Human Rheumatoid Factors with Restrictive Specificity for Rabbit Immunoglobulin G: Auto- and Multi-reactivity, Diverse Vh Gene Segment Usage and Preferential Usage of VxIb

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Summary
To determine the molecular and functional properties of human rheumatoid factors (RF), we established stable hybridomas and Epstein-Barr virus-transformed B cell lines from the synovial fluid or peripheral blood of three patients with rheumatoid arthritis and one patient with systemic lupus erythematosus. 17 cell lines were obtained that produced high-titer immunoglobulin M (IgM) RF that reacted exclusively with rabbit but not human IgG or IgG of other mammalian species. Certain anti-rabbit IgG RF also had specificity for other mammalian antigens (Ag), including cytoskeletal proteins and intracellular proteins found in HeLa cells, as well as for Ag present in an extract prepared from the cell wall of group A streptococci. 13 of the 17 RF contained λ-type light (L) chains, of which 12 were classified serologically as members of the λ-λ chain variable region (Vλ) subgroup, designated VλIII. The heavy chain V region (VH) and Vλ sequences of nine of these IgMRF were determined at the cDNA level. Five Vh genes in three Vh families were used by these antibodies (Ab), including Vh1 (dp21/1-4b and dp10 [51p1/hv1051]), Vh3 (dp38/3-15 and dp77/13-21), and Vh4 (dp70/4-4b). The deduced V gene-encoded amino acid sequences of the λ chains of these IgMRF confirmed their serological classification as xII, and they were further classified as members of the relatively uncommon VλIII subgroup, designated VλIIIb. Based on cDNA analyses, nine were the product of three different VλIIIb germline genes. Two such genes, designated hsiggll150 and hsiggll29s, were cloned and sequenced from genomic DNA. Unique combinations of these Vh and VxIIIb genes could be related to distinctive patterns of reactivity among the IgMRF. Although the Vh and Vx regions of these Abs were expressed primarily as germline-encoded sequences, four of nine multireactive Abs had extensive V region mutation, indicative of an Ag-driven process. The finding that xIIIb L chains are preferentially found among anti–rabbit IgG RF, and that some of these Ab have specificity for other protein, cellular, and bacterial Ag, provides new insight into the pathogenesis of RA and related diseases.

Rheumatoid arthritis (RA)1 is a chronic inflammatory disorder primarily affecting joints, although extra-articular organs are frequently involved during the course of the disease (1, 2). Humoral, cellular, and nonimmunological factors have all been implicated in the pathological process that results in joint destruction and other manifestations characteristic of RA (3–6). In the case of humoral immunity, a variety of autoantibodies, particularly RF, interact with Ag in synovial tissue, fluid, and cartilage. This interaction, which

1 Abbreviations used in this paper: FR, framework region; MAP, microtubule-associated protein; RF, rheumatoid factor.
gives rise to the formation of immune complexes, complement activation, and the recruitment of neutrophils and macrophages, is responsible for the intensive inflammatory reaction and resultant morbidity.

The biological function and pathological role of RF have been the subject of extensive investigation (for reviews see references 7 and 8). The specificity of these Ab, which are most often of the IgM class, is directed towards IgG and in particular, the Fc-related epitopes. Based on reactivity patterns, Williams and Kunkel (9) showed that IgM RF could be divided into three groups: those that recognized solely (a) rabbit IgG; (b) human IgG; or (c) both. RF were initially detected by the Rose-Waaler test, which employs SRBC coated with rabbit IgG Ab. A positive Rose-Waaler test has been considered more specific for RA than the bentonite or latex flocculation tests, which employ human IgG as the coating protein (7). In this regard, it is also of interest to note that RF in diseases other than RA appear to be more reactive with human IgG (10-12).

Considerable progress in identifying and characterizing the genes encoding the H and L chain V regions (VH and VL, respectively) of anti-IgG Ab has come through study of RF-secreting hybridomas and immortalized B cell lines (for a review see reference 13). Studies to date involving human IgM RF that have specificity for human IgG or both human and rabbit IgG have indicated that these V genes are diverse and often mutated. However, limited information is available regarding the antigenic specificity and V region gene usage by RF reactive with rabbit but not human IgG (14).

As part of a long-standing study to elucidate the molecular structure of RF, we have prepared hybridomas and EBV-transformed B cell lines from synovial cells and PBL obtained from three patients with RA and one with SLE. Many of these hybridomas and cell lines secreted IgM RF that had exclusive specificity for rabbit IgG. These RF differed from anti–human IgG RF in that they contained predominately λ-type L chains that, remarkably, were the product of a relatively uncommon Vλ gene family, designated Vl11b (15). Our studies have also shown that some of these anti–rabbit IgG RF were multireactive and recognized additional Ag including cytoskeletal and other intracellular proteins, as well as streptococcal cell wall components.

Materials and Methods

Generation of Human Monoclonal RF-secreting Cell Lines. EBV-transformed B cell lines were established from peripheral blood (PB) and synovial non-T cells from three patients with RA (LBR, PHB, and DGA) and one patient with SLE (KE5) as described previously (16). Cell lines of interest were fused with K6H6/B5 (American Type Culture Collection, Rockville, MD) according to Carroll et al. (17). The hybridomas were repeatedly cloned by limiting dilution until all clones were positive for RF activity. KE5-643 was cloned twice without being fused with K6H6/B5 using irradiated human T cells as feeder cells. The cell lines were shown to be monoclonal, as evidenced by PCR amplification of unique VH and Vλ transcripts.

ELISA. The methods used for the ELISA were previously described (16). Coating Ag used for RF determination included human and rabbit IgG isolated by NH₄SO₄ precipitation and DEAE ion exchange chromatography, IgG of horse, goat, sheep, bovine, dog, and rat (Sigma Chemical Co., St. Louis, MO), and Fe and F(ab')₂ fragments of rabbit IgG from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA). Other Ag were tetanus toxoid and diphtheria toxoid from the State Laboratory Institute (Jamaica Plain, MA), thyroglobulin, double stranded (ds)DNA, human insulin, DNP-hemocyanin, bovine type II collagen, bovine muscle myosin, porcine muscle actin, and human epidermal keratin from Sigma Chemical Co. and bovine glial fibrillary acidic protein from ICN Biomedicals Inc. (Costa Mesa, CA). Porcine brain tubulin and microtubule-associated proteins (MAPs) were purified as described previously (18).

For inhibition, human IgG at 50 mg/ml in PBS was heat aggregated at 60°C for 30 min and then centrifuged at 2,000 rpm to remove insoluble and large aggregates. The aggregated IgG was incubated at room temperature for 2 h with appropriately diluted RF-containing cell supernatants. The mixtures were used in ELISA as described above.

The Vλ-subgroup nature of the λ-type IgM anti–rabbit IgG RF was determined by ELISA using murine mAbs specific for the Vλ I, Vλ II, Vλ IV, and Vλ VI subgroups and the Vλ IIIa, Vλ IIIb, and Vλ IIIc sub-subgroups (19). The ELISA for RF-binding to streptococcal extract was kindly performed by Dr. Madeleine W. Cunningham (University of Oklahoma, Oklahoma City, OK) (20). The affinity constants of RF to rabbit IgG and myosin were measured by ELISA as described by Friguet et al. (21).

Immunofluorescence and Immunoblotting. Indirect immunofluorescence to HeLa cells (16) and SDS-PAGE and immunoblots (22) were done as previously described. Porcine MAPs (12 μg/lane) and homogenized HeLa cells (106 cells/lane) were electrophoresed on 5-25% gradient SDS-PAGE gels. After transferring, the blots were washed, blocked with 5% milk for 1 h, and incubated overnight at 4°C with supernatants diluted with 0.5% BSA in PBS. The blots were then treated with reagents contained in the Vectastain ABC anti–human IgM kit (Vector Laboratories, Inc., Burlingame, CA) and analyzed by enhanced chemiluminescence reagents (Amerham Corp., Arlington Heights, IL).

RNA Isolation, cDNA Synthesis, and Anchored PCR for Amplification of VH and VL. Total RNA was prepared from hybridoma cell lines using either the single-step technique (23) or the RNAzol™ B method (Biotecx Laboratories, Houston, TX). 5 μg of total RNA was reverse transcribed with oligo (dT) primer and Moloney murine leukemia virus reverse transcriptase. A poly (dG) tail was added to the 3′ end of the cDNA with terminal deoxynucleotidyl transferase. 2G-tailed cDNA was amplified using anchored PCR (24, 25) modified by the “touchdown” method (26). Oligonucleotide primers for the amplification of VH were PAN (5′-CACCTGCA-AGGGGCCCCGCGGCTGA) and PANpoly C (5′-CACCTGCA-AGGGGCCCCGCGGCTGA), and for the amplification of VL, the following primers were used: Sar (5′-CTACGAGCTACGAGCGGCCC-GCGGACC) and SANpoly C (5′-CTACGAGCTACGAGCGGCCC-GCGGACC) with an SstI cloning site, and HMCl (5′-CGCAAGTATTATCTCACAGACGAG) with a HindIII cloning site. In the case of KE5-643, because the Vλ had an internal SstI site, PAN and PANpoly C primers were used.

DNA Isolation and PCR for Amplification of VH Germline Counterparts. High molecular weight DNA was extracted from the PB granulocytes of PHB, a patient with Alzheimer’s disease (MRE), and two normal donors and from the T cell fraction of LBR PBMNC according to Blin and Stafford (27). Briefly, frozen cell pellets were
ground into a fine powder in liquid nitrogen and incubated at 55°C for 3 h in 0.5 M EDTA, pH 8.0, containing 0.5% sodium dodecyl sulfate and 100 µg/ml proteinase K. After RNase treatment, the DNA was purified by phenol extraction and the concentration in the dialyzed fractions determined spectrophotometrically. When necessary for PCR reaction, the DNA was concentrated by HindIII digestion and ethanol precipitation. A 5’ primer, GLUNA (5’-CAGGTCGACGGYTCAGGAGGCAGARCTC), was designed from the untranslated regions of the LBR150 and LBR295 V<sub>λ</sub> genes. The 3’ primer for the LBR150 germline gene was GLCDA (5’-CCGGATCTTACCCACTGTGTTCGCTGATTG), designed from CDR3 of the LBR150 V<sub>λ</sub> gene. The 3’ primer for the LBR295 germline gene was GLCDB (5’-GCAGGYYCACAGGCGACARCTC), designed from CDR3 of the LBR295 V<sub>λ</sub> gene. These primers contained SalI and EcoRI cloning sites, respectively. The PCR amplification procedure was essentially that described by Sotagil et al. (28).

**Cloning and Sequencing of the Amplified DNA.** The amplified DNA was digested with the appropriate enzymes and separated by electrophoresis on 1% agarose gels (Sea Plaque; FMC Corp., Rockland, ME). To sequence the V region in both directions, the gel-purified DNA was directionally cloned into both M13mp18 and M13mp19. The cloned single-stranded (ss)DNA was sequenced by the dideoxynucleotide chain termination method (29) using the Sequenase Version 2.0 kit (United States Biochemical Corp., Cleveland, OH). Each given sequence represented the consensus residues of eight clones generated from two independent PCR products. The DNA sequence data were analyzed using the EuGene software program (Molecular Biology Information Resource, Baylor Medical College, Houston, TX) linked to major data banks via the University of Virginia Computer Center.

### Results

**Preferential Usage of V<sub>III</sub> Gene Segments by IgM RF Reactive with Rabbit IgG.** 17 IgM anti-IgG Ab were identified by screening the supernatants of EBV-transformed B cell lines from RA patients LBR (1,351 from synovial tissue and 620 from PB), PHB (2,281, PB), and DGA (311, synovial tissue) and SLE patient KES (705, PB). These RF reacted strongly with rabbit IgG but not with human IgG or the IgG from six other mammalian species (Table 1). They were solely Fc reactive. That all 17 RF had exclusively anti-rabbit IgG reactivity was further evidenced by the demonstration that heat-aggregated, human pooled IgG up to 5 mg/ml were not inhibitory. In contrast, aggregated human IgG at 0.1 mg/ml readily inhibited our other IgM RF, reactive either with human IgG or with both human and rabbit IgG.

Serologically, 13 of the 17 RF were typed as IgM<sub>λ</sub>. This

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**Table 1. 17 IgM RF Specific for Rabbit IgG Show a Predominant Usage of λ L chain (13/17) and Utilize VA III Subgroup (12/13)**

| Cell lines | Tissue | Rabbit IgG | Human IgG* | ELISA OD<sub>450</sub> | L chain<sup>†</sup> | Class | V<sub>λ</sub> subgroup |
|------------|--------|------------|------------|-------------------------|-----------------|-------|-------------------|
| LBR 150 | ST      | 50<sup>†</sup> | Nonreactive<sup>†</sup> | 40 | λ | λIIla/b |
| 271       | "       | 80         | "          | 80 | " | " |
| 295       | "       | 160        | "          | 40 | " | " |
| 962       | "       | 160        | "          | 40 | " | " |
| 1045      | "       | 50         | "          | 160 | " | " |
| 1189      | "       | 160        | "          | 80 | " | " |
| 318       | "       | 20         | "          | 20 | " | " |
| 328       | "       | 20         | "          | 20 | " | " |
| 1039      | "       | 20         | "          | 20 | " | " |
| 1052      | "       | 50         | "          | 640 | " | " |
| P255      | PB      | 20         | "          | 20 | " | " |
| PHB 1213 | "       | 40         | "          | 40 | " | " |
| 1540      | "       | 20         | "          | 20 | " | " |
| 1650      | "       | 40         | "          | 40 | " | " |
| KES 643   | "       | 640        | "          | " | " | " |
| DGA 127  | ST      | 20         | "          | " | " | " |

<sup>*</sup> Also nonreactive with IgG from horse, goat, sheep, bovine, dog, and rat.

<sup>†</sup> VA determination was done by ELISA and V<sub>κ</sub> were determined by cDNA sequence analysis.

<sup>§</sup> OD<sub>450</sub> multiplied by the dilution factor i.e., OD<sub>450</sub> = 1 at 1:50 dilution. The OD<sub>450</sub> values were the averages of three determinations.

<sup>∥</sup> OD<sub>450</sub> <0.1 at 1:10 dilution.

ST, synovial tissue.
Table 2. IgM RF Specific for Rabbit IgG Are Reactive with Cytoskeletal Proteins and other Ag

| RF    | Isotype | MAPs  | Keratin | Myosin | GFAP | Actin | Tubulin | Other Ag* | Strept. | HeLa± |
|-------|---------|-------|---------|--------|------|-------|---------|-----------|---------|-------|
| LBR 295 | μλ      | 18± | - ** | 2      | -     | -     | -       | -         | ++      |       |
| 962    |         |      |         |        |      |       |         |           |         |       |
| 1045   |         |      |         |        |      |       |         |           |         |       |
| 1189   |         |      |         |        |      |       |         |           |         |       |
| LBR 150 | μλ      | 4    | 19      | 5      | 7    | 5     | 29      | -         | ++      | ++    |
| 271    |         |      |         |        |      |       |         |           |         |       |
| LBR 1104 | μκ    | -    | -       | -      | -    | -     | -       | -         | -       | + +   |
| PHB 1213 | μλ    | 14   | 20      | 19     | 26   | 13    | 56      | 7 (TT), 2 (DT) | + +      | + +   |
| KES 643 | μλ      | 19   | 7       | 8      | 13   | 10    | 22      | -         | + +     | + +   |
| LBR 1052 | μκ    | -    | -       | -      | -    | -     | -       | -         | -       |       |
| PHB 1540 | μκ    | -    | -       | -      | -    | -     | -       | -         | -       | + +   |

* dsDNA, insulin, thyroglobulin, type II collagen, DNP-hemocyanin, TT, and DT.
† Group A streptococcal cell wall extract: (+ +) OD₄₀₅ ≥2 at 1:10 dilution; (-) OD₄₀₅ <0.1 at 1:10 dilution.
§ Intracellular staining by indirect immunofluorescence: (+ +) staining; (-) nonstaining.
‖ Similar results have been obtained with LBR295, LBR962, LBR1045, and LBR1189 and only data on LBR295 is presented. Similarly, only data on LBR150 is presented.
*** OD₄₀₅ multiplied by the dilution factor, i.e., = 1 at 1:18 dilution. The OD₄₀₅ values were the averages of three determinations.
** Negative, OD₄₀₅ <0.1 at 1:10 dilution.
DT, diptheria toxoid, GFAP, glial fibrillary acidic protein, TT, tetanus toxoid.

biased usage of λ-L chains was in marked contrast to the predominance of κ-L chains among anti-human IgM RF. In our collection of 63 IgM RF reactive with either human IgG or with both human and rabbit IgG, 54 were typed to be κ. Of the 13 IgMRF, 10 were classified serologically as VₓIIIb and one as VₓI. Two remaining RF were reactive with both anti-VₓIIIa and anti-VₓIIIb mAb.

It is of considerable interest to note that the B cells which were responsible for the secretion of these RF were markedly enriched in the synovium. This was best documented in the case of LBR whose blood and synovial tissue were available for EBV transformation and immortalization; 11 out of 1,351 synovial B cell lines secreted RF of desired specificity whereas only 1 of 620 PB B cell lines did so.

Autoreactivity of IgM RF. Stable hybridomas were formed with 10 of the 17 EBV-transformed B cell lines fused with K6H5/B5 cells. KES643 was cloned without fusion and proved to be a stable, high IgM secretor. The RF produced by these 11 hybridomas and B cell lines were studied further regarding their reactivities with other Ag and V region usage.

With the exception of the IgMκ RF LBR1052 and PHB1540, the other nine λ-type IgM RF listed in Table 2 reacted with one or more types of cytoskeletal proteins, and, of these, LBR1104 also reacted weakly with tetanus and diphtheria toxoids. LBR1104 reacted weakly with BSA, indicating that it was an mAb with a tendency for nonspecific binding in ELISA. The predominant reactivity of one group (LBR295, LBR962, LBR1045, and LBR1189) was with MAPs and that of a second group (LBR150 and LBR271) was with keratin and tubulin. Other patterns of reactivity were found. LBR1104 had major reactivity with tubulin but also recognized other cytoskeletal proteins, as did KES643. PHB1213 was most strongly reactive with myosin and keratin. Western blot anal-

![Figure 1. Immunoblot of LBR295 (lane 1) and LBR150 (lane 2) against MAPs. Lane 2 is an unreactive IgM antibody.](image-url)
Figure 2. Immunofluorescent staining of methanol-fixed HeLa cells by IgM RRF. LBR150 and LBR271 stained HeLa cells in an identical pattern. LBR295, LBR962, LBR1045, and LBR1189 also stained HeLa similarly. LBR1104, PHB1213, and KES643 stained HeLa with distinct patterns. Three of the RF staining are presented: (A) LBR150, (B) PHB1213, and (C) LBR295. Although the staining is predominantly intracellular and cytoplasmic, their nuclear staining patterns differ from each other. LBR150 stains nucleoli. Filamentous staining is apparent over the nuclei in the case of PHB1213. The nuclear staining of LBR295 looks speckled.

Further analyses revealed that five of the IgM RRF were reactive with a group A streptococcal cell wall extract. All nine of the IgM RRF and one of the two IgMc RRF stained HeLa cells intracellularly. The staining patterns of HeLa appeared to be discernable for each group of the RF (Fig. 2). By Western blotting using an extract of HeLa cells as substrate, these RF also had unique reactivity patterns. As shown in Fig. 3, LBR150 (lane 3) and LBR295 (lane 2) both stained a 17-kD band. However, these two RF recognized two other different cytoplasmic HeLa constituents: LBR150 reacted strongly with a 40-kD component, whereas LBR295 reacted comparably with a 29-kD band. Similar results were obtained when the HeLa extract was electrophoresed under nonreducing conditions. KES643, PHB1213, and LBR1104 blotted faintly different bands than those stained by LBR150 and LBR295.

Despite the multireactive nature of these RF, their affinity constants for rabbit IgG were relatively high, ranging from $10^{-6}$ to $10^{-7}$ M. For the LBR150 and LBR295 groups, the affinity constants were $2.8 \times 10^{-7}$ M and $2.5 \times 10^{-6}$ M, respectively. For PHB1213, the affinity constant for rabbit IgG was $1.6 \times 10^{-6}$ M. In contrast, it had a higher binding constant for myosin ($6.3 \times 10^{-8}$ M).

In an attempt to demonstrate circulating RF or RF in synovial fluid with sole reactivity to rabbit IgG, the sera of the studied patients and those of six additional patients as well as six synovial fluid samples with high RF titers to both human and rabbit IgG were selected for absorption with human IgG linked to Sepharose 4B. None of these samples had demonstrable titer to rabbit IgG after extensive absorptions. These negative results may be due to in vivo absorptions and removal of the RF of interest by reactive autoantigens.

Usage of Multiple $V_\mu$ Genes by IgM RRF. The $V$ gene utilization of the IgM RRF was determined from sequence analyses of the cDNA encoding the $V_\mu$ and $V_\lambda$ portions of these Ab. One group, LBR150 and LBR271, had identical $V_\mu$ and $V_\lambda$ sequences. They were also grouped together in
the preceding section by virtue of similar Ag reactivity. The Vᵦ-encoded regions of a second group, LBR295, LBR962, LBR1045, and LBR1189, were also identical, as were their Vᵦ regions. Thus, these latter four RF were related, and the B cells secreting them were the progeny of a single progenitor B cell. They also exhibited essentially identical binding specificities as described above.

Based on published data on human Vᵦ genes (30, 31), the germline counterparts of the Vᵦ segments utilized by the IgMₐ RF have been identified. The cDNA sequences encoding the Vᵦ segments of the RF have been submitted to the GenBank. Only the Vᵦ cDNA sequence of RF LBR295, which is highly mutated, is shown.

The Vᵦ-encoded portions of LBR150 and LBR271 were 100% homologous with that of the Vᵦ₃ germline dp38 (30). This gene is identical to the gene designated as 3-15 by Matsuda et al. (31). However, to date, the GenBank database contains no germline gene with a leader sequence identical to those of LBR150 and LBR271. The D and Jᵦ regions of the LBR150 H chain were classified as D4 (32) and J₄, respectively.

The Vᵦ sequence of LBR295 was homologous to the Vᵦ₁ germline hsvi41b which was originally designated as 1-4b by Shin et al. (33). It had a leader sequence identical to that of the germline gene (Fig. 4). The Vᵦ region of hsvi41b is most homologous to dp21 (30) with the exception of two substitutions in FR3. They are likely to be allelic genes. Thus dp21/1-4b is likely to be the germline for LBR295 Vᵦ. The Vᵦ region of LBR295 contained a DN₄ segment (34) and a J₄ region with a silent nucleotide substitution. There were 15 nucleotide substitutions that resulted in 13 amino acid substitutions (mostly in CDR1 and FR3).

The leader and Vᵦ sequences of LBR1104 were identical to those of hswgh16g (35). This gene has been designated as 3-12 by Matsuda et al. (31). The Vᵦ of LBR1104 was also completely identical to dp77 (30). Both DN₁ (34) and D21/10 (32) segments were identified in its CDR3. It used a J₄ segment.

The 5' untranslated and leader sequences of PHB1213 were highly homologous to those of the germline gene humhv1051 (36). A single mutation was identified at the 5' untranslated region. The Vᵦ segment of PHB1213 was homologous to that encoded by the germline genes humhv1051 and hsigdp10 except for a single mutation in FR3. It appears that the Vᵦ of PHB1213 was slightly mutated. It contained a DXP₁ region (34) and a J₆ region. The Vᵦ segment of this RF was homologous to the fetal Vᵦ cDNA 5P₁ (37) which is completely homologous to the coding regions of humhv1051 and dp10.

The leader sequence of the H chain of KES643 was identical to that of hsigv79 (38), a germline gene homologous with two other Vᵦ₄ germline genes, dp70 (30) and hsvh419 (39). The Vᵦ germline gene hsigv79 has been designated as 4-4b by Shin et al. (33). It appears that hsigv79/4-4b and dp70/hsvh419 are allelic genes and that the Vᵦ of KES643 is in germline configuration. KES643 also contained a DN₁ (34) segment and a J₄ segment.

Figure 4. Identical Vᵦ regions are utilized by LBR295 (highv295), LBR962 (highv962), LBR104 (highv104), and LBR1189 (highv1189). They are derived from the germline hsvi41b/hsigdp21 which is a member of the Vᵦ₁ family. Highv1295 is shown here (4). The leader and CDR are assigned and marked by a solid line, Jᵦ is in bold, and D is denoted by asterisks. The intron of hsvi41b has been deleted. (4) The position of the deleted intron of the germline. The deduced amino acid sequences are included (8). The identity between the leader sequences of the germline and the cDNA of these Vᵦ indicates that the assignment is likely to be correct. There are 15 substitutions in the Vᵦ, which result in 13 amino acid substitutions. Most substitutions are in CDR1 and CDR3. The sequence data of highv295 are available from EMBL/GenBank/DDBJ under accession number L29117.

The Vᵦ, D, and Jᵦ region usage of the nine IgMₐ RF is summarized in Table 3.

Preferential Usage of VᵦIIIb Genes by IgMₐ Anti-Rabbit IgG RF. The V regions of the λ chains of the nine IgMₐ RF serologically classified as VᵦIIIb were sequenced in the cDNA level (Figs. 5–8). LBR150 and LBR271 contained identical 5' untranslated, leader, Vᵦ, and Jᵦ gene segments. PHB1213 differed only in that nine additional nucleotides (TATCCAGGG) were present at the 3' end of the CDR3 segments (Fig. 5). With this exception, these three λ chains were most likely derived from a single Vᵦ gene. The cDNA sequences of the λ chains of LBR295, LBR962, LBR1045, and LBR1189 shared identical untranslated, leader, CDR3, and Jᵦ gene segments (Fig. 6). They differed from one another by zero to three nucleotide substitutions and were also seemingly the product of the same germline gene, as was KES643, which had untranslated and leader sequences identical to those of the LBR295 gene and remarkably homologous framework regions (FR) and CDR. The J segment of KES643 was J₄/2/3 (Figs. 6 and 7). The Vᵦ cDNA sequence of LBR1104 had completely different untranslated and leader sequences from those of the LBR150 and LBR295 groups but also contained a J₄/2/3 segment.

The L chain sequences of the Vᵦ-encoded portion of all
Table 3. Five Vα Genes Are Used by Nine IgMA RF. Eight of Them Use Jα4

| RF      | Vα designation | Vα family | Corresponding germline gene | Distance of Vα from Jα | D | Jα |
|---------|----------------|-----------|-----------------------------|------------------------|---|---|
| LBR 295 | hsigvl295      | 1         | dp21*/1-4b*                 | kβ                     |   |   |
| LBR 926 | hsigvl962      |           | "                           | 155†                   | D4†| 4 |
| LBR 1045 | hsigvl1045    |           | "                           | "                      |   |   |
| LBR 1189 | hsigvl1189    |           | "                           | "                      |   |   |
| LBR 150 | hsigvl150      | 3         | dp38*/3-15f                 | 280f                   | D4†| 4 |
| LBR 271 | hsigvl271      |           | "                           | "                      |   |   |
| LBR 1104 | hsigvl1104    | 3         | dp77*/3-21†                 | 360†                   | D4†| 4 |
| PHB 1213 | hsigvp1213    | 1         | dp10 (51P1)*/hv1051†        | >800†                  | D21/10†| 6 |
| KES 643 | hsigvk643      | 4         | dp70*/4-46††                | 145†                   | DN15| 4 |

* Tomlinson et al. (30).
† Matsuda et al. (31).
‡ Ichihara et al. (34).
§ Buluwela et al. (32).
¶ Yang et al. (36).
** Shin et al. (33).

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Figure 5. LBR150 (hsigvl150) and LBR271 (hsigvl271, not shown) use identical untranslated, leader, Vα, and Jα1 gene segments. PHB1213 (hsigvp1213) differs from this sequence only in the addition of nine extra nucleotides in CDR3. These cDNA use the same unmutated germline gene hsiggl150. The leader and CDR are assigned and marked by a solid line. The deletion of the intron. The cDNA, ch4Vα from Wong et al. (14) also uses this Vα germline but is Jα2/3. The differing Jα has been deleted for our comparison. Accession numbers: L29165 for hsiggl150, L29153 for hsigvl150, and L29163 for hsigvp1213.

nine IgMA RF, as deduced from cDNA analyses, were >90% homologous with the serologically and chemically defined sub-subgroup of αIII proteins designated αIIIb by Eulitz et al. (15). In contrast, these L chains were <80% homologous to proteins classified as αIIIa or αIIIc. These findings, in general agreement with the serological classification, indicated that the L chains of these anti-rabbit IgM RF were encoded by VαIIIb-related genes.

Identification of Two VαIIIb Germline Genes. The germline gene counterparts of the Vα cDNA sequences of the anti-rabbit IgG RF were not identified in the database of the GenBank. We used the method of Sota-Gil et al. (28) to generate the germline genes encoding these αIII chains by PCR from the genomic DNA of patient LBR. Two of the three germline genes, designated hsiggl150 and hsiggl295, were identified. As indicated in Fig. 5, LBR150, PHB1213, and LBR271 (not shown) represented the unmutated products of hsiggl150, whereas three substitutions have been found in the FR of the L chain (ch4Vα) in mAb H4, another anti-rabbit IgG IgMα RF. In the case of hsiggl150, we have also identified an identical germline, by PCR, in the genome of two unrelated normal individuals. In contrast to the LBR150 group, the cDNAs of LBR295, LBR962, LBR1045, and LBR1189, when compared with their
Figure 7. The V\(\alpha\) cDNA sequence of KES643 (higgl\(\kappa\)443) is homologous to germline higgl\(\kappa\)295 with two substitutions in CDR2, one in FR2, and three in CDR3. The leader and CDR are assigned and marked by a solid line. J\(\alpha\) is in bold. (1) The deletion of the germline intron. (2) The three substitutions that are identical to those found in the cDNAs of LBR295, LBR962, LBR1045, and LBR1189. Accession number: L29164 for higgl\(\kappa\)443.

Discussion

A large number of IgM RF specific for the Fc region of rabbit IgG have been obtained through generation of multiple EBV-transformed B cell lines and hybridomas established from PB and synovial tissue of patients with RA and related disorders. These anti-rabbit IgG RF had relatively high binding affinity constants \((10^{-6} \text{ to } 10^{-7} \text{ M})\) and contained predominantly \(\lambda\) L chains. The nonreactivity of these Ab line gene higgl\(\lambda\)11 (\(\lambda\)III.1) (40) which show the greatest homology with the L chain of LBR1104 among the L chain germine sequences currently available in the GenBank.

The \(V\alpha\) and J\(\alpha\) region usage of the nine IgMAbRF is summarized in Table 4.
Table 4. Three V_{IIIb} Genes Are Used by Nine IgM RF. Both J_1 and J_{2/3} Are Used

| RF  | V_{\alpha} designation | V_{\alpha} sub-family | Corresponding germline gene | J_{\alpha} |
|-----|------------------------|-----------------------|-----------------------------|-----------|
| LBR 295 | hsigvl295 | IIIb | hsiggl295 Highly mutated | 1 |
| LBR 962 | hsigvl962 | " | " | " |
| LBR 1045 | hsigvl1045 | " | " | " |
| LBR 1089 | hsigvl1189 | " | " | " |
| LBR 150 | hsigvl150 | " | hsiggl1150 Unmutated | 1 |
| LBR 271 | hsigvl271 | " | " | " |
| LBR 1104 | hsigvl1104 | " | hsiggl1104 (Not yet identified) | 2/3 |
| PHB 1213 | hsiglp1213 | " | hsiggl1150 Unmutated | 1 |
| KES 643 | hsiglk643 | " | hsiggl295 Mutated | 2/3 |

with human IgG was established by inhibition studies using heat-aggregated IgG. Despite the lack of reactivity with other Ag often recognized by anti-human IgM RF including IgG from other mammalian species, these RF recognized epitopes associated with cytoskeletal proteins and HeLa cells (Table 2). Additionally, the reactivity with a group A streptococcal cell wall extract of certain anti-rabbit IgG RF is of considerable interest because of the role of streptococcal infections in rheumatic fever and the poststreptococcal autoimmune process related to the production of antistreptococcal Ab cross-reactive with human and mouse tissue (41). We have also found that 21 supernatants of our LBR cell lines derived from synovial tissue were highly reactive with whole streptococcal extracts (Cunningham, M. W., and S. M. Fu, unpublished results). The multireactivity of anti-rabbit IgG RF may have pathogenic importance in that these Ab, which are found in patients with RA and other related diseases, may be induced by a variety of cellular autoantigens, most notably cytoskeletal proteins, and by streptococcal infection. In this regard, it is of interest to note that a higher affinity constant to myosin was obtained in the case of PHB1213. The preferential homing of the B cells related to these RF to the synovium adds support to this thesis.

The five patterns of reactivity noted among the anti-rabbit IgG RF (Table 2) could be related to specific V_{\alpha} or V_{\gamma} gene usage. These five reactivity patterns are due to unique combinations of five V_{\alpha} and three V_{\gamma} genes. Whereas the inciting Ag and its epitopes are not known, this observation suggests that different epitopes select unique V_{\alpha} and V_{\gamma} combinations. This has been demonstrated in a study by Stark and Caton (42). mAb that are specific for a single amino acid interchange in a defined epitope of the influenza virus hemagglutinin use structurally distinct V regions. Thus, the use of diverse V_{\alpha} and V_{\gamma} gene segments by human autoantibodies (recently reviewed in 13, 43–46) is to be expected because diverse epitopes are present in most autoantigens commonly studied. With better defined epitopes, the use of V_{\alpha} and V_{\gamma} by autoantibodies will undoubtedly be more restricted.

Five distinct V_{\alpha} gene segments, including V_{\alpha}1, V_{\alpha}3, and V_{\alpha}4, were identified among our nine anti-rabbit IgG \lambda-type IgM RF (Table 3). Another RF with similar reactivity, H4 (14), contained V_{\alpha}26, a V_{\alpha}3 gene designated as 3-23 (31) and located ~395 kb from the J_{\alpha}. Thus, these V_{\alpha} genes are scattered throughout the V_{\alpha} locus. As stated previously in the Results section, several of these V_{\alpha} genes are encountered in fetal repertoire, and all of them have been utilized by autoantibodies of various specificities. The V_{\alpha}1, V_{\alpha}3, and V_{\alpha}4 families have a large number of genes (30, 31), and members of these families are well presented in the repertoire of human autoantibodies and fetal repertoire (13, 43–46). Recently, we have identified a V_{\alpha}2 RF (47) even though V_{\alpha}2 is a small family. Thus, our data add support to the conclusions that multiple V_{\alpha} genes are used by RF and other autoantibodies and that the V_{\alpha} gene representation in autoantibodies reflects the normal human B cell repertoire (45).

Serologically, 10 of the 13 \lambda L chains of the anti-rabbit IgG RF were typed as members of the \lambdaIIIb sub-subgroup. Two others, serologically typed to be \lambdaIIIa/b, were classified to be \lambdaIIb by amino acid sequence (deduced from their cDNA sequences) analysis. The overwhelming presence of \lambdaIIIb L chains among these Ab is in marked contrast to the
The preferential usage of V\textsubscript{\textalphaIIb}-related genes by these RF was confirmed through cDNA sequence analyses of nine such Ab. This remarkable restriction in V\textsubscript{\textalpha} gene utilization by anti-rabbit IgG RF extends the observation of Wong et al. (14), who found that H4, a RF monospecific for rabbit IgG, also contained \textalphaIIb L chains. These data provide the first evidence that particular L chain genes, as a result of antigenic stimulus or genetic factors, are selectively utilized by certain types of RF.

Our studies have also provided new information on the human \textlambda-L chain genome. Two heretofore undescribed V\textsubscript{\textalphaIIb}-related germline genes were identified that encoded the L chain V gene segments of certain anti-rabbit IgG RF. Based on cDNA sequence analyses of our nine RF, this gene family consists of at least three different members. Remarkably, two of these three were used by RF derived from two different individuals. Based on nucleotide sequence homology, the \textalphaIIb L chains of the anti-rabbit IgG RF H4 (14) were most closely related to those of LBR150 and, thus, were also products of the germline hsigg\textsubscript{1150} gene. Ermel et al. (49) have recently reported that two other synovial cell-derived anti-rabbit IgG RF from another RF patient contained \textalphaIIb L chains identical to those of RF H4 (14). The predominance of \textalphaIIb L chains among RF with comparable specificity, especially those that are related to the hsigg\textsubscript{1150} V\textsubscript{\textalphaIIb} germline gene, implies the functional importance of this gene in certain patients with RA and related diseases.

The demonstration that the V\textalpha or V\textbeta portions of certain RF are derived from unmutated germline genes and that others have extensive somatic mutations (13) is of pathogenic importance. Among our anti-rabbit IgG RF, four (LBR295, LBR962, LBR1045, and LBR1189) had extensive FR and CDR mutations as compared with their germline counterparts. These RF are among the multireactive Ab with the most mutated V regions. It is also of considerable interest that the L chain of KES643 was derived from a germline similar to that of LBR 295 with identical nucleotide substitutions at three identical positions in CDR2 and FR3. These identical substitutions were unlikely to be attributable to a random event and were likely under selective pressure, reflecting an Ag-driven process. Thus, multireactive Ab should not be excluded from the repertoire generated by an Ag-driven process.

In summary, we have demonstrated that the L chains of IgM Ab with exclusive specificity for rabbit IgG, were predominantly products of the relatively uncommon V\textalphaIIb-gene family. Additionally, some of these RF reacted with a variety of proteins, cellular, and bacterial Ag. The findings of selective L chain gene usage and the multireactivity of such Ab provide new information on the molecular properties of RF and further insight into the etiology of RA and other autoimmune diseases.

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