Modern Pathology (2009) 22, 833–838

www.modernpathology.org

Use of similar immunoglobulin *VH* gene segments by MALT lymphomas of the ocular adnexa

David W Bahler[1], Philippe Szankasi[2], Sucheta Kulkarni[3], Raymond R Tubbs[3], James R Cook[3] and Steven H Swerdlow[4]

[1]*Department of Pathology, University of Utah, Salt Lake City, UT, USA;* [2]*ARUP Laboratories, University of Utah, Salt Lake City, UT, USA;* [3]*Department of Clinical and Molecular Pathology, Cleveland Clinic, Cleveland, OH, USA and* [4]*Department of Pathology, University of Pittsburgh, Pittsburgh, PA, USA*

Extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue type (MALT lymphomas) develop from acquired reactive infiltrates directed against external or autoantigens. Although some European cases of ocular adnexal MALT lymphoma have been associated with *Chlamydia psittaci* infections, *C. psittaci* has not been detected in large studies of US-based cases. To evaluate whether the growth of US-based ocular adnexal MALT lymphomas may be promoted by a similar antigen, we identified and analyzed the expressed immunoglobulin *VH* genes in 10 cases. Interestingly, the *VH* genes in two cases used the same *VH1* family *V1-2* gene segment, and three cases used the same *VH4* family *V4-34* gene segment. The other five cases all used different gene segments *V4-31*, *V5-51*, *V3-23*, *V3-30*, and *V3-7.* All of the *VH* genes were mutated from germ line, with percent homologies ranging between 96.9 and 89.0%. The distribution of replacement and silent mutations within the *VH* genes was nonrandom consistent with the maintenance of immunoglobulin function and also strongly suggestive of antigen selection in the six *VH* genes with highest mutation loads. The CDR3 sequences in two of three *VH-34* cases were the same size (15 amino acids) and had similar sizes in the two *VH1-2* cases (18 and 16 amino acids). In conclusion, US-based MALT lymphomas of the ocular adnexa preferentially express a limited set of *VH* gene segments not frequently used by other MALT lymphomas and consistent with some recognizing similar antigens. Analysis of somatic mutations present within the *VH* genes is also consistent with antigen binding stimulating the growth of these lymphomas.
Modern Pathology (2009) **22,** 833–838; doi:10.1038/modpathol.2009.42; published online 3 April 2009

Keywords: MALT lymphoma; ocular adnexa; immunoglobulin *VH* gene

Extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue type (MALT lymphomas) are typically indolent B-cell neoplasms that develop out of acquired reactive infiltrates associated with localized long-standing autoimmune diseases or chronic infections.[1,2] The growth of early MALT lymphomas is thought to be dependent on antigen stimulation but may become antigen independent over time with the acquisition of additional genetic abnormalities.[1,3] Antigen stimulation of MALT lymphoma growth is strongly supported by studies of gastric MALT lymphomas that develop out of infiltrates associated with chronic *Helicobacter pylori*-associated infections and can often be cured with *H. pylori*-eradicating antibiotics.[4–6] Studies by an Italian group have suggested *Chlamydia psittaci* may have a similar function in ocular adnexal MALT lymphoma and reported tumor regressions in patients receiving *C. psittaci*-eradicating antibiotic treatment.[7,8] However, studies of US-based cases have not found evidence for *C. psittaci* in ocular adnexal MALT lymphomas using sensitive PCR techniques.[9,10]

Analysis of expressed immunoglobulin heavy chain variable region (*VH*) genes can provide strong support for surface immunoglobulin-mediated direct antigen stimulation of lymphoma growth without prior identification of the reactive MALT antigenic trigger.[11–15] For example, studies of salivary gland MALT lymphoma *VH* genes have demonstrated that approximately 60% of cases use a single *VH* gene segment, *V1-69*, which is suggestive of antigen selection because there are

Correspondence: Dr DW Bahler, MD, PhD, Department of Pathology, University of Utah, JMR Building, Room 2100, 15 North Medical Drive, Salt Lake City, UT 84112, USA.
E-mail: david.bahler@path.utah.edu
Received 12 September 2008; revised and accepted 19 November 2008; published online 3 April 2009

approximately 40 different functional *VH* gene segments that can potentially be used.[16] In addition, antigen selection of salivary gland MALT lymphoma growth is further supported from finding nonrandom distributions of point mutations typically present in the expressed *VH* genes and stretches of similar CDR3 amino-acid sequences in *VH* genes from different patients.[12,16] Evidence supporting antigen stimulation of low-grade B-cell neoplasm growth is not limited to MALT lymphomas as recent studies of chronic lymphocytic leukemias (CLLs) have documented nearly identical expressed heavy and light chain variable genes from different patients, suggesting that some of these cases recognize the same antigens or epitopes.[14,17–19]

In this study, we identified and analyzed the *VH* genes used by 10 US-based ocular adnexal MALT lymphoma cases to look for evidence of antigen stimulation. Our findings provide support that direct antigen stimulation by a common antigen is important in the growth of these neoplasms that are not associated with *C. psittaci*.

Materials and methods

Cases

Cases of MALT lymphoma involving the ocular adnexa as defined by the WHO[2] with available frozen tissue were identified by searching the pathology files of the University of Pittsburgh Medical Center and the Cleveland Clinic. Of the identified 13 cases, 10 were included in our previous study of ocular MALT lymphoma[10] and 3 are new to this study. The hematoxylin-and-eosin-stained slides were reviewed on all cases. Immunohistochemistry and flow cytometry studies (done on all cases) preformed as described[10] were also reviewed. Two independent real-time nested PCR assays for detection of *C. psittaci* were previously performed on 7 of the cases as described.[10] The research use of these specimens was approved by the institutional review boards of the University of Pittsburgh (no. 0505102), the Cleveland Clinic (no. 81008), and the University of Utah (no. 13172).

VH Gene Analysis

Genomic DNA was prepared from frozen tissues using the Gentra Puregene kit (Qiagen Inc., Valencia, CA, USA) or the Genomic DNA Purification kit (Gentra Systems, Minneapolis, MN, USA) following the manufacturer's directions. Rearranged immunoglobulin heavy chain variable regions were amplified from the isolated DNA using a reverse 3′ *JH* primer and seven different 5′ *VH* leader region primers, six of which were previously described,[20] with an additional primer that perfectly matches the leader sequence of the *VH3-21* gene segment, 5′-CCATGGAACTGGGGCTCCGC. Genomic DNA (20 ng) was amplified in 1 × GoTaq flexi PCR buffer (Promega Corp., Madison, WI, USA), 3 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 μM leader prime, 0.2 μM J primer, and 1 U GoTaq Flexi DNA polymerase in a final volume of 20 μl. Cycling conditions were 2 min at 94°C followed by 35 cycles of 20 s at 94°C, 10 s at 55°C, 30 s at 72°C followed by a hold 2 min at 72°C and cool down. The PCR reactions were run on a 2% agarose gel and the DNA was visualized with ethidium bromide (0.5 μg/ml). Before sequencing, primers and dNTP were removed from the reactions with the most prominent bands by mixing 10 μl of reaction with 2 μl ExoSAP-IT (USB Corp., Cleveland, OH, USA) and incubating at 37°C for 45 min followed by 15 min at 85°C. The treated samples were appropriately diluted in water and 6 μl was mixed with 8 μl of the appropriate leader primer (0.8 μM) and subjected to DNA sequence analysis using BigDye Terminator chemistry (Applied Biosystems Inc., Foster City, CA, USA). The readable DNA sequences were searched against the IMGT database (http://imgt.cines.fr/) to identify the most closely related *VH*, *DH*, and *JH* germ-line segments and to confirm a productive *VDJ* rearrangement.

Results

Of the 13 ocular adnexal MALT lymphoma cases initially identified with available frozen tissue, the expressed *VH* genes were successfully obtained from the 10 cases further described in Table 1. The

Table 1 Ocular MALT lymphoma case information

Case	*Age/sex*	*Biopsy site*	*Light chain*	Chlamydia psittaci *status*
1	54/M	R lacrimal	Kappa	Negative
2	74/F	R orbit	Kappa	Negative
3	55/F	L orbit	Lambda	Negative
4	81/M	L orbit	Kappa	ND
5	82/M	L orbit	Kappa	ND
6	76/M	L orbit	Kappa	Negative
7	43/F	R orbit	Kappa	Negative
8	68/M	L orbit	Lambda	Negative
9	58/F	R orbit/lacrimal	Kappa	ND
10	80/M	L orbit	Kappa	Negative

ND, not determined.

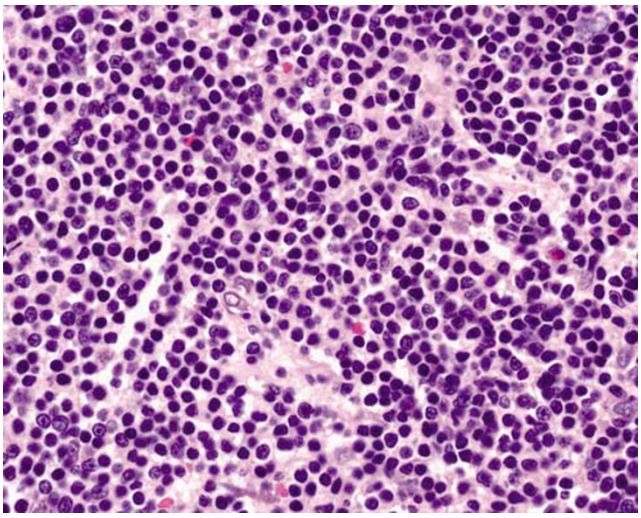

Figure 1 Hematoxylin-and-eosin-stained section of a representative case (no. 4) showing morphological features typical of ocular adnexal MALT lymphoma.

patients mean age was 60 years, range 43–82 years, and included 6 men and 4 women. Evaluated biopsies were from orbital soft tissue in nine cases, the lacrimal gland in two cases, with one case (no. 9) having both orbit and lacrimal gland biopsies. All showed morphological features typical of orbital MALT lymphoma, namely a predominance of small lymphocytes admixed with variable numbers of monocytoid appearing cells and sometimes smaller numbers of plasma cells (Figure 1). Flow cytometry that was performed in all cases revealed populations of CD5-negative, CD10-negative B cells with monotypic expression of κ (8 of 10 cases) or λ (2 of 10 cases) light chains. Sensitive PCR testing for *C. psittaci* was negative in all seven cases evaluated. Only one patient (no. 4) had a prior history of lymphoma, reportedly a diffuse large-cell lymphoma of the small bowel treated with R-CHOP 2 years before the orbit biopsy, and 9 months later a cutaneous low-grade lymphoma with a follicular growth pattern treated with radiation. Slides were not available for review from either biopsy, but the patient was clinically thought to represent a stage 1 orbital MALT lymphoma and received only radiation treatment.

A single rearranged functional *VH* gene without stop codons was identified in the 10 cases by directly sequencing PCR products generated by amplifying lymphoma DNA with *VH* leader and *JH* primers. Comparing the lymphoma *VH* genes to germ-line genes in the IMGT database revealed that two used *VH* gene segments from the *VH1* family, four used *VH4* family gene segments, three used *VH3* family gene segments, and one used a *VH5* family segment (Table 2). Interestingly, the two *VH1* family cases used the same *V1-2* gene segment, whereas three of the four *VH4* family cases used the same *V4-34* gene segment. The three *VH3* family cases all used different *V3* gene segments.

Table 2 Ocular MALT lymphomas use of *VH* gene segments

Case	*Family*	*Name*	*No. of mutations*	*Homology (%)*[a]
1	VH1	V1-2	19	93.5
2	VH1	V1-2	20	93.1
3	VH4	V4-34	17	94.2
4	VH4	V4-34	32	89.0
5	VH4	V4-34	19	93.5
6	VH4	V4-31	9	96.9
7	VH3	V3-30	21	92.9
8	VH3	V3-74	24	91.8
9	VH3	V3-23	10	96.9
10	VH5	V5-51	23	91.8

[a]Relative to the most closely related germ-line gene.

Table 3 Distribution of mutations in lymphoma *VH* segments

Case	*CDR1 and 2*				*FWR1, 2, and 3*			
	R	*S*	*R/S*	*Random R/S*	*R*	*S*	*R/S*	*Random R/S*
1	8	1	8.0	4.3	6	4	1.5	3.0
2	5	3	1.7	4.3	5	7	0.7	3.0
3	3	1	3.0	4.5	9	11	0.8	2.7
4	4	4	1.0	4.5	13	11	1.2	2.7
5	4	3	1.3	4.5	6	6	1.0	2.7
6	3	0	3	4.1	3	3	1	2.7
7	9	3	3	3.9	6	3	2	2.9
8	5	3	1.7	4.2	11	5	2.2	2.8
9	4	1	4	3.6	3	2	1.5	2.9
10	6	5	1.2	3.5	5	7	0.7	3.3

Columns headed by 'R' or 'S' give the number of replacement or silent mutations, respectively, relative to the most closely related germ-line *VH* gene segment. 'Random R/S' refers to the ratio of all possible replacement to silent mutations.

All of the lymphoma *VH* gene segments showed significant numbers of point mutations from the most closely related germ-line counterparts, with percent homologies ranging between 96.9 and 89.0%, mean percent homology $93.4 \pm 2.4\%$. The mutation locations, complementarity determining regions (CDRs) or framework regions (FWRs), and type, those causing an amino-acid replacement (R) or not, ie silent (S), are listed in Table 3. All of the lymphoma *VH* genes segments had fewer replacement mutations in the FWRs than would be expected by chance alone,[21] ie random mutation, consistent with the maintenance of immunoglobulin functionality.[22] Five cases with higher numbers of mutations (no. 2, 4, 5, 8, and 10) also had fewer replacement mutations than would be expected by chance alone in the CDRs, suggestive of negative selection or selection against replacement mutations in these areas. In only one case (no. 1) were mutations above what would be expected by chance alone in the CDRs that could be suggestive of positive selection.

The CDR3 regions of the lymphoma *VH* genes appeared to be encoded by a variety of different *D* segments and to have variable sizes (Table 4). Most

Table 4 Ocular lymphoma *VH* gene CDR3 sequences

Case	D_H	J_H	*Amino-acid sequence*
1	2-2	4b	GPRQCSSITCLSYFHY
2	3-16	6b	AARVVRLFNPDRSSGMDV
3	6-13	6b	GEAVTGPPRSDDMDV
4	5-5	4b	TGGPHLGNSDGVTHS
5	2-15	4b	ITEIIDVHYCSGGDCNEGGFDY
6	4-4	6b	VHSNYYYRMDV
7	3-9	4b	QYDILTDYYKWGVFDY
8	1-1	4b	GNGY
9	5-24	4b	AWAYIAGNYFDY
10	2-21	3b	PYCGGDCYSGDAPFHI

D_H and J_H refer to the germ-line heavy chain diversity and joining segments used in the CDR3, respectively.

Table 5 Differential use of *V1-2* and *V4-34* segments

Cell types	*V1-2*	*V4-34*
Peripheral blood B cells	3%[a]	3%[a]
Mutated CLL	7% (3/46)[b]	20% (9/46)[b]
Unmutated CLL	8% (3/38)[b]	3% (1/38)[b]
Splenic marginal zone lymphoma	42% (20/48)[c]	15% (7/48)[c]
Primary CNS lymphoma	0% (0/15)[d]	60% (9/15)[d]
Other MALT lymphomas (gastric, salivary gland, lung)	1% (1/76)[e]	4% (3/76)[e]

[a]Brezinschek *et al*.[23]
[b]Hamblin *et al*.[25]
[c]Bahler *et al*[29] and Algara *et al*.[28]
[d]Thompsett *et al*[27] and Montesions-Rongen *et al*.[26]
[e]Bende *et al*,[12] Sakuma *et al*,[31] and Lenze *et al*.[30]

used either *J4* or *J6* joining segments, which are also used in the majority of normal B-cells.[23] No conserved amino-acid motifs in the CDR3 regions were appreciated among the different cases.

Discussion

The development and early growth of MALT lymphomas appears to be dependent on antigen stimulation, which can be direct, mediated by surface immunoglobulin, and/or indirect and mediated by T cells. The antigens having key functions in MALT lymphomagenesis are mostly unknown, but are likely dependent on the type of tissue the lymphomas arise from and could also vary with different geographical areas. We analyzed the *VH* genes expressed by 10 cases of ocular adnexal MALT lymphoma to look for evidence of direct antigen stimulation. An important finding along this line was observing preferential use of certain *VH* gene segments with two cases using the same *V1-2* gene segment and three other cases using the same *V4-34 VH* gene segment. Because there are approximately 40 different functional *VH* gene segments that can potentially be used,[23,24] the probability that 2 or 3 of the 10 cases evaluated would use the same *VH* gene segments by chance alone is 0.023 and 0.002, respectively. By contrast, approximately 3% of normal peripheral blood B cells use *V1-2* and *V4-34*, which is the expected frequency if use is random.[23] Of the other 10 cases, 1 expressed a *VH4* family gene segment different from *V4-34, V4-31*, which further suggests orbital MALT lymphomas may also display preferential use of *VH4* family gene segments.

Nonrandom or preferential use of certain *VH* gene segments suggests that these encode important determinants that have been selected by an antigen binding to surface immunoglobulin leading to stimulation of B-cell growth. Besides our study, preferential use of *V4-34* and *V1-2* has been reported for other types of low-grade B-cell neoplasms. The *V4-34* gene segment is used by approximately 20% of CLL cases with mutated *VH* genes,[25] and by approximately 60% of cases of primary central nervous system lymphoma[26,27] (Table 5). Preferential use of *V1-2* is seen in splenic marginal zone lymphomas and used by approximately 40% of cases.[28,29] It is interesting that the expression of *V1-2* and *V4-34* does not appear to be increased above random use levels in MALT lymphomas that occur at other sites such as the stomach, lung, or salivary gland.[12,30,31] This is consistent with the concept that antigens stimulating MALT lymphoma growth in the ocular adnexal will be different than those at other MALT lymphoma sites. It may be significant regarding the antigen specificity of ocular adnexal MALT lymphomas that both *V1-2* and *V4-34* are used by autoantibodies, and almost all cold agglutinin autoantibodies use *V4-34*.[32]

Our findings of preferential use of *V1-2* and *V4-34* differ from the two earlier studies of ocular adnexal MALT lymphoma *VH* genes. In the study by Coupland *et al*[33] that involved 26 cases, 2 (8%) used *V4-34* and only 1 (4%) used *V1-2*, whereas none of the 12 cases analyzed by Hara *et al*[34] used *V1-2* or *V4-34*. Because the cases analyzed in Coupland's study were European and those in Hara's study were Japanese, it is possible that antigens driving ocular adnexal MALT lymphomagenesis vary depending on geographical location. This possibility is also supported by reports showing an association of *C. psittaci* with mostly Italian orbital MALT lymphoma cases,[7,8,35] whereas US-based orbital MALT cases using sensitive PCR techniques appear to be *C. psittaci* negative.[9,10,35] Technical considerations could also have complicated positive lymphoma *VH* gene identification in the study by Coupland *et al*[33] because paraffin-extracted DNA was used in nested PCR reactions involving consensus FW1 and FW2 primers and 65 total cycles of amplification.

All of the lymphoma *VH* genes identified in this study were mutated relative to their most closely related germ-line counterparts consistent with the proposed post-germinal center B-cell origin. In addition, the level of mutation we observed in the orbital MALT lymphoma *VH* gene segments (mean

percent homology to germ line of 93 ± 2.4%) is also close to the mutation load values reported for MALT lymphomas that occur at other locations.[12,16] Analysis of the point mutation types (replacement or silent) and distribution (FWRs or CDRs) within many of the *VH* gene segments suggested there is selection against mutations occurring in CDR1 and CDR2, or negative selection. Finding evidence of negative selection in these CDRs was not an artifact related to analyzing small numbers of mutations because the five cases showing clear negative selection also had some of the highest mutation loads. Negative selection observed in the CDRs of these cases is similar to the negative selection that was observed in the FWRs of these cases, which is an indicator of functionality in that many FWR residues cannot withstand replacement mutations if immunoglobulin function is to be maintained.[22] Selection against replacement mutations in the CDRs is also a feature of *VH* genes expressed by MALT lymphomas that occur at other sites such as the salivary gland,[36,37] and could function in preventing the disruption of antigen-mediated signaling that may be detrimental to lymphoma cell survival.[38,39] Negative selection is also seen with antibodies that display reactivity towards autoantigens and may have a function in preventing the emergence of high-affinity variants.[40]

The CDR3 nucleotide sequence, made up of randomly templated nucleotides and diversity and joining segments that have been shortened by nuclease trimming, is the most variable part of a *VH* gene and serves as a clonal marker.[41] Because of the tremendous variability, antibodies that recognize the same antigens typically do not have similar CDR3 sequences.[42–44] As such, not finding similarities of CDR3 sequences among our MALT lymphoma cases does not alter the possibility that some may recognize similar or common antigens. Because the size of the CDR3 sequence also has an important function in antigen binding,[45] it may be significant that the CDR3 of two cases using *V4-34* are the same size, whereas the CDR3 sequences for the two *V1-2* cases differ by only two amino acids.

In summary, our study suggests that US-based ocular adnexal MALT lymphomas that are not associated with *C. psittaci* preferentially express a limited set of *VH* gene segments not frequently used by MALT lymphomas that occur at other sites. This finding is consistent with these cases recognizing a common antigen that is responsible for selecting and promoting the transformation of B cells with the frequently expressed *VH* gene segments through immunoglobulin-mediated growth stimulation. Although the nature of the antigen is unknown, frequent use of the *V4-34* and *V1-2* gene segments as well as selection against replacement point mutations occurring in the CDRs, favors these cases recognizing a self-antigen or autoantigen.

Acknowledgement

This study was supported by the ARUP Institute of Pathology.

Conflict of interest

The authors have no financial interests or conflicts with anything mentioned in this article.

References

1 Isaacson PG, Du MQ. MALT lymphoma: from morphology to molecules. Nat Rev Cancer 2004;4:644–653.
2 Jaffe ES, Harris NL, Stein H, *et al.* WHO Classification: Tumors of Haematopoietic and Lymphoid Tissues. IARC Press: Lyon, France, 2001.
3 Suarez F, Lortholary O, Hermine O, *et al.* Infection-associated lymphomas derived from marginal zone B cells: a model of antigen-driven lymphoproliferation. Blood 2006;107:3034–3044.
4 Bayerdorffer E, Neubauer A, Rudolph B, *et al.* Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of *Helicobacter pylori* infection. Lancet 1995;345:1591–1594.
5 Parsonnet J, Hansen S, Rodriguez L, *et al. Helicobacter pylori* infection and gastric lymphoma. N Engl J Med 1994;330:1267–1271.
6 Wotherspoon AC, Doglioni C, Diss TC, *et al.* Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet 1993;342:575–577.
7 Ferreri AJ, Guidoboni M, Ponzoni M, *et al.* Evidence for an association between *Chlamydia psittaci* and ocular adnexal lymphomas. J Natl Cancer Inst 2004;96:586–594.
8 Ferreri AJ, Ponzoni M, Guidoboni M, *et al.* Regression of ocular adnexal lymphoma after *Chlamydia psittaci*-eradicating antibiotic therapy. J Clin Oncol 2005;23:5067–5073.
9 Rosado MF, Byrne Jr GE, Ding F, *et al.* Ocular adnexal lymphoma: a clinicopathologic study of a large cohort of patients with no evidence for an association with *Chlamydia psittaci*. Blood 2006;107:467–472.
10 Ruiz A, Reischl U, Swerdlow SH, *et al.* Extranodal marginal zone B-cell lymphomas of the ocular adnexa: multiparameter analysis of 34 cases including interphase molecular cytogenetics and PCR for *Chlamydia psittaci*. Am J Surg Pathol 2007;31:792–802.
11 Bahler DW, Zelenetz AD, Chen TT, *et al.* Antigen selection in human lymphomagenesis. Cancer Res 1992;52:5547s–5551s.
12 Bende RJ, Aarts WM, Riedl RG, *et al.* Among B cell non-Hodgkin's lymphomas, MALT lymphomas express a unique antibody repertoire with frequent rheumatoid factor reactivity. J Exp Med 2005;201:1229–1241.
13 Stevenson F, Sahota S, Zhu D, *et al.* Insight into the origin and clonal history of B-cell tumors as revealed by analysis of immunoglobulin variable region genes. Immunol Rev 1998;162:247–259.
14 Chiorazzi N, Ferrarini M. B cell chronic lymphocytic leukemia: lessons learned from studies of the B cell

antigen receptor. Annu Rev Immunol 2003;21: 841–894.
15 Lossos IS, Tibshirani R, Narasimhan B, *et al.* The inference of antigen selection on Ig genes. J Immunol 2000;165:5122–5126.
16 Miklos JA, Swerdlow SH, Bahler DW. Salivary gland mucosa-associated lymphoid tissue lymphoma immunoglobulin V(H) genes show frequent use of V1-69 with distinctive CDR3 features. Blood 2000;95:3878–3884.
17 Stamatopoulos K, Belessi C, Moreno C, *et al.* Over 20% of patients with chronic lymphocytic leukemia carry stereotyped receptors: pathogenetic implications and clinical correlations. Blood 2007;109:259–270.
18 Tobin G, Thunberg U, Johnson A, *et al.* Chronic lymphocytic leukemias utilizing the VH3-21 gene display highly restricted Vlambda2-14 gene use and homologous CDR3s: implicating recognition of a common antigen epitope. Blood 2003;101:4952–4957.
19 Widhopf II GF, Goldberg CJ, Toy TL, *et al.* Nonstochastic pairing of immunoglobulin heavy and light chains expressed by chronic lymphocytic leukemia B cells is predicated on the heavy chain CDR3. Blood 2008;111:3137–3144.
20 Bahler DW, Campbell MJ, Hart S, *et al.* Ig VH gene expression among human follicular lymphomas. Blood 1991;78:1561–1568.
21 Chang B, Casali P. The CDR1 sequences of a major proportion of human germline Ig VH genes are inherently susceptible to amino acid replacement. Immunol Today 1994;15:367–373.
22 Shlomchik MJ, Aucoin AH, Pisetsky DS, *et al.* Structure and function of anti-DNA autoantibodies derived from a single autoimmune mouse. Proc Natl Acad Sci USA 1987;84:9150–9154.
23 Brezinschek HP, Brezinschek RI, Lipsky PE. Analysis of the heavy chain repertoire of human peripheral B cells using single-cell polymerase chain reaction. J Immunol 1995;155:190–202.
24 Lefranc MP, Lefranc G. The Immunoglobulin Factsbook. Academic Press: London, UK, 2001.
25 Hamblin TJ, Davis Z, Gardiner A, *et al.* Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 1999;94:1848–1854.
26 Montesinos-Rongen M, Kuppers R, Schluter D, *et al.* Primary central nervous system lymphomas are derived from germinal-center B cells and show a preferential usage of the V4-34 gene segment. Am J Pathol 1999;155:2077–2086.
27 Thompsett AR, Ellison DW, Stevenson FK, *et al.* V(H) gene sequences from primary central nervous system lymphomas indicate derivation from highly mutated germinal center B cells with ongoing mutational activity. Blood 1999;94:1738–1746.
28 Algara P, Mateo MS, Sanchez-Beato M, *et al.* Analysis of the IgV(H) somatic mutations in splenic marginal zone lymphoma defines a group of unmutated cases with frequent 7q deletion and adverse clinical course. Blood 2002;99:1299–1304.
29 Bahler DW, Pindzola JA, Swerdlow SH. Splenic marginal zone lymphomas appear to originate from different B cell types. Am J Pathol 2002;161:81–88.
30 Lenze D, Berg E, Volkmer-Engert R, *et al.* Influence of antigen on the development of MALT lymphoma. Blood 2006;107:1141–1148.
31 Sakuma H, Nakamura T, Uemura N, *et al.* Immunoglobulin VH gene analysis in gastric MALT lymphomas. Mod Pathol 2007;20:460–466.
32 Li Y, Spellerberg MB, Stevenson FK, *et al.* The I binding specificity of Human V_H4-34 (V_H4-21) encoded antibodies is determined by both V_H framework region 1 and complementarity determining region 3. J Mol Biol 1996;256:577–589.
33 Coupland SE, Foss HD, Anagnostopoulos I, *et al.* Immunoglobulin VH gene expression among extranodal marginal zone B-cell lymphomas of the ocular adnexa. Invest Ophthalmol Vis Sci 1999;40: 555–562.
34 Hara Y, Nakamura N, Kuze T, *et al.* Immunoglobulin heavy chain gene analysis of ocular adnexal extranodal marginal zone B-cell lymphoma. Invest Ophthalmol Vis Sci 2001;42:2450–2457.
35 Decaudin D, de Cremoux P, Vincent-Salomon A, *et al.* Ocular adnexal lymphoma: a review of clinicopathologic features and treatment options. Blood 2006;108: 1451–1460.
36 Bahler DW, Miklos JA, Swerdlow SH. Ongoing Ig gene hypermutation in salivary gland mucosa-associated lymphoid tissue-type lymphomas. Blood 1997;89: 3335–3344.
37 Bahler DW, Swerdlow SH. Clonal salivary gland infiltrates associated with myoepithelial sialadenitis (Sjogren's syndrome) begin as nonmalignant antigen-selected expansions. Blood 1998;91:1864–1872.
38 Friedman DF, Cho EA, Goldman J, *et al.* The role of clonal selection in the pathogenesis of an autoreactive human B cell lymphoma. J Exp Med 1991;174:525–537.
39 Lam KP, Kuhn R, Rajewsky K. *In vivo* ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. Cell 1997;90:1073–1083.
40 Borretzen M, Randen I, Zdarsky E, *et al.* Control of autoantibody affinity by selection against amino acid replacements in the complementarity-determining regions. Proc Natl Acad Sci USA 1994;91: 12917–12921.
41 Sanz I. Multiple mechanisms participate in the generation of diversity of human H chain CDR3 regions. J Immunol 1991;147:1720–1729.
42 Caton AJ, Brownlee GG, Staudt LM, *et al.* Structural and functional implications of a restricted antibody response to a defined antigenic region on the influenza virus hemagglutinin. Embo J 1986;5:1577–1587.
43 Borretzen M, Chapmen C, Stevenson FK, *et al.* Structural analysis of VH4-21 encoded human IgM allo- and autoantibodies against red blood cells. Scand J Immunol 1995;42:90–97.
44 Mariette X, Brouet JC, Danon F, *et al.* Nucleotide sequence analysis of the VL and VH domains of five human IgM directed to lamin B. Arthritis Rheum 1993;36:1315–1324.
45 Rock EP, Sibbald PR, Davis MM, *et al.* CDR3 length in antigen-specific immune receptors. J Exp Med 1994; 179:323–328.