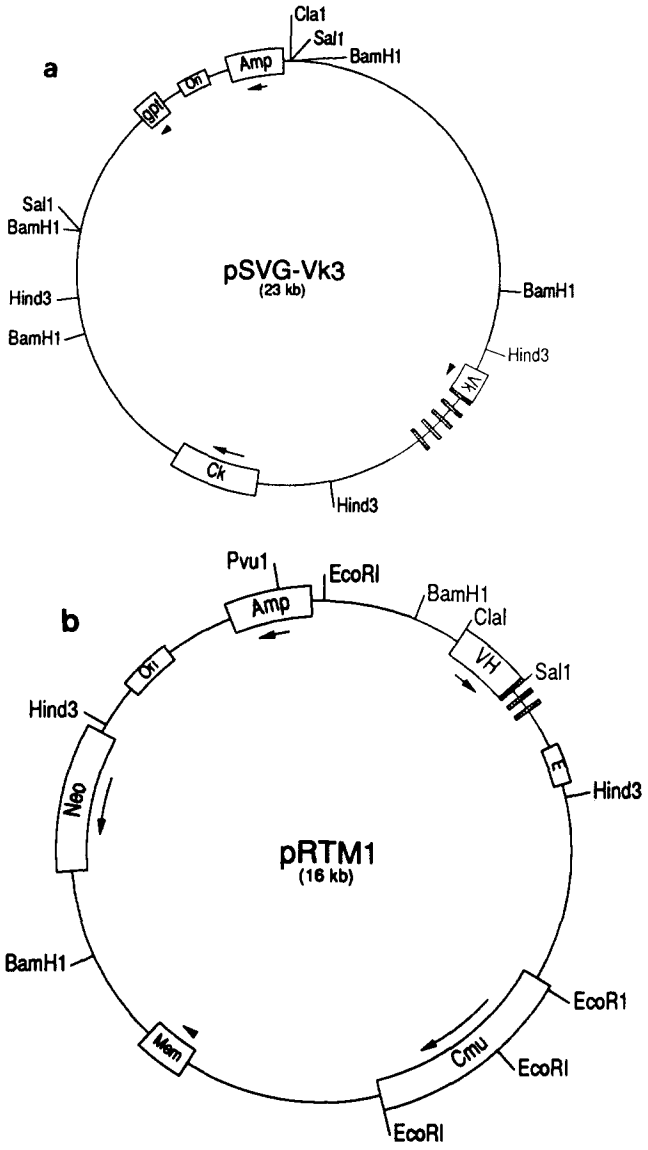

Figure 1. Structure of pSVG-Vk3 and pRTM1 plasmids. (←) Directions of gene transcription; (*open labeled boxes*) important regions. (*a*) pSVG-Vk3 contains the functionally rearranged Humkv325 V_κ gene and C_κ exon of SMI (17.8 kb) that was inserted into the BamHI site of pSVG-gpt (27, 28). Vκ, Humkv325 of SMI rearranged with $J_\kappa 1$; C_κ, κ L chain constant region exon; gpt, *Ecogpt* and SV40 origin of replication: Ori, pBR322 origin of replication; Amp, ampicillin resistance gene. (*b*) pRTM1 contains the modified and functionally rearranged V_H gene of SMI and Ig H chain enhancer (3.4 kb) that was inserted between the EcoRI-HindIII sites of pN· ×-μTNP (30), that previously flanked the functionally rearranged mouse V_HTNP gene. VH, the $V_H 1$ gene expressed by SMI that is functionally rearranged to $J_H 4$, and modified via site-directed mutagenesis to have unique flanking restriction enzyme sites ClaI and SalI; E, the Ig H chain enhancer region; Cμ, exons encoding the human C_μ constant region; Mem, C_μ membrane exon; Neo, the bacterial gene conferring neomycin resistance, the SV40 origin and SV40 early region; Ori, pBR322 origin of replication; Amp, ampicillin resistance gene.

CLL (8, 18, and unpublished observations). H chain constructs L26, L30, L33, and L42 were derived from the rearranged $V_H 1$ genes expressed by G6$^+$ tonsillar B cells (17). From amino acid positions 1–94, L30 shares complete homology with SMI (Fig. 2). All other $V_H 1$ genes have a GAA codon instead of an AAA codon at amino acid position 73, resulting in a lysine to glutamine substitution in the third framework region (Fig. 2). In addition, BRA has two other nonconservative nucleic acid base differences from SMI that result in serine to asparagine substitutions at amino acid positions 35 and 76. For comparison, the amino acid sequence of the H chain variable region of the G6$^+$ IgM$_\kappa$ RF paraprotein BOR (35) differs from SMI by nine residues (Fig. 2).

In contrast to the relative uniformity in the V_H gene-encoded protein sequences, the rearranged V_H genes differ markedly in the CDR3s (Fig. 2). In addition, the lengths of the CDR3s vary greatly, ranging from 6–25 codons (L26–SIC). Finally, rearranged J_H gene segments $J_H 3$, $J_H 4$, $J_H 5$, and $J_H 6$ are represented in this collection of G6$^+$ H chain variable regions (Fig. 2).

To generate transfectomas expressing each of these H chains with a 17.109$^+$ L chain, we generated pSVG-V$_\kappa$3. This vector contains the genomic DNA of the rearranged κ L chain gene complex of SMI that encodes a 17.109$^+$ L chain (Fig. 1 *A*). Two other G6$^+$ neoplastic B cell populations represented in this study, SIC and BRA, also originally expressed 17.109$^+$ κ L chains (15). In addition, the reference IgM$_\kappa$ RF paraprotein BOR also bears the 17.109 CRI (35). The deduced or actual protein sequences of these κ variable regions are highly homologous, reflecting expression of the conserved V_κ gene, Humkv325, with little or no somatic mutation (Fig. 3) (7, 15, 16).

Cotransfection with pSVG-V$_\kappa$3 and the different constructs of pRTM1 generated transfectomas that each secreted intact human IgM$_\kappa$ protein. Sample concentrations were determined via ELISA using monoclonal IgM$_\kappa$ paraproteins, ME591 and/or BOR, as standards. The amounts of measured IgM$_\kappa$ protein in the supernatants varied between different transfectoma cell lines, ranging from a few hundred nanograms to several micrograms of antibody per ml. However, the Ig concentration of each supernatant assessed using plates coated with anti-human μ chain mAb was comparable to that determined using plates coated with G6, 17.109, or mAbs specific for human κ L chains (data not shown). Column chromatography of transfectoma proteins SMI, HEN, SIC, or L26 revealed the measured IgM$_\kappa$ or Ig κ activity to have a molecular size similar to that of IgG (data not shown). Collectively, these results indicate that each transfectoma apparently produces intact monomeric IgM$_\kappa$ molecules that bear both 17.109 and G6 CRIs.

Although each transfectoma secretes intact G6$^+$/17.109$^+$ IgM$_\kappa$, only the cell lines generated using the Ig H and L chain genes of SMI produced IgM$_\kappa$ with significant RF activity. Serial dilutions of concentrated IgM$_\kappa$ from each sample were assayed for binding activity to human IgG. The IgM$_\kappa$ produced by the transfectoma expressing both Ig chains of the SMI leukemia cell population (designated SMI) has detectable RF activity at concentrations below 1 μg/ml (Fig. 4). At any one IgM$_\kappa$ concentration, the RF activity of SMI is lower than that of the multimeric IgM$_\kappa$ RF paraprotein, BOR (Fig. 4). However, except for L30, none

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                                    CDR1                    CDR2                                         CDR3            JH SEGMENT
     1                  20          +---+   40         +---------60----+            80              94+-------------------------+103
SMI  QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCAR GGNYDYIWGSYRSND           AFDIWGQGTMVTVSS JH3
SIC  ·························································································E························ N·YCGGDCY·RWELLRTDFSED   ······P········ JH3
HEN  ·························································································E························ PHASIDDFW·GYYPN       YYYYGM·V·····T····· JH6
AND  ·························································································E························ VSIFGVVQH            YYYYYYM·V··K··T····· JH6
NEI  ·························································································E························ ·PRLLADVLLWFGELS          E··Y·····L····· JH4
BRA  ··································N······································E··N····················· D·CSGGSCYFW               GW··P·····L····· JH5
HUR  ·························································································E························ VP·PLFFAV                 GM·V·····T····· JH6
L26  ·························································································E························ ·D                        NW··P·····L····· JH5
L30  ·································································································································· ·RTRVSVSTL·D·SGYYDFSG YYGM·V·····T····· JH6
L33  ·························································································E························ ·                    YYYYYGM·V·····T····· JH6
L42  ·························································································E························ ··VAGRPHFD                   Y·····L····· JH5

BOR  E···················T·····D····S·························P·············T·E········V·········L····· E·RRMA·NP                    ··Y·····L····· JH4/5
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Figure 2. Sequence comparison of the deduced or actual amino acid sequences of G6+ Ig H chain variable regions. (*Right*) Code name for each sequence. The 20-letter amino acid code represents the deduced sequence of SMI. All other sequences are compared with that of SMI. (·) Homology between the compared sequences and SMI. Otherwise, letters correspond to the discordant amino acids. Spaces are introduced to maximize sequence homology in CDR3. Listed above SMI, amino acid residues are numbered according to Kabat et al. (51). Also listed are labels indicating the first, second, and third CDRs, and the region encoded by the J_H gene segment. (*left*) Deduced J_H gene segments used to encode the fourth framework of the H chain variable regions. At the bottom of the figure is the actual amino acid sequence of BOR (35) compared to that of SMI.

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                                 CDR1                   CDR2                                    CDR3     JK
     1                      24--------34           50---56                                 89-----97---------107
SMI  EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPPAFGQGTKVEIKR JK1
SIC    ···········································································································  NT··G·S······· JK4
BRA    ····························································································································RYT············ JK2

BOR  ···································································································V···········QT············ JK1
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Figure 3. Sequence comparison of the deduced or actual amino acid sequences of 17.109+ Ig L chain variable regions. The amino acid sequences are described, and compared to SMI as in Fig. 2

of the other transfectomas IgM_κ proteins had any detectable RF activity, even at their highest concentrations (>2 μg/ml for all except for AND [0.6 μg/ml]) (Fig. 4). Furthermore, although L30 had slight reactivity with human IgG, its RF activity only was detectable at concentrations exceeding 1 μg/ml (Fig. 4).

The SMI IgM_κ RF is isotype specific. SMI has binding activity for IgG1 or IgG4 myeloma proteins that is similar to that for total human IgG (Fig. 5). In contrast, SMI binds poorly to IgG2 and IgG3. The same hierarchy of binding activity for IgG1 > IgG4 >> IgG2 > IgG3, is noted using either of two sets of purified myeloma proteins representing each of the IgG isotypes. As such, the reactivity of the SMI IgM_κ for IgG1 or IgG4 cannot be ascribed to an idiosyncratic binding activity for a particular myeloma protein of a single donor. Of note, the BOR IgM_κ RF paraprotein also reacts primarily with IgG of the IgG1 isotype (data not shown).

In contrast to BOR and each of the other transfectoma proteins, the SMI Ig also has polyspecific antigen binding activity. The SMI IgM_κ reacts strongly with myoglobin, actin, or ssDNA, and, by comparison, weakly with thyroglobulin (Fig. 6). That the ssDNA binding activity cannot be ascribed to trace residual chromatin is demonstrated by SMI's reactivity with synthetic polydeoxyinosine (poly dI) or polydeoxythymidine (poly dT) (Fig. 6). In every case, the binding activity of SMI for each of these antigens is detectable at IgM_κ concentrations below 1 μg/ml. In contrast, none of the other transfectomas IgM_κ proteins, including L30, had detectable binding activity to any of these antigens, even

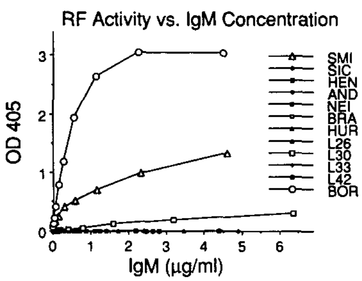

Figure 4. RF activity of each transfectoma protein compared with BOR. Ordinate is OD at 405 nm of the ELISA for RF activity measured on plates coated with total human IgG. The abscissa describes the IgM_κ concentration (μg/ml) of the assayed sample. Symbols representing titration of each sample are indicated. In particular, (○) BOR, (△) SMI, and (□) binding activity of L30.

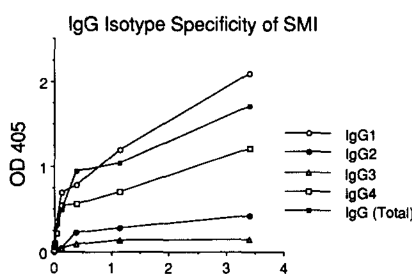

Figure 5. IgG isotype specificity of SMI. Graph labeled as in Fig. 4. Symbols represent titration of SMI on plates coated with IgG1 (○), IgG2 (●), IgG3 (△), IgG4 (□), or total IgG (■), respectively.

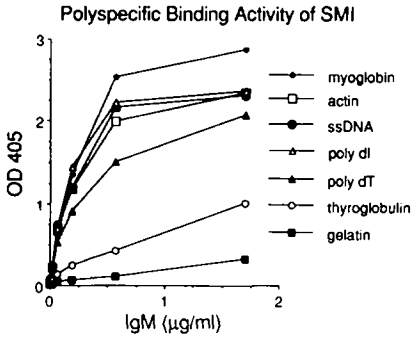

Figure 6. Polyspecific binding activity of SMI. Graph labeled as in Fig. 4. Symbols represent titration of SMI on plates coated with myoglobin (●), actin (□), single-stranded DNA (●), poly deoxyinosine (Δ), poly deoxythymidine (▲), thyroglobulin (O), or gelatin (■), respectively.

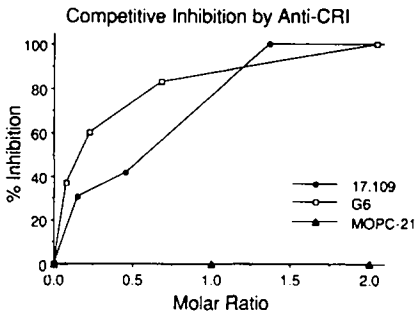

Figure 8. Inhibition of SMI antigen binding activity by anti-CRI mAb. Ordinate lists percent inhibition of SMI binding to myoglobin-coated plates by preincubation of SMI with either 17.109 (●), G6 (□), or MOPC-21 (Δ), calculated as described in Materials and Methods. The measured values are plotted against the molar ratio of anti-idiotypic or MOPC-21 inhibitor to SMI IgM_κ.

at the highest tested concentration of >2 μg/ml for all except AND (1.6 μg/ml) (data not shown). In addition, the IgM_κ RF, BOR, also did not have any detectable binding activity for any of these antigens, even at concentrations in excess of 4 μg/ml. None of the transfectoma proteins, including SMI, had significant binding activity for bovine gelatin (data not shown, and Fig. 6).

We size fractionated the SMI IgM_κ via FPLC and evaluated each fraction for antigen binding activity by ELISA. These analyses revealed that the predominant IgM actin-binding activity of SMI is similar in size to monomeric IgG (Fig. 7). Similarly, the IgM antimyoglobin binding activity of SMI also comigrated with human IgG (data not shown). These results demonstrate that the observed polyspecific antigen-binding activity of SMI is not dependent on a multimeric structure.

Finally, the polyspecific binding activity of SMI could be inhibited specifically by anti-CRI mAbs. Varying amounts of IgG murine mAbs were added to individual wells containing SMI IgM_κ at a final concentration of 2 μg/ml. After a 30-min incubation at room temperature, the samples were assessed for their ability to bind myoglobin via ELISA. The percent inhibition of antigen binding were plotted versus the molar ratios of murine IgG inhibitor to SMI IgM. We find that either 17.109 or G6 can completely inhibit SMI antigen binding activity at molar ratios in slight excess of unity (Fig. 8). In contrast, MOPC-21, a control mouse myeloma IgG1 protein of unknown specificity, did not inhibit SMI antigen binding activity (Fig. 8).

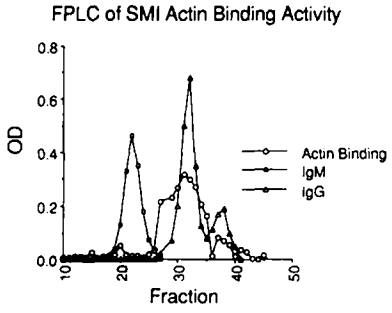

Figure 7. Size chromatography of SMI antigen binding activity. The SMI IgM_κ was size fractionated via FPLC. Each fraction was assayed for human IgM anti-actin activity by ELISA. The ELISA OD at 405 of SMI (O) is plotted against the fraction sample number. Also shown are ODs at 280 nm of the sample fractions of ME591, a pentameric Waldenstrom's IgM_κ paraprotein (□), or monomeric IgG (Δ).

Discussion

This study demonstrates that the random pairing of different $G6^+$ Ig H chains with a single 17.109^+ κ L chain generally does not produce an IgM autoantibody. Of 11 murine transfectomas that express IgM_κ proteins bearing both the G6 and 17.109 CRIs, only the native Ig H and L chain pair had significant autoantibody activity. This cannot be attributed to defective Ig synthesis by the other transfectomas. The culture supernatants of each transfectoma were found to have stoichiometric concentrations of Ig μ chains and κ L chains by ELISA. Furthermore, ELISA plates coated with either anti-κ L chain antibodies or anti-CRI mAbs captured amounts of transfectoma IgM that were similar to those of anti-hu-IgM-coated plates. Finally, column-chromatography of representative samples of the IgM antibodies from those with or without RF activity indicated that these proteins have a molecular size similar to that of monomeric IgG. Collectively, these results indicate that each transfectoma produces intact monomeric human IgM_κ molecules bearing both 17.109 and G6 CRIs. We conclude that the random pairing of different $G6^+$ Ig H chains with 17.109^+ κ L chains generally does not generate an Ig with antiself reactivity.

The H chain constant regions of the transfectoma IgM proteins examined in this study are encoded by human C_μ exons derived from an IgM expression vector, designated pN· ×-μTNP. In previous studies, Boulianne et al. noted that Sp2/0 cells cotransfected with pN· ×-μTNP and an Ig L chain expression vector secreted mostly multimeric IgM (30). However, as noted above, we found that most of the IgM secreted by each transfectoma comigrates with monomeric human IgG

in FPLC. Conceivably, cell culture conditions and/or processing procedures may have influenced the ratio of IgM monomers to IgM multimers that is secreted by our transfectomas (Dr. Marc J. Shulman, personal communication). Alternatively, a mutation(s) may have occurred in the human C_μ exons during the construction of pRTM1, resulting in a vector that encodes Ig μ chains that are inefficient in forming multimers (36, 37). Restriction mapping studies of pRTM1 and pN· ×-μTNP, however, failed to reveal any differences between the two vectors in the regions containing the Ig C_μ exons (data not shown). In any case, all transfectomas used in this study possessed the same Ig C_μ exons, were cultured under similar conditions and apparently secreted mostly monomeric IgM. Furthermore, most of IgM autoantibody activity of SMI also chromatographically comigrates with human IgG (Fig. 7). Therefore, the differences in the binding activities of the transfectoma proteins bearing both 17.109 and G6 CRIs cannot be ascribed to simple differences in Ig receptor valency.

The V_H1 exon of SMI differs from those of most other transfectomas by a single nonconservative base substitution at amino acid position 73, resulting in a glutamine to lysine substitution (Fig. 2). Although this substitution may be due to somatic mutation, this difference also may reflect a genetic polymorphism in the V_H1 gene encoding the G6-CRI. Possibly, the lysine residue at position 73 contributes to the RF binding activity of the SMI transfectoma protein. In this regard, it should be noted that L30, the only other transfectoma IgM with lysine at amino acid position 73 (Fig. 2), also is the only other transfectoma protein that has binding activity for IgG that is above background (Fig. 4). It is not likely that this substitution enhances the RF activity of G6-reactive IgM proteins, in general, however, as most other G6-reactive RF autoantibodies and CLL Ig do not have a lysine at this position (e.g., BOR, Fig. 2, and references 12, 13, 35). Another possibility is that the CDR3 of the SMI L chain may have been selected for its ability to generate an RF Ig when paired with a G6-reactive H chain that has a lysine at this position. Evaluation of the relative contribution of this lysine residue to the RF activity of SMI may require site-directed mutagenesis studies and/or further chain mixing experiments.

In any case, the present study delineates the importance of the CDR3 to the polyspecific binding activity of Ig bearing these autoantibody-associated CRIs. Each of the $G6^+$ H chains has a unique CDR3 sequence. However, except for the lysine to glutamine substitution at position 73, the V_H gene-encoded protein sequences for all but one of the transfectoma proteins are identical. Moreover, L30 shares comparable homology with SMI in the V_H gene-encoded protein sequence. Since the pairing of L30 with the 17.109^+ SMI L chain does not produce an Ig with detectable polyspecific autoantibody reactivity, this amino acid substitution alone cannot account for the absence of such binding activity in the other transfectoma proteins. Collectively, these data argue that the somatically generated CDR3 may contribute significantly to the polyspecific autoantibody activity of certain $G6^+$/ 17.109^+ IgM proteins.

This indicates that such polyspecific binding activity of natural autoantibodies may be a selected specificity. In the primary follicles of human fetal spleen, high proportions of B cells express autoantibody-associated CRIs, such as G6 and 17.109 (38). Genetic mechanisms may favor the frequent rearrangement of Ig V genes that have the capacity to encode CRI-bearing IgM autoantibodies (39). However, as with our transfectoma studies, the random pairing of such Ig L and H chains during ontogeny may infrequently give rise to Ig with polyspecific antiself-reactivity. Conceivably, B cells that chance express functional and self-reactive receptors may be stimulated to mature into functional B lymphocytes. Such selection may contribute to the noted high frequency of B cell clones expressing such polyreactive natural autoantibodies during early development (40, 41). Indeed, recent data indicate that most peripheral B cells in mice may be ligand selected (42). The selection for self-reactive autoantibody-expressing B cells during B cell ontogeny may be similar to that of T cells in the thymus, where only the cells expressing receptors with low affinity antiself-reactivity are selected for subsequent T cell maturation (43–47).

With regard to CLL, it is not certain whether the apparent selection for polyreactive autoreactivity merely reflects its cytogenesis, or if it actually plays a role in leukemogenesis (48, 49). Recent studies indicate that most patients with CLL have leukemia cells that express IgM autoantibodies that, like the SMI transfectoma IgM_κ, react with a variety of different self antigens (3–6). The frequency of CLL patients that have leukemia B cells that express such polyreactive autoantibodies apparently greatly exceeds the noted frequency of polyreactive B cells in normal embryonic tissues, cord blood or adult peripheral blood. Whatever the origin, Guigou et al. (50) recently noted that 11–16% of the EBV-transformed Ig-secreting B cell clones expressed polyspecific antibodies when tested on a panel of nine antigens, including self-antigens. Conceivably, B cells selected for expression of sIg with autoantibody activity during early B cell ontogeny could be stimulated constitutively by self-antigen, thus increasing the risk for malignant transformation to CLL.

Finally, chance somatic mutation in the V genes encoding such self-reactive antibodies may enhance the affinity of the expressed Ig for self-antigen, contributing to augmented cell stimulation and B cell maturation. Unlike CLL, the neoplastic cells in patients with Waldenstrom's macroglobulinemia and/or mixed cryoglobulinemia have differentiated into Ig secreting cells. The IgM_κ RF paraprotein BOR was isolated from the plasma of one such patient. It is important to note that the Ig H chain of the RF paraprotein BOR differs from the deduced amino sequence of 51P1 or SMI by several residues (35). Conceivably, these differences are secondary to somatic mutations that result in loss of polyspecific autoreactivity and higher affinity for the driving self-antigen(s), possibly human IgG. Further comparative studies on the structure-function relationships of such IgM autoantibodies may help elucidate factors involved in the etiopathogenesis of these lymphoproliferative diseases.

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References

1. Preud'homme, J.L., and M. Seligmann. 1972. Anti-human immunoglobulin G activity of membrane-bound immunoglobulin M in lymphoproliferative disorders. *Proc. Natl. Acad. Sci. USA.* 69:2132.
2. Kipps, T.J., S. Fong, E. Tomhave, P.P. Chen, R.D. Goldfien, and D.A. Carson. 1987. Immunoglobulin V gene utilization in CLL. *In* Chronic Lymphocytic Leukemia: Recent Progress and Future Direction. R.P. Gale, and K.R. Rai, editors. Alan R. Liss, New York. pg. 115.
3. Youinou, P., L. Mackenzie, B.M. Broker, D.I. Isenberg, A. Drogou-Lelong, A. Gentric, and P.M. Lydyard. 1988. The importance of CD5-positive B cells in nonorgan-specific autoimmune diseases. *Scand. J. Rheumatol. Suppl.* 76:243.
4. Broker, B.M., A. Klajman, P. Youinou, J. Jouquan, C.P. Worman, J. Murphy, L. Mackenzie, R. Quartey-Papafio, M. Blaschek, P. Collins, S. Lai, and P.M. Lydyard. 1988. Chronic lymphocytic leukemic (CLL) cells secrete multispecific autoantibodies. *J. Autoimmun.* 1:469.
5. Sthoeger, Z.M., M. Wakai, D.B. Tse, V.P. Vinciguerra, S.L. Allen, D.R. Budman, S.M. Lichtman, P. Schulman, L.R. Weiselberg, and N. Chiorazzi. 1989. Production of autoantibodies by CD5-expressing B lymphocytes from patients with chronic lymphocytic leukemia. *J. Exp. Med.* 169:255.
6. Borche, L., A. Lim, J.L. Binet, and G. Dighiero. 1990. Evidence that chronic lymphocytic leukemia B lymphocytes are frequently committed to production of natural autoantibodies. *Blood.* 76:562.
7. Kipps, T.J., S. Fong, E. Tomhave, P.P. Chen, R.D. Goldfien, and D.A. Carson. 1987. High-frequency expression of a conserved kappa light-chain variable-region gene in chronic lymphocytic leukemia. *Proc. Natl. Acad. Sci. USA.* 84:2916.
8. Kipps, T.J., B.A. Robbins, A. Tefferi, G. Meisenholder, P.M. Banks, and D.A. Carson. 1990. CD5-positive B-cell malignancies frequently express cross-reactive idiotypes associated with IgM autoantibodies. *Am. J. Pathol.* 136:809.
9. Kipps, T.J., B.A. Robbins, P. Kuster, and D.A. Carson. 1988. Autoantibody-associated cross-reactive idiotypes expressed at high frequency in chronic lymphocytic leukemia relative to B-cell lymphomas of follicular center cell origin. *Blood.* 72:422.
10. Carson, D.A., and S. Fong. 1983. A common idiotope on human rheumatoid factors identified by a hybridoma antibody. *Mol. Immunol.* 20:1081.
11. Carson, D.A., P.P. Chen, T.J. Kipps, V. Radoux, F.R. Jirik, R.D. Goldfien, R.I. Fox, G.J. Silverman, and S. Fong. 1987. Idiotypic and genetic studies of human rheumatoid factors. *Arthritis. Rheum.* 30:1321.
12. Mageed, R.A., M. Dearlove, D.M. Goodall, and R. Jefferis. 1986. Immunogenic and antigenic epitopes of immunoglobulins XVII - - Monoclonal antibodies reactive with common and restricted idiotopes to the heavy chain of human rheumatoid factors. *Rheumatol. Int.* 6:179.
13. Crowley, J.J., R.D. Goldfien, R.E. Schrohenloher, H.L. Spiegelberg, G.J. Silverman, R.A. Mageed, R. Jefferies, W.J. Koopman, D.A. Carson, and S. Fong. 1988. Incidence of three cross-reactive idiotypes on human rheumatoid factor paraproteins. *J. Immunol.* 140:3411.
14. 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. Implications for etiology and immunotherapy. *J. Exp. Med.* 167:840.
15. Pratt, L.F., L. Rassenti, J. Larrick, B. Robbins, P. Banks, and T.J. Kipps. 1989. Immunoglobulin gene expression in small lymphocytic lymphoma with little or no somatic hypermutation. *J. Immunol.* 143:699.
16. 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.
17. Kipps, T.J., and S.F. Duffy. 1991. Relationship of the CD5 B cell to human tonsillar lymphocytes that express autoantibody-associated cross-reactive idiotypes. *J. Clin. Invest.* 87:2087.
18. Kipps, T.J., E. Tomhave, L.F. Pratt, S. Duffy, P.P. Chen, and D.A. Carson. 1989. Developmentally restricted VH gene expressed at high frequency in chronic lymphocytic leukemia. *Proc. Natl. Acad. Sci. USA.* 86:5913.
19. Schroeder, H.W., Jr., J.L. Hillson, and R.M. Perlmutter. 1987. Early restriction of the human antibody repertoire. *Science (Wash. DC).* 238:791.
20. Sanz, I., P. Casali, J.W. Thomas, A.L. Notkins, and J.D. Capra. 1989. Nucleotide sequences of eight human natural autoantibody VH regions reveals apparent restricted use of VH families. *J. Immunol.* 142:4054.
21. Yancopoulos, G.D., and F.W. Alt. 1986. Regulation of the assembly and expression of variable region genes. *Annu. Rev. Immunol.* 4:339.
22. Desiderio, S.V., G.D. Yancopoulos, M. Paskind, E. Thomas, M.A. Boss, N. Landau, F.W. Alt, and D. Baltimore. 1984. In-

sertion of N regions into heavy-chain genes is correlated with expression of terminal deoxytransferase in B cells. *Nature (Lond.).* 311:752.
23. Maruyama, S., H. Kubagawa, and M.D. Cooper. 1985. Activation of human B cells and inhibition of their terminal differentiation by monoclonal anti-mu antibodies. *J. Immunol.* 135:192.
24. Axelrod, O., G.J. Silverman, V. Dev, R. Kyle, D.A. Carson, and T.J. Kipps. 1991. Idiotypic cross reactivity of immunoglobulins expressed in Waldenstroms' macroglobulinemia, chronic lymphocytic leukemia, and mantle zone lymphocytes of secondary B cell follicles. *Blood.* 77:1484.
25. Kohler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature (Lond.).* 256:495.
26. Frischauf, A.M., H. Lehrach, A. Poustka, and N. Murray. 1983. Lambda replacement vectors carrying polylinker sequences. *J. Mol. Biol.* 170:827.
27. Mulligan, R.C., and P. Berg. 1980. Expression of a bacterial gene in mammalian cells. *Science (Wash. DC).* 209:1422.
28. Southern, P.J., and P. Berg. 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. *J. Mol. Appl. Genet.* 1:327.
29. Oi, V.T., S.L. Morrison, L.A. Herzenberg, and P. Berg. 1983. Immunoglobulin gene expression in transformed lymphoid cells. *Proc. Natl. Acad. Sci. USA.* 80:825.
30. Boulianne, G.L., N. Hozumi, and M.J. Shulman. 1984. Production of functional chimaeric mouse/human antibody. *Nature (Lond.).* 312:643.
31. Rabbitts, T.H., A. Forster, and C.P. Milstein. 1981. Human immunoglobulin heavy chain genes: evolutionary comparisons of C mu, C delta, and C gamma genes and associated switch sequences. *Nucleic. Acids Res.* 9:4509.
32. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
33. Kearney, J.F., A. Radbruch, B. Liesegang, and K. Rajewsky. 1979. A new myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. *J. Immunol.* 123:1548.
34. Potter, H., L. Weir, and P. Leder. 1984. Enhancer-dependent expression of human kappa immunoglobulin introduced into mouse pre-B lymphocytes by electroporation. *Proc. Natl. Acad. Sci. USA.* 81:7161.
35. Newkirk, M.M., R.A. Mageed, R. Jefferis, P.P. Chen, and J.D. Capra. 1987. Complete amino acid sequences of variable regions of two human IgM rheumatoid factors, BOR and KAS of the Wa idiotypic family, reveal restricted use of heavy and light chain variable and joining region gene segments. *J. Exp. Med.* 166:550.
36. Shulman, M.J., C. Heusser, C. Filkin, and G. Kohler. 1982. Mutations affecting the structure and function of immunoglobulin M. *Mol. Cell. Biol.* 2:1033.
37. Davis, A.C., C. Collins, M.I. Yoshimura, G. D'Agostaro, and M.J. Shulman. 1989. Mutations of the mouse mu H chain which prevent polymer assembly. *J. Immunol.* 143:1352.
38. Kipps, T.J., B.A. Robbins, and D.A. Carson. 1990. Uniform high frequency expression of autoantibody-associated cross reactive idiotypes in the B cell follicles of human fetal spleen. *J. Exp. Med.* 171:189.
39. Rassenti, L.Z., L.F. Pratt, P.P. Chen, D.A. Carson, and T.J. Kipps. 1991. Autoantibody-encoding kappa light chain genes frequently rearranged in lambda light chain expressing chronic lymphocytic leukemia. *J. Immunol.* 147:1060.
40. Dighiero, G., P. Lymberi, D. Holmberg, I. Lundquist, A. Coutinho, and S. Avrameas. 1985. High frequency of natural autoantibodies in normal newborn mice. *J. Immunol.* 134:765.
41. Dighiero, G., P. Lymberi, B. Guilbert, T. Ternynck, and S. Avrameas. 1986. Natural autoantibodies constitute a substantial part of normal circulating immunoglobulins. *Ann. NY Acad. Sci.* 475:135.
42. 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.
43. Kisielow, P., H.S. Teh, H. Bluthmann, and H. von Boehmer. 1988. Positive selection of antigen-specific T cells in thymus by restricting MHC molecules. *Nature (Lond.).* 335:730.
44. Benoist, C., and D. Mathis. 1989. Positive selection of the T cell repertoire: where and when does it occur. *Cell.* 58:1027.
45. Fink, P.J., M.J. Blair, L.A. Matis, and S.M. Hedrick. 1990. Molecular analysis of the influences of positive selection, tolerance induction, and antigen presentation on the T cell receptor repertoire. *J. Exp. Med.* 172:139.
46. Blackman, M.A., P. Marrack, and J. Kappler. 1989. Influence of the major histocompatibility complex on positive thymic selection of V beta 17a$^+$ T cells. *Science (Wash. DC).* 244:214.
47. Sha, W.C., C.A. Nelson, R.D. Newberry, J.K. Pullen, L.R. Pease, J.H. Russell, and D.Y. Loh. 1990. Positive selection of transgenic receptor-bearing thymocytes by Kb antigen is altered by Kb mutations that involve peptide binding. *Proc. Natl. Acad. Sci. USA.* 87:6186.
48. Gu, H., I. Forster, and K. Rajewsky. 1990. Sequence homologies, N sequence insertion and JH gene utilization in VHDJH joining: Implication for the joining mechanism and the ontogenetic timing of Ly1 B cell and B-CLL progenitor generation. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:2133.
49. Kipps, T.J. 1989. The CD5 B cell. *Adv. Immunol.* 47:117.
50. Guigou, V., B. Guilbert, D. Moinier, C. Tonnelle, L. Boubli, S. Avrameas, M. Fougereau, and F. Fumoux. 1991. Ig repertoire of human polyspecific antibodies and B cell ontogeny. *J. Immunol.* 146:1368.
51. Kabat, E., T.T. Wu, M. Reid-Miller, H.M. Perry, and K.S. Gottesmann. 1987. Sequences of Proteins of Immunological Interest. US Government Printing Office, Bethesda, MD.