Expression of the Immunoglobulin V_H Gene 51p1 Is Proportional to Its Germline Gene Copy Number

Eric H. Sasso,* Todd Johnson,‡ and Thomas J. Kipps‡

**Division of Rheumatology, University of Washington, Seattle, Washington 98105; and ‡Department of Medicine, University of California at San Diego, La Jolla, California 92093-0663*

Abstract

51p1 is an immunoglobulin V_H gene that is frequently expressed in B cell chronic lymphocytic leukemia and early in B cell ontogeny. The 51p1 gene locus is highly polymorphic, consisting of 13 alleles that can be classified as being either 51p1-related or hv1263-related, based on distinctive sequence motifs in the second complementarity determining region. Two of the 51p1-related genes usually occur as a linked pair on the same haplotype, resulting from gene duplication. Consequently, a person can have a total of zero to four copies of 51p1-related genes. These genes are detectable in genomic DNA by sequence-specific RFLP analysis using oligonucleotide probes. Ig encoded by nonmutated 51p1-related genes can be detected by G6, a murine antiidiotypic mAb. We have now studied lymphocytes from 35 human tonsils to examine the relation between the number of 51p1-related germline gene copies and the proportion of IgD-bearing tonsillar B cells that react with G6. All subjects who had zero copies of 51p1-related genes lacked any G6-reactive B cells, whereas those with four copies of 51p1-related genes had the highest proportions of G6-positive IgD B cells, up to 11.4%. Subjects with intermediate gene doses had intermediate proportions of G6-reactive B cells. Over the entire data set, the percentage of IgD-bearing B cells that reacted with G6 was proportional to the 51p1-related gene copy number ($r = 0.92$, $P < 0.001$), with each copy accounting for 2.4–4.0% of the IgD-bearing B cells. We conclude that 51p1-related genes are expressed by a relatively large percentage of IgD+ tonsillar B cells and this percentage is proportional to the germline copy number of 51p1-related genes. (*J. Clin. Invest.* 1996. 97:2074–2080.)

Key words: B lymphocytes • gene dosage • gene expression • genes, immunoglobulin V_H germline • RFLP

Introduction

There are approximately 90 V_H segments in the major Ig heavy chain gene complex on chromosome 14, of which about 50 are functional (1, 2). These gene segments rearrange with the D and J_H gene segments to form the V_H-D-J_H exon that ultimately can encode the variable region of the antibody heavy chain. If each functional Ig V_H gene had an equal chance of undergoing rearrangement and expression, then a single allele of a V_H gene should encode ~ 1% of all Ig heavy chains.

Several studies indicate, however, that Ig V_H gene use is not random (3–8). For example, the 51p1 gene of the V_H1 gene family appears to be expressed by a disproportionately high number of adult and fetal B cells (3, 5, 9). Furthermore, 51p1 is a common source of paraprotein IgM rheumatoid factors (RF)[1] (10) and may be the most frequently used gene in human B cell chronic lymphocytic leukemia (CLL) (11–14). Biased use of this or other Ig V_H genes could reflect preferential gene rearrangement and expression and/or somatic selection.

Immunoglobulins encoded by nonmutated 51p1 genes react with G6, a murine antiidiotypic mAb (9, 11, 15). The G6 mAb detects a high proportion of B cells in the primary follicles of human fetal spleen (5), and in the mantle zones of adult tonsil (9), but the exact proportions of B cells that react with G6 vary between individuals. While the mantle zone B cells of some subjects have up to 12% G6-reactive cells, the mantle zones of others have fewer or, in some subjects, no B cells that react with G6 (9). Variation in the G6-reactivity of mantle zone B cells is probably not due to somatic mutation, because mantle zone B lymphocytes are generally IgD+, IgM+ B cells that express nonmutated Ig V_H genes (16–18). IgD+ cells comprise from 37 to 87% of all tonsillar B cells (9).

The wide range in the proportions of B cells that react with G6 may reflect genetic polymorphism at the 51p1 locus. Indeed, the 51p1 locus, also known as 1-69 (2), consists of at least 13 alleles that can be classified as being either 51p1-related or hv1263-related, based on distinctive sequence motifs in the second complementarity determining region (CDR2) (19). The 51p1-related alleles encode the G6 idiotype, whereas it appears the hv1263-related alleles do not (9). In a predominantly Caucasian population, the 51p1-related alleles and the hv1263-related alleles were present in 85 and 58% of subjects, respectively (19). Two of the most prevalent 51p1-related genes, Nos. 1 and 7, nearly always occurred as a linked pair, due to gene duplication. As such, they comprise a two-gene haplotype that is allelic to the other alleles of the 51p1 locus (19). Other two-gene haplotypes of the 51p1 locus were rare, and null alleles were not identified. Thus, all genomes were found to contain a diploid total of two, three, or four genes from the 51p1 allelic set, of which zero to four were 51p1-related and zero to two were hv1263-related (19).

We have now studied the lymphocytes from 35 human tonsils to examine the relation between the number of 51p1-related germline gene copies, and the proportion of IgD-positive mantle zone B cells that react with G6. We find that the percentage of G6-positive B cells is proportional to the germline gene copy number of 51p1-related genes. This study indi-

Address correspondence to Eric H. Sasso, Harborview Medical Center, Box 359785, 325 Ninth Avenue, Seattle, WA 98104. Phone: 206-731-5988; FAX: 206-731-8787.

Received for publication 14 November 1995 and accepted in revised form 6 March 1996.

J. Clin. Invest.

0021-9738/96/05/2074/07 $2.00
Volume 97, Number 9, May 1996, 2074–2080

1. *Abbreviations used in this paper:* CDR, complementarity determining region; CLL, chronic lymphocytic leukemia; RF, rheumatoid factor.

cates that V_H gene copy number can play a major role in shaping the human IgD-positive B cell repertoire.

Methods

Patient materials. All lymphoid study material was obtained from surgical tonsillectomy specimens. Some donors have been previously described elsewhere (9). Most were young adults with acute or chronic tonsillitis. Handling of specimens and preparation of mononuclear cells by Ficoll-Hypaque gradient centrifugation were as described (9). The genomic DNA control sample is described elsewhere, as Virginia Mason Research Center subject #3116 (19).

Antibodies and fluorescence probes. G6, a murine IgG1 mAb reactive with an autoantibody heavy chain cross-reactive idiotype (15), was provided by Drs. Rizgar Mageed and Roy Jefferis (University of Birmingham, Birmingham, England). IgD was detected with Ab dTA4-1, an IgG3 anti–human delta heavy chain (20, 21). Antibodies were prepared for use in flow cytometric analysis by previously described methods (9).

Flow cytometric analysis. Three- and four-color flow cytometric analyses were performed using a FACScan® (Becton, Dickinson & Co., San Jose, CA) equipped with a Consort30® (Hewlett-Packard Co., Palo Alto, CA) and a MicroVAX® computer (Digital Equipment Corp., Marlboro, MA), as described (20). Previously described methods were used to label cells with flourochrome-conjugated mAbs and to assess cell viability, which generally exceeded 90% (9).

Preparation of gels. Aliquots of genomic DNA, prepared from unfractionated tonsillar mononuclear cells of experimental subjects, or peripheral blood leukocytes of the control subject, were digested with TaqI, purified with phenol, choroform, and ethanol precipitation, then size separated on 1% agarose gels, 12 μg per lane. Methods are described in detail elsewhere (22).

Oligonucleotide probes. Gels were hybridized with 21-mer deoxyoligonucleotide probes H111 and M27 which target 5′ CDR2 and mid-CDR2 of the 51p1 sequence, respectively, and M28, which targets mid-CDR2 of the hv1263 sequence (19). Probes were synthesized at the Biopolymer Synthesis Laboratory of the Howard Hughes Medical Institute, University of Washington, Seattle, WA and were the generous gift of Dr. Eric Milner, Virginia Mason Research Center, Seattle, WA.

In situ genomic hybridization analysis with oligonucleotide probes. Dried down agarose gels were hybridized overnight to ^{32}P-end-labeled oligonucleotide probes by previously described methods (22). Hybridization temperatures were 52 to 57°C for all probes. The final postwashing step, in tetramethylammonium chloride, was performed at 57°C. This step confers absolute specificity to each probe for its target sequence, except for terminal or next-to-terminal nucleotide mismatches, which occur infrequently among the 51p1 alleles. Under these conditions, all alleles of the 51p1 locus are detectable (19). As described, gels were denatured and neutralized before each reuse (22).

Characterization of hybridization bands. Hybridization bands were characterized according to their TaqI restriction fragment size, and the detection profile with probes M27, H111, and M28 (19). Bands detected by probe M27 were designated 51p1-related. Bands detected by probe M28 were designated hv1263-related.

Results

Frequency of G6-positive tonsillar B lymphocytes. Tonsillar lymphocytes from 35 unrelated subjects were studied by flow cytometric analysis to identify IgD-positive B cells and to determine the frequency of B cells expressing the G6 idiotype. Nearly all cells detected with G6 were also IgD-positive, consistent with previous reports (Fig. 1) (9). In 29 of the subjects, G6 reactivity was detected in 2.9–11.4% of the IgD-positive B cells. In the other six subjects, no G6 reactivity was detected, even though comparable numbers of IgD-positive cells were present (Fig. 1, Table I).

Genotypes of the 51p1 locus. The genotype of the 51p1 locus in each of the 35 subjects was determined by hybridization of TaqI-digested genomic DNA with oligonucleotide probes to CDR2 of 51p1 (probes M27 and H111) and hv1263 (probe M28) (Fig. 2). Probes M27 and M28 target mutually exclusive sites in 51p1 and hv1263, respectively, and thereby define the genes they detect as either 51p1-related or hv1263-related (19). Probe H111 detects the same genes as probe M27, except for rare sequence variants that are considered 51p1-related when M27+, H111−, and M28− and hv1263-related when M27−, H111+, and M28+. Fig. 3 shows a hybridization result with probe H111, demonstrating genomes that contain 1, 2, 3, or 4 copies of 51p1-related genes.

In 30 of 35 subjects, exactly two haplotypes from the 51p1 locus were detected, if the 51p1-related 7.5- and 4.0-kb TaqI fragments (i.e., genes Nos. 1 and 7) are assumed to occupy the same chromosome 14 (Table I). Four of the remaining subjects (E1, V1, H1, and C2, Table I) had combinations of three or four 51p1 locus genes that indicated the existence of rare duplications, distinct from the 7.5/4.0-kb pair. The genome of the

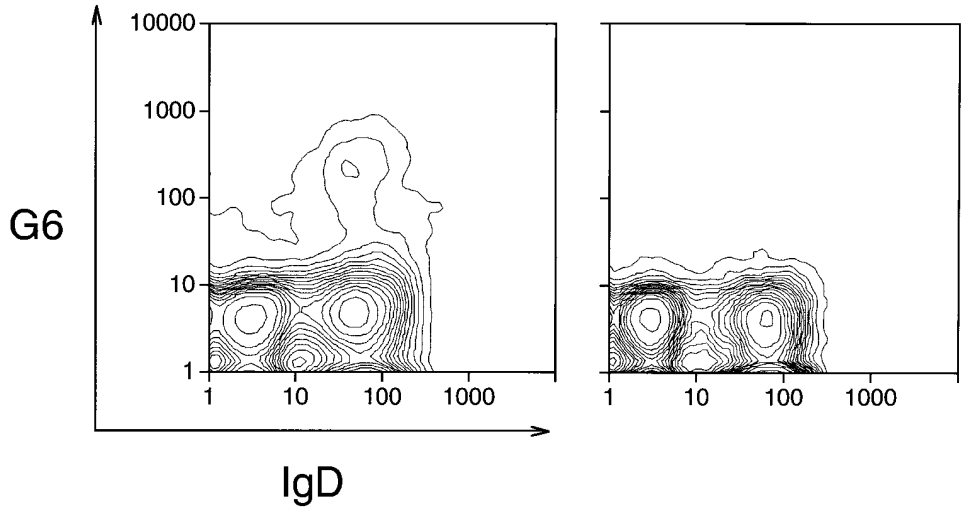

Figure 1. FACScan immunofluorescence analyses of tonsillar lymphocytes from a G6-positive subject (*left*, subject C3) and a representative G6-negative subject (*right*). Lymphocytes from each subject were labeled simultaneously, with PE-conjugated G6 and FITC-conjugated anti-IgD. Numbers on axes correspond to the relative red (*ordinate*) or green (*abscissa*) fluorescence intensity of the cells when excited at 488 nm. Contour lines are drawn with a 6% probability distribution.

Table I. Expression of G6 Idiotype by Human Tonsillar IgD+ B Lymphocytes and Germline Alleles of the 51p1 Locus in 35 Subjects

		51p1-related alleles‡		hv1263-related alleles§	
Subject	Percent G6+*	No.‖	RFLP¶	No.‖	RFLP¶
			kb		*kb*
B2	0	0	—	2	6.5, 4.0
B4	0	0	—	2	3.9, 3.9
E1	0	0	—	3	6.5, 4.0, 4.0
M2	0	0	—	2	6.5, 3.9
M3	0	0	—	2	6.5, 3.9
M5	0	0	—	2	4.0, 3.9
M4	2.9	1	4.3	1	4.0
H2	3.4	1	4.3	1	4.0
G1	3.6	1	4.3	1	4.0
B1	3.7	1	4.3	1	4.3
F1	3.7	1	4.3	1	4.3
M7	3.8	1	4.3	1	4.0
K1	3.9	1	4.3	1	4.0
M6	4.1	1	4.3	1	6.5
V1	4.3	1	4.3	2	6.5, 4.3
P1	5.1	1	7.1	1	3.9
R1	5.4	1	4.3	1	3.9
W3	5.6	2	7.5, 4.0	0	—
C1	6.3	2	7.5, 4.0	1	4.0
B7	6.4	2	7.5, 4.0	1	6.5
L1	6.7	2	4.3, 4.3	0	—
W2	7.1	2	7.5, 4.3	0	—
A1	7.4	2	4.3, 4.3	0	—
O1	7.4	2	4.3, 4.3	0	—
B5	7.7	2	4.3, 4.3	0	—
H1	5.2	3	6.9, 6.5, 4.0	1	4.2
J1	7.3	3	7.5, 4.3, 4.0	0	—
L3	7.8	3	7.5, 4.3, 4.0	0	—
B3	8.4	3	7.5, 4.3, 4.0	0	—
C2	8.4	3	7.5, 6.5, 4.0	1	4.2
P2	8.9	3	7.5, 4.3, 4.0	0	—
W4	9.3	3	7.5, 4.3, 4.0	0	—
L2	7.8	4	7.5, 7.5, 4.0, 4.0	0	—
W1	9.3	4	7.5, 7.5, 4.0, 4.0	0	—
C3	11.4	4	7.5, 7.5, 4.0, 4.0	0	—

*Percentage of IgD-positive tonsillar lymophocytes detected by fluorescence cytometry with G6 antiidiotype Ab. ‡Germline genes detected with probe M27. All were also detected by probe H111. 51p1-related genes at 7.1 kb (P1) and at 6.9 kb (H1) have not been previously reported (19). §Germline genes detected with probe M28. All were negative with probe H111, except the 6.5-kb fragments in subjects E1, Vl, and B7. The hv 1263-related genes at 6.5 kb (several) and at 4.2 kb (H1, C2) have not been previously reported (19). ‖Total germline copy numbers of 51p1-related genes (column 3) or hv1263-related genes (column 5). ¶TaqI restriction fragment sizes, in kb, of genes enumerated in preceding column.

fifth remaining subject, W3, is noteworthy because either the 51p-related genes on its 7.5- and 4.0-kb TaqI fragments occupy opposite haplotypes, or it has a 51p1 locus null allele. Both explanations invoke a rare event but do not change the tally of two 51p1-related genes in W3 (Table I). Consistent with previous evidence that null alleles are rare or nonexistent at the 51p1 locus (19), none of the other 34 subjects in this study appeared to have a 51p1 locus null allele.

Gene dose of 51p1-related alleles. 51p1-related alleles were detected with probes M27 and H111 in 29 subjects (82%), of whom 11 had one, 8 had two, 7 had three, and 3 had four copies of 51p1-related genes (Table I). In seven of these subjects (L1, A1, B5, O1, L2, W1, and C3), homozygosity for a 51p1-related gene was presumed, based in each case on the absence of any hv1263-related genes and the increased intensity of the M27/H111-positive hybridization band(s) (e.g., Fig. 3, lane *6*). All other 51p1-related genes were counted as one gene copy per hybridization band (40 hybridization bands, each detected by probe M27 and probe H111). In each of the six subjects having no 51p1-related genes, two or more hv1263-related genes were detected, consistent with a previous study (Table I) (19).

The percentage of G6-reactive B cells correlates with the copy number of 51p1-related genes. A comparison of the frequency of G6 reactivity among IgD-positive B lymphocytes and the copy number of 51p1-related germline genes revealed a strong linear correlation ($r = 0.92$, $P < 0.001$) (Fig. 4). Absence of G6-positive, IgD-positive cells occurred in all subjects with no 51p1-related germline genes, and no others (Fig. 4, Table I). The highest levels of G6-reactive cells occurred in the subjects with four 51p1-related germline genes, the maximum possible dose. Intermediate values of 51p1-related gene dose also correlated with frequency of G6 reactivity, although some overlap was seen. The mean values of percent G6-reactive cells per 51p1-related gene copy ranged from 4.0%, for subjects with one gene copy, to 2.4%, for subjects with four gene copies (Table II).

Discussion

In this study, we demonstrate that the frequency of IgD-positive tonsillar B lymphocytes expressing the G6 idiotype is proportional to the number of 51p1-related gene copies in the germline. The finding is particularly striking in view of the fact that the study population was composed of 35 unrelated subjects who have different Ig V_H repertoires and different histories of immune development. This result suggests that, on average, such interindividual differences have relatively little total effect on the measured levels of Ig V_H gene expression. Rather, the copy number of 51p1-related germline genes appears to be the major determinant of how many IgD-positive B cells will express the G6 idiotype.

An important assumption underlying this study is that the G6 idiotype is encoded by all prevalent 51p1-related genes, and only by 51p1-related genes. Several lines of evidence, from past reports and the present data, support this conclusion. Previously, we noted that the expressed V_H sequences obtained from IgD-positive/G6-positive tonsillar B cells of one subject shared 99.4–100% homology to 51p1 (12 clones) or to the sequence of 51p1 variant No. 7 (2 clones) (9). No transcripts from non-51p1 loci were found. The germline repertoire of that subject (Fig. 3, lane *2*; Table I, L3) contained M27+ TaqI fragments at 7.5, 4.3, and 4.0 kb, which represent the most prevalent 51p1-related genes, Nos. 1, 5, and 7, respectively (19). Genes No. 1 and No. 5 have exact 51p1 sequences, and gene No. 7 differs by one nucleotide in framework region 3 (19).

CODON		2	6	31 (CDR1)					50												60	73
Gene	51p1	GTG	CAG	AGC	TAT	GCT	ATC	AGC	GGG	ATC	ATC	CCT	ATC	TTT	GGT	ACA	GCA	AAC	TAC	GCA		GAA
Gene	hv1263	--C	---	---	---	---	---	---	A--	---	---	---	---	C--	---	-T-	---	---	---	---		A--
Probe	H111								---	---	---	---	---	---	---							
Probe	M27													---	---	---	---	---	---	---		
Probe	M28													C--	---	-T-	---	---	---	---		
Allele	RFLP																					
# 1	M27 7.5	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		---
# 2	M27 6.5	--C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		---
# 5	M27 4.3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		---
# 6	M27 4.3	--C	---	---	---	---	---	---	A--	---	---	---	---	C--	---	---	---	---	---	---		---
# 7	M27 4.0	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		A--
# 8	M27 4.0	--C	--A	---	---	A--	---	---	A--	---	---	---	---	C--	---	---	---	---	---	---		A--
#11	M27 3.9	--C	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---		---
# 3	M28 5.8	--C	---	---	---	---	---	---	A--	---	---	---	---	C--	---	-T-	---	---	---	---		A--
# 4	M28 4.3	---	---	---	---	---	---	---	A--	---	---	---	---	C--	---	-T-	---	---	---	---		A--
# 9	M28 4.0	--C	---	---	---	---	---	---	A--	---	---	---	---	C--	---	-T-	---	---	---	---		A--
#10	M28 4.0	--C	--A	---	---	A--	---	---	A--	---	---	---	---	C--	---	-T-	---	---	---	---		A--
#12	M28 3.9	--C	---	---	---	---	---	---	---	---	---	---	---	C--	---	-T-	---	---	---	---		A--
#13	M28 3.9	--C	--A	---	---	A--	---	---	A--	---	---	---	---	C--	---	-T-	---	---	---	---		A--

Figure 2. Oligonucleotide probes and alleles of the 51p1 locus. Nucleotide sequences of genes 51p1 (3) and hv1263 (23) are depicted in the regions that distinguish the alleles of the 51p1 locus. Below, are sequences of oligonucleotide probes H111, M27, and M28, and the abbreviated sequences of 13 alleles of the 51p1 locus. Each of these sequences is designated by its previously assigned number, #1–13 (19), and by its RFLP, which is identified by the detecting probe (M27 or M28) and the TaqI restriction fragment size, in kb. The 51p1-related alleles, detected by M27, are grouped above, the hv1263-related alleles, detected by M28, are grouped below. Nucleotides of hv1263, of all probes, and of all alleles are specified only where different from gene 51p1. In regions not shown, sequences of hv1263 and of all alleles are identical to 51p1, except allele No. 12 at codon 17 (19). Codon numbering and location of CDR1 and CDR2 (shown only in part) are according to Kabat et al. (24).

Thus, the hybridization and sequence data establish that G6-positive Ig are encoded by the germline configurations of 51p1-related gene No. 7, and probably both of genes No. 1 and No. 5 (9, 15). They also indicate that genes outside the 51p1 locus rarely, if ever, encode G6-positive Ig in subject L3.

The conclusion that G6 is encoded only by 51p1-related alleles is also supported by the previous finding that two G6-reactive CLL B cells expressed V_H sequences that were identical to 51p1, except for a terminal-codon nucleotide in each (11). Moreover, G6-bearing IgM paraproteins, Bor, Kas (25), and RF-TS1 (26), each have protein sequences that appear to be encoded by a 51p1-related gene.

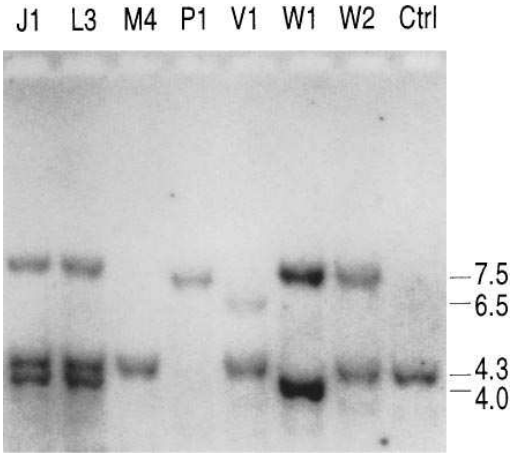

Figure 3. Detection of 51p1-related germline genes. DNA samples extracted from tonsillar lymphocytes of seven experimental subjects (J1, L3, M4, P1, V1, W1, W2, lanes *1–7*, respectively) and from peripheral blood leukocytes of a control subject (lane *8*) were digested with TaqI and separated on a 1% agarose gel. The gel was dried down and prepared for hybridization. The hybridization result, with probe H111, shows experimental subjects with one (lanes *3–5*), two (lane *7*), three (lanes *1* and *2*), or four (lane *6*) copies of 51p1-related germline genes. The upper band in lane *5* (6.5 kb), was M27−/M28+ and is therefore hv1263-related, rather than 51p1-related. TMACl postwash temperature was 57°C. The film was exposed for 11 d. Molecular weight sizes appear to the right, in kb.

In the present study, no G6-positive cells were detected among IgD-positive tonsillar B cells of subjects having no 51p1-related genes. This finding indicates that the G6 idiotype is not encoded by hv1263-related genes, which were present in all six of these subjects. It is possible that somatic substitutions might occasionally generate a G6-positive Ig from a non–51p1-related gene (27, 28). However, such B cells should not have affected this study because G6 analysis was restricted to IgD-positive cells, whose expressed Ig V genes generally have not incurred somatic mutations (16–18).

Our data provide evidence that, in addition to 51p1-related gene No. 5, and the duplication pair of genes 1 and 7, G6 appeared to be encoded by some of the less prevalent 51p1-related alleles, a novel M27+ gene on a 7.1-kb restriction fragment (subject P1), and gene 2, on a 6.5-kb fragment (subject C2, and perhaps H1) (19). Although gene 5 (on the 4.3-kb band) was the most prevalent, the data were not dominated by its expression alone, because comparable levels of G6 expression were seen in many subjects who had other 51p1-related alleles (Table I). Thus, among IgD-positive B cells, G6-positive Ig derive from many of the 51p1-related genes, and the G6 idiotype is a specific marker for expression of these genes. Consequently, the data demonstrate that the expression of 51p1-related genes in the IgD+ B cell repertoire is proportional to their copy number in the germline.

Although these data are the first to directly demonstrate that germline copy number has an important influence on the frequency of human Ig V_H gene expression, suggestive evidence exists in other reports. The finding by Stewart et al., that the VH26 gene is expressed, on average, two to three times as frequently as the 56p1 gene is consistent with the usual copy numbers of VH26 and 56p1 in the germline (6, 22, 29). A study of blood B cells by Suzuki et al. showed that the frequencies of cells carrying rearrangements of eight V_H3 genes were relatively constant over time in the same individual but differed between subjects by increments consistent with their different germline repertoires (8). Thus, it might be generally true that a given Ig V_H gene is expressed by IgD+ B cells at a frequency directly proportional to its germline copy number. Further studies are needed to confirm this hypothesis.

The incremental contribution of an Ig V_H gene to the IgD+

repertoire, per gene copy, is probably an intrinsic property that differs from one V_H gene to the next but is relatively constant for a given V_H gene in different individuals. Several V_H genes, such as VH26, 56p1, and V_H 4.21, are said to be "overexpressed" because their expression repeatedly exceeds the predicted level for random V_H gene use: about 1% per allele, or 2% per diploid gene pair (3–8). In most studies of Ig V_H gene use, interpretation of the level of expression is limited by not knowing the copy number of the corresponding germline V_H gene. Furthermore, studies of Ig V_H gene expression usually assessed the expression of single V_H genes relative to that of other members of the same V_H gene family. Such analysis does not measure the frequency of gene use in the entire Ig V_H repertoire. In the case of 51p1, our data indicate that when a single gene copy is present, it contributes $\sim$ 3–5% of the expressed repertoire of IgD-positive B cells, and that with four copies of 51p1-related germline genes, over 11% is possible. As there are $\sim$ 100 functional V_H genes in the human diploid genome, these data indicate that the frequency of rearrangement and/or expression of 51p1-related genes is relatively high.

The calculated increments of G6 expression by 51p1-related alleles depend somewhat upon whether the data are interpreted linearly or nonlinearly. A ratio of $\sim$ 3% G6-reactive B cells per gene copy is obtained from the linear regression analysis of our data (Fig. 4). Alternatively, the mean G6 frequency declined from 4.0% per gene copy, for subjects with one 51p1-related allele, to 2.4% per gene copy, for subjects with four copies (Table II). This nonlinear trend might be related to the different alleles that predominate in the different copy number categories. Subjects with one 51p1-related gene virtually always had the 4.3-kb band (gene No. 5), whereas

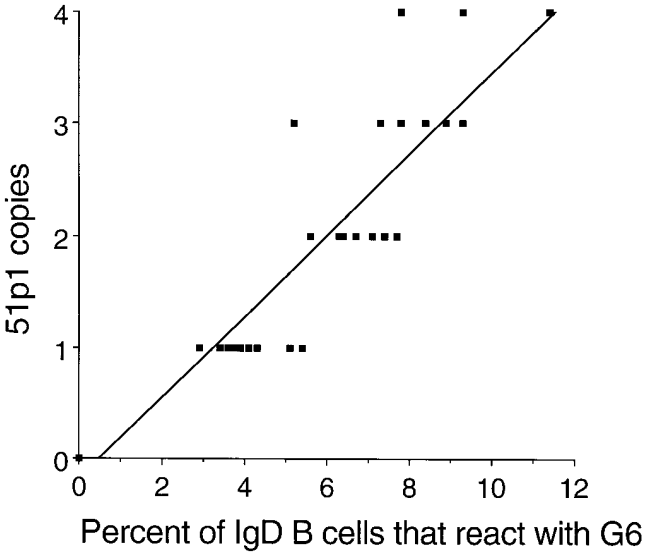

Figure 4. Correlation of gene expression with genotype for the 51p1-related alleles. Each point depicts the percentage of G6-positive cells among IgD-positive tonsillar B lymphocytes (*abscissa*) versus the number of 51p1-related germline genes for the 35 study subjects (*ordinate*). Data are from Table I. In the data clusters for zero, one, and two copies of 51p1-related alleles, the points at 0.0, 3.7, and 7.4%, respectively, represent 6, 2, and 2 subjects. All other points represent one subject. The line ($y = -0.1153 + 0.3469\,x$) was calculated from the entire data set by linear regression analysis; $r = 0.92$ ($P < 0.001$).

*Table II. Frequency of G6 Idiotype Expression per 51p1-related Gene Copy**

n	Copy number of 51p1-related genes	Percent G6-reactive IgD+ B cells	
		Mean (range)	per gene copy‡
6	0	0.0 (all 0.0)	—
11	1	4.0 (2.9–5.4)	4.0
8	2	6.8 (5.6–7.7)	3.4
7	3	7.9 (5.2–9.3)	2.6
3	4	9.5 (7.8–11.4)	2.4

*Derived from data in Table I. ‡Calculated as mean percent G6+ (column 3) divided by copy number of 51p1-related genes (column 2).

subjects with a total of four copies were always homozygous for the 7.5/4.0 kb pair (genes Nos. 1 and 7). Subjects with two or three copies had mixtures of these variants and, occasionally, the rarer 51p1-related genes (Table I). We found that the percentage of G6-positive B cells was lower in the 2-gene-copy subjects who had the 7.5/4.0 kb pair (5.6–6.4%) than those having two copies of the 4.3-kb gene (6.7–7.7%). The data therefore suggest that the genes of the 7.5/4.0 kb pair result in a lower measured value of percent G6 positivity per gene copy than the other 51p1-related alleles. This effect might be due to the fact that the gene duplication the 7.5/4.0 kb pair represents is part of an 80-kb multigene chromosomal insertion (30, 31). For example, a haplotype that has two copies of another V_H gene, 1.9III, is the product of a 50-kb insertion containing five additional functional V_H genes (32). In genomes containing this sort of insertion, the repertoire of functional Ig V_H germline genes is enlarged, and the relative contribution from any single V_H gene could be reduced.

However, another reason that the ratio of the percentage of G6-positive cells to the number of 51p1-related genes declined, as the gene copy number increased, is that different 51p1-related alleles might have different propensities to be expressed in the mantle zone B cell repertoire. If so, the 4.3-kb 51p1-related gene may have a slightly greater propensity to be expressed than the 7.5- and/or 4.0-kb genes. In addition, unexpectedly low G6 expression occurred with two subjects, H1 and L2. They appear as outliers in their respective 3-gene-copy and 4-gene-copy groups and might reflect the existence of 51p1-related genes that have poorly functional regulatory regions (Table I and Fig. 4). While extreme underexpression of certain 51p1-related genes might sometimes occur, it must be rare, because G6 values of 2.9–5.4% were measured in all 11 subjects of the 1-gene-copy subset and because the correlation coefficient for the total data set was very high ($r = 0.92$). Thus, our data indicate that, in the population studied, several prevalent 51p1-related genes appear to be expressed similarly, each at a high level. Moreover, the percent of G6-positive mantle zone B cells is proportional to the total number of 51p1-related genes in the diploid genome.

The potential to have from zero to four copies of 51p1-related genes may explain the wide variation seen in the expression of 51p1 in fetal development and in some pathological states. Exact 51p1 sequences were seen in 14 (3), 0 (4), 12.5, and 0% (33) of transcripts from four different second trimester fetal expression libraries (104–134 d gestation). Conceivably, 51p1-related alleles may have been absent in two of these sub-

jects, and the existing alleles (likely hv1263-related) were either not expressed or expressed below the level of detection. Failure to detect expression of 51p1 may have also been due to the limited number of clones examined in these studies.

In human CLL, ~ 20% of cases express the G6 idiotype (11, 14) or have rearranged the 51p1 locus (34). Our data suggest that patients whose leukemia cells express 51p1 may tend to have high copy numbers of 51p1 germline genes. Additional disease-related factors favoring the 51p1 locus could also be involved, however. In acute lymphocytic leukemia, a pro/pre B cell malignancy that is ontologically less mature than CLL, 51p1 expression was not seen (13). This difference between CLL and acute lympocytic leukemia suggests that the intrinsic propensity for rearrangement of 51p1 genes, or the pressures selecting for 51p1 gene products, or both, might increase during early B cell development.

In human paraprotein IgM rheumatoid factors, the G6 idiotype is common and is almost invariably associated with a V_L chain encoded by the Ig V_κ gene, kv325 (10, 28). Thus, autospecificity for Fc appears favored in the germline-encoded V_H/V_L combination of a 51p-related gene plus kv325, even though D and J_H variations sometimes modify RF activity (35). In this regard, our data imply that individuals lacking 51p1-related genes will be generally unable to produce G6-positive IgM RF and that people with only one or two 51p1-related gene copies might be less likely to do so than those with three or four.

In conclusion, we have demonstrated that the percentage of IgD-positive tonsillar lymphocytes that bear the G6 idiotype is proportional to the germline copy number of 51p1-related genes. The example provided by this study of the 51p1 gene indicates that, for at least some Ig V_H loci, the frequency with which a V_H gene is expressed in the IgD+ B cell repertoire is the product of its germline copy number and its gene-specific propensity for rearrangement and expression. Germline gene copy number is thus a major factor governing the differential expression of Ig V_H genes.

Acknowledgments

This work was supported in part by National Institutes of Health Grants AR40561 and RR05432 to E.H. Sasso, and R37 CA49870-08 to T.J. Kipps.

Reference

1. Matsuda, F., E. Shin, H. Nagaoka, R. Matsamura, M. Haino, Y. Fukita, S. Taka-Ishi, T. Imai, J. Riley, R. Anand, et al. 1993. Structure and physical map of 64 variable segments in the 3′ 0.8-megabase region of the human immunoglobulin heavy-chain locus. *Nat. Genet.* 3:88–94.

2. Cook, G., I. Tomlinson, G. Walter, H. Riethman, N. Carter, L. Buluwela, G. Winter, and T. Rabbits. 1994. A map of the human immunoglobulin V_H locus completed by analysis of the telomeric region of chromosome 14q. *Nat. Genet.* 7:162–168.

3. Schroeder, H., Jr., J. Hillson, and R. Perlmutter. 1987. Early restriction of the human antibody repertoire. *Science (Wash. DC).* 238:791–793.

4. Schroeder, H.W., Jr., and J.Y. Wang. 1990. Preferential utilization of conserved immunoglobulin heavy chain variable gene segments during human fetal life. *Proc. Natl. Acad. Sci. USA.* 87:6146–6150.

5. Kipps, T.J., B.A. Robbins, and D.A. Carson. 1990. Uniform high frequency expression of autoantibody-associated crossreactive idiotypes in the primary B cell follicles of human fetal spleen. *J. Exp. Med.* 171:189–196.

6. Stewart, A.K., C. Huang, B.D. Stollar, and R.S. Schwartz. 1993. High-frequency representation of a single V_H gene in the expressed human B cell repertoire. *J. Exp. Med.* 177:409–418.

7. Klein, U., R. Küppers, and K. Rajewsky. 1994. Variable region analysis of B cell subsets derived from a 4-year-old child: somatically mutated memory B cells accumulate in the peripheral blood already at young age. *J. Exp. Med.* 180:1383–1393.

8. Suzuki, I., L. Pfister, A. Glas, C. Nottenberg, and E.C.B. Milner. 1995. Representation of rearranged V_H gene segments in the human adult antibody repertoire. *J. Immunol.* 154:3902–3910.

9. Kipps, T., and S. Duffy. 1991. Relationship of the CD5 B cell to human tonsillar lymphocytes that express autoantibody-associated cross reactive idiotypes. *J. Clin. Invest.* 87:2087–2096.

10. Crowley, J.J., R.J. Goldfein, R.E. Schrohenloher, H.L. Speigelberg, G.J. Silverman, R.A. Mageed, R. Jefferis, W.J. Koopman, D.A. Carson, and S. Fong. 1988. Incidence of three cross-reactive idiotypes on human rheumatoid factor paraproteins. *J. Immunol.* 140:3411–3418.

11. Kipps, T.J., E. Tomhave, L.F. Pratt, S. Duffy, P.P. Chen, and D.A. Carson. 1989. Developmentally restricted immunoglobulin heavy chain region expressed at high frequency in chronic lymphocytic leukemia. *Proc. Natl. Acad. Sci. USA.* 86:5913–5917.

12. Kipps, T.J., B.A. Robbins, A. Tefferi, G. Meisenholder, P.M. Banks, and D.A. Carson. 1990. CDR-positive malignancies frequently express cross-reactive idiotypes associated with IgM autoantibodies. *Am. J. Pathol.* 136:809–816.

13. Deane, M., and J.D. Norton. 1991. Preferential rearrangement of developmentally regulated immunoglobulin VH1 genes in human B-lineage leukemias. *Leukemia.* 5:646–650.

14. Shokri, F., R.A. Mageed, P. Richardson, and R. Jefferis. 1993. Modulation and high frequency expression of autoantibody-associated cross-reactive idiotypes linked to the VHI subgroup in CD5-expressing B lymphocytes from patients with chronic lymphocytic leukemia (B-CLL). *Scand. J. Immunol.* 37: 673–679.

15. Mageed, R.A., M. Dearlove, D.M. Goodall, and R. Jefferis. 1986. Immunogenic and antigenic epitopes of immunoglobulins XVII. Monoclonal antibodies reactive with common restricted idiotopes to the heavy chain of human rheumatoid factors. *Rheumatol. Int.* 6:179–183.

16. Klein, U., R. Küppers, and K. Rajewsky. 1993. Human IgM+IgD+ B cells, the major B cell subset in the peripheral blood, express Vk genes with little or no somatic mutation throughout life. *Eur. J. Immunol.* 23:3272–3277.

17. Pascual, V., Y.-J. Liu, A. Magalski, O. de Bouteiller, J. Banchereau, and J.D. Capra. 1994. Analysis of somatic mutation in five B cell subsets of human tonsil. *J. Exp. Med.* 180:329–339.

18. Nicholson, I.C., M.J. Brisco, and H. Zola. 1995. Memory B lymphocytes in human tonsil do not express surface IgD. *J. Immunol.* 154:1105–1113.

19. Sasso, E.H., K. Willems van Dijk, A. Bull, and E.C.B. Milner. 1993 A fetally expressed immunoglobulin V_H1 gene belongs to a complex set of alleles. *J. Clin. Invest.* 91:2358–2367.

20. Kubagawa, H., W.E. Gathings, D. Levitt, J.F. Kearney, and M.D. Cooper. 1982. Immunoglobulin isotype expression of normal pre-B cells as determined by immunofluorescence. *J. Clin. Immunol.* 2:264–269.

21. Parks, D.R., L.L. Lanier, and L.A. Herzenberg. 1986. Flow cytometry and fluorecence activated cell sorting (FACS). *In* Handbook of Experimental Immunology. D.M. Weir, L.A. Herzenberg, and C. Blackwell, editors. Blackwell Scientific Publications, Oxford. 29.1–29.21.

22. Sasso, E., K. Willems van Dijk, and E. Milner, 1990. Prevalence and polymorphism of human V_H3 genes. *J. Immunol.* 145:2751–2757.

23. Chen, P.P., M.-F. Lio, C.A. Glass, S. Sinha, T.J. Kipps, and D.A. Carson. 1989. Characterization of two immunoglobulin V_H genes that are homologous to human rheumatoid factors. *Arthritis Rheum.* 32:72–76.

24. Kabat, E.A., T.T. Wu, H.M. Perry, K.S. Gottesman, and C. Foeller. 1991. Sequences of proteins of immunological interest. U.S. Department of Health and Human Services, USPHS, NIH, U.S. Government Printing Office, Bethesda, MD.

25. Newkirk, M.M., R.A. Mageed, R. Jefferis, P.P. Chen, and J.D. Capra. 1987. The 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: 550–564.

26. Pascual, V., I. Randen, K. Thompson, M. Sioud, O. Forre, J. Natvig, and J.D. Capra. 1990. The complete nucleotide sequences of the heavy chain variable regions of six monospecific rheumatoid factors derived from Epstein-Barr virus–transformed B cells isolated from the synovial tissue of patients with rheumatoid arthritis. Further evidence that some autoantibodies are unmutated copies of germ line genes. *J. Clin. Invest.* 86:1320–1328.

27. Andrews, D.W., and J.D. Capra. 1981. Complete amino acid sequence of variable domains from two monoclonal human anti-gamma globulins of the Wa cross-idiotypic group: suggestion that the J segments are involved in the structural correlate of the idiotype. *Proc. Natl. Acad. Sci. USA.* 78:3799–3803.

28. Silverman, G.J., R.D. Goldfein, P. Chen. R.A. Mageed, R. Jefferis, F. Goni, B. Frangioni, S. Fong, and D.A. Carson. 1988. Idiotypic and subgroup analysis of human monoclonal rheumatoid factors. Implications for structural and genetic basis of autoantibodies in humans. *J. Clin. Invest.* 82:469–475.

29. Sasso, E.H., J.A. Buckner, and L.A. Suzuki. 1995. Ethnic differences in polymorphism of an immunoglobulin V_H3 gene. *J. Clin. Invest.* 96:1591–1600.

30. Walter, M.A., U. Surti, M.H. Hofker, and D.W. Cox. 1990. The physical

organization of the human immunoglobulin heavy chain gene complex. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:3303–3313.

31. Cook, G.P., and I.M. Tomlinson. 1995. The human immunoglobulin V_H repertoire. *Immunol. Today.* 16:237–242.

32. Walter, G.I., I.M. Tomlinson, G.P. Cook, G. Winter, T.H. Rabbits, and P.H. Dear. 1993. HAPPY mapping of a YAC reveals alternative haplotypes in the human immunoglobulin V_H locus. *Nucleic Acids Res.* 21:4524–4529.

33. Hillson, J., I. Opplinger, E. Sasso, E. Milner, and M. Wener. 1992. Emerging human B cell repertoire. Influence of developmental stage and inter-individual variation. *J. Immunol.* 149:3741–3752.

34. Kipps, T.J., L.Z. Rassenti, S. Duffy, T. Johnson, R. Kobayashi, and D.A. Carson. 1992. Immunoglobulin V gene expression in CD5 B-cell malignancies. *Ann. NY Acad. Sci.* 651:373–383.

35. Martin, T., R. Crozier, J.-C. Weber, T.J. Kipps, and J.-L. Pasquali. 1994. Structure-function studies on a polyreactive (natural) autoantibody. Polyreactivity is dependent on somatically generated sequences in the third complementarity-determining region of the antibody heavy chain. *J. Immunol.* 152:5988–5996.