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Published in:
Journal of Experimental Medicine

DOI:
10.1084/jem.20050068

Citation for published version (APA):
Bende, R. J., Aarts, W. M., Riedl, R. G., de Jong, D., Pals, S. T., & van Noesel, C. J. M. (2005). Among B cell non-Hodgkin's lymphomas, MALT lymphomas express a unique antibody repertoire with frequent rheumatoid factor reactivity. Journal of Experimental Medicine, 201(8), 1229-1241. DOI: 10.1084/jem.20050068
Among B cell non-Hodgkin’s lymphomas, MALT lymphomas express a unique antibody repertoire with frequent rheumatoid factor reactivity

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We analyzed the structure of antigen receptors of a comprehensive panel of mature B non-Hodgkin’s lymphomas (B-NHLs) by comparing, at the amino acid level, their immunoglobulin (Ig)VH-CDR3s with CDR3 sequences present in GenBank. Follicular lymphomas, diffuse large B cell lymphomas, Burkitt’s lymphomas, and myelomas expressed a CDR3 repertoire comparable to that of normal B cells. Mantle cell lymphomas and B cell chronic lymphocytic leukemias (B-CLLs) expressed clearly restricted albeit different CDR3 repertoires. Lymphomas of mucosa-associated lymphoid tissues (MALTs) were unique as 8 out of 45 (18%) of gastric- and 13 out of 32 (41%) of salivary gland-MALT lymphomas expressed B cell antigen receptors with strong CDR3 homology to rheumatoid factors (RFs). Of note, the RF-CDR3 homology without exception included N-region–encoded residues in the hypermutated IgVH genes, indicating that they were stringently selected for reactivity with auto-IgG. By in vitro binding studies with 10 MALT lymphoma–derived antibodies, we showed that seven of these cases, of which four with RF-CDR3 homology, indeed possessed strong RF reactivity. Of one MALT lymphoma, functional proof for selection of subclones with high RF affinity was obtained. Interestingly, RF-CDR3 homology and t(11;18) appeared to be mutually exclusive features and RF-CDR3 homology was not encountered in any of the 19 pulmonary MALT lymphomas studied.

B cell non-Hodgkin’s lymphomas (B-NHLs) comprise >85% of malignant lymphomas worldwide. They are in majority of germinal center (GC) or post-GC phenotype and often harbor chromosomal translocations typically involving immunoglobulin (Ig) loci (1). In spite of their genetic defects, most B-NHLs do not replicate spontaneously in vitro, indicating that they still depend on environmental stimuli for their growth. To date, these external factors are ill defined. Evidence exists that B cell antigen receptor (BCR) ligands have, as in normal B cell development, a pivotal role in the pathogenesis of at least some B-NHLs. For example, the architecture and cellular composition of follicular lymphomas (FLs) is highly reminiscent of normal GCs. Furthermore, extranodal marginal zone B cell lymphoma (MZBCL) of mucosa-associated lymphoid tissue (MALT) arise at sites of antigenic stimulation due to organ-specific autoimmunity; e.g., Sjögren’s salaladenitis (2) and Hashimoto’s thyroiditis (3), or infection like Helicobacter pylori gastritis (4, 5) and Borrelia burgdorferi dermatitis (6, 7), respectively. Similarly, a role of hepatitis C virus (HCV) infection has been inferred in the development of malignant B cell proliferation, including splenic MZBCL and MALT lymphoma (8, 9). Most recently, it has been claimed that ocular adnexal MALT lymphoma and immunoproliferative small intestinal disease (also known as α-heavy chain disease) are associated with Chlamydia psittaci and Campylobacter jejuni infections, respectively (10, 11). The low tendency of MALT lymphomas to spread beyond the environment in which they evolve may be related to the expression of certain homing and chemokine receptors, such as α4β7 and CXC.CR3 (12, 13). In addition, it has been proposed that even during the tumor

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stage, antigen (Ag) plays a growth-sustaining role. This notion is strongly supported by the observation that a proportion of low grade gastric MALT lymphomas (14, 15) and skin MZBCLs (7) are curable by bacterial eradication alone, while interferon–α-2b treatment can cause regression of HCV-associated MZBCLs (16, 17).

Analysis of the Ig variable (IgV) genes supported the concept of Ag-driven lymphomagenesis in FL and MALT lymphoma. The IgV heavy (IgV_H) and IgV light (IgV_L) chain genes of these malignancies are heavily mutated, compatible with a GC or post-GC derivation (18–22). The mutation patterns unequivocally indicate that Ag-based selection occurs at some stage of their development; despite high mutation loads, the overall structure of the Ig is generally being preserved in these lymphomas often during years of disease. Apparently, selective forces prevent the outgrowth of BCR lymphoma mutants.

Although many studies on FL and MALT lymphomas allude to a role for Ag in the pathogenesis of these lymphomas, only sporadic data exist on the exact ligands that these B cell neoplasms might recognize. Although the obvious candidate ligand for gastric MALT lymphoma was H. pylori, in vitro cultures revealed that the tumor B cells were not directly stimulated by H. pylori, but indirectly by CD40/CD40L-mediated help of intratumoral, H. pylori–specific T cells (23). Hussell et al. (24) and Greiner et al. (25) observed reactivity of MALT lymphoma–derived Abs with follicular DCs (FDCs), various epithelia, or postcapillary venules of Peyer’s patches, but no specific Ags were molecularly defined. It is well documented that a significant proportion of B cell chronic lymphocytic leukemias (B-CLLs) express Ig (poly) reactive with a diversity of autoantigens (26–31). In contrast, with other B-NHL entities, autoreactivity has only sporadically been reported (32–34). Finally, viral antigens of HTLV-I and HCV have been implicated as BCR ligands of individual cases of B-CLL (35) and diffuse large B cell lymphoma (DLBCL; reference 36), respectively.

To address the issue of antigen-receptor specificity of B-NHL, we performed a systematic analysis of the antigen binding sites of 132 extranodal MZBCL (24 from our laboratory and 108 from literature) and, for comparison, from a comprehensive panel of 478 other B-NHL. We provide ev-

### Table I. Clinical presentation, immunohistochemistry, and genetics of 24 MALT lymphomas

| Patient | Sex | Age | Location | Clinical presentation | Immunohistochemistry | PCR |
|---------|-----|-----|----------|-----------------------|----------------------|-----|
|         |     |     |          |                       |                      |     |
| M4      | f   | 60  | thyroid gland | Hashimoto’s thyroiditis | IgM k | – | – | t(11;18) |
| M5      | f   | 64  | parotid gland | Sjögren’s syndrome | IgM k | + | + | – |
| M6’95   | f   | 55  | stomach     | gastritis            | IgM k | + | + | – |
| M6’96   | f   | 56  | stomach     | gastritis            | IgM k | + | + | ND |
| M8      | m   | 44  | parotid gland | unknown              | IgM k | – | + | – |
| M9      | f   | 58  | tonsil      | unknown              | IgM k | – | + | – |
| M11     | m   | 38  | parotid gland | unknown              | IgM k | + | + | – |
| M13     | m   | 63  | ileum       | unknown              | IgM k | + | + | – |
| M14     | f   | 70  | parotid gland | Sjögren’s syndrome | IgA k | + | + | – |
| M15     | f   | 45  | lacrymal gland | Sjögren’s syndrome | IgA k | + | + | – |
| M19     | f   | 74  | lung        | unknown              | IgM k | + | + | – |
| M20     | m   | 39  | lung        | unknown              | IgM/ IgA k | + | + | – |
| M21     | f   | 64  | parotid gland | Sjögren’s syndrome | IgM k | + | + | – |
| M22a    | m   | 71  | groin lymph node | unknown              | IgA k | + | + | – |
| M23     | m   | 52  | lung        | unknown              | IgG k | + | + | – |
| M30     | f   | 60  | stomach     | gastritis            | NC k | ND | ND | – |
| M41     | f   | 55  | stomach     | gastritis            | ND | ND | ND | ND |
| M45     | m   | 81  | stomach     | gastritis            | ND | ND | ND | ND |
| M46     | m   | 65  | stomach     | gastritis            | ND | ND | ND | ND |
| M55     | m   | 41  | lung        | unknown              | ND | ND | ND | ND |
| M56     | m   | 78  | stomach     | gastritis            | IgM k | + | + | – |
| M57     | m   | 81  | stomach     | gastritis            | IgM k | + | + | – |
| M58     | m   | 74  | stomach     | gastritis            | IgM k | + | + | – |
| M60     | m   | 71  | stomach     | gastritis            | IgM k | + | + | – |
| M61     | m   | 38  | stomach     | gastritis            | IgM k | + | + | – |

*a Initially located in salivary gland.
NC, not clear.
idence that among the various B cell neoplasms, gastric- and salivary gland-MALT lymphomas express a distinctive Ig repertoire and frequently originate from precursor B cells clonally selected for auto-IgG binding capacity. The fact that B-NHL entities express qualitatively different Igs points toward different roles of the BCRs in their pathogenesis.

RESULTS

IgV<sub>H</sub> and IgV<sub>L</sub> sequence analysis of MALT lymphomas

A panel of 24 MALT lymphomas was analyzed (Table I). All lymphomas were CD20<sup>+</sup> and virtually all expressed the chemokine receptor CXCR3 and the mucosal homing integrin α4β7. The pulmonary lymphomas M19, M20, and M23 and the gastric lymphomas M56, M57, M58, M60, and M61 carried the t(11;18), involving the API2 and MALT1 genes (Table I). Except for M60, all IgV<sub>H</sub> and IgV<sub>L</sub> genes analyzed were somatically mutated with means of 21 (range 0–68) and 18 mutations (range 1–58) per IgV gene, respectively (Tables II and III). Of M6, biopsies were available of two time points spanning a 9 mo interval. At relapse (M6<sub>96</sub>), 15 somatic mutations were found in the expressed V3-7 IgV<sub>H</sub> gene while at presentation (M6<sub>95</sub>) an additional replacement mutation in codon 13 had been present (Table II). Immunohistochemistry (Table I) and RT-PCR (Table II) indicated that the lung lymphoma M20 contained both IgM- and IgA-expressing tumor cells. The IgM- and the IgA-related IgV<sub>H</sub> sequences were identical and contained 5 mutations (Table II). In 17 of the 24 lymphomas (71%), the replacement versus silent (R/S) mutation ratios in the framework regions (FRs) of the IgV<sub>H</sub> genes, were significantly <1.5, implying that, in spite of the high mutation frequencies, selective forces had preserved the BCR in these lymphomas (unpublished data). Intraclonal variation (ICV) was determined for 16 IgV<sub>H</sub> genes and for 8 IgV<sub>L</sub> genes (18). In 10 out of the 16 (63%) MALT lymphomas.

### Table II. Immunoglobulin variable heavy chain genes of 24 MALT lymphomas

| Patient | Ig isotype | VH family | Closest VH germline gene | No. of mutations (%) | D gene | JH gene | Intraclonal variation<sup>a</sup> |
|---------|------------|-----------|--------------------------|----------------------|--------|---------|-------------------------------|
| M4      | γ          | VH3       | V3-23 (DP47)             | 68 (23)              | NA     | JH4b    | 0.8 (5)                       |
| M5      | μ          | VH3       | V3-7 (DP54)              | 17 (5.8)             | D3-3   | JH3b    | 0.8 (5)                       |
| M6'95   | μ          | VH3       | V3-7 (DP54)              | 16 (5.4)             | D3-22  | JH3b    | ND                            |
| M6'96   | μ          | VH3       | V3-7 (DP54)              | 15 (5.1)             | D3-22  | JH3b    | 1.0 (5)                       |
| M8      | μ,δ<sup>b</sup> | VH3   | V3-30 (DP49)             | 26 (8.8)             | D5-24  | JH5     | ND                            |
| M9      | μ,δ<sup>b</sup> | VH1   | V1-69 (DP10)             | 8 (2.7)              | NA     | JH4b    | ND                            |
| M11     | μ          | VH1       | V1-69 (DP10)             | 11 (3.7)             | D6-13  | JH4b    | 3.0 (7)                       |
| M13     | μ          | VH4       | V4-31 (DP65)             | 14 (4.7)             | D5-24  | JH4     | 1.8 (5)                       |
| M14     | α          | VH3       | V3-23 (DP47)             | 43 (15.0)            | NA     | JH6     | 2.3 (6)                       |
| M15     | α          | VH1       | V1-18 (DP14)             | 46 (16.0)            | NA     | JH4b    | <0.4 (5)                      |
| M19     | μ,δ<sup>b</sup> | VH3   | V3-53                   | 29 (10.0)            | NA     | JH4b    | ND                            |
| M20     | μ,α<sup>c</sup> | VH4   | V4-30.4 (DP78)           | 5 (1.7)              | NA     | JH3b    | ND                            |
| M21     | μ,δ<sup>b</sup> | VH3   | V3-23 (DP47)             | 23 (7.8)             | D2-2   | JH4b    | 0.7 (11)                      |
| M22     | α          | VH1       | V1-69 (DP88)             | 14 (4.6)             | D4-17  | JH4b    | <0.4 (15)                     |
| M23     | γ          | VH1       | V1-69 (DP88)             | 7 (2.4)              | NA     | JH4b    | <0.4 (7)                      |
| M30     | γ          | VH2       | V2-5 (VII-5)             | 33 (11.1)            | NA     | JH1     | 0.7 (7)                       |
| M41     | ND         | VH1       | V1-69 (DP10)             | 19 (6.5)             | D3-22  | JH4b    | <0.4 (7)<sup>d</sup>         |
| M45     | ND         | VH1       | V1-3 (DP25)              | 22 (7.5)             | D3-10  | JH4b    | ND                            |
| M46     | ND         | VH3       | V3-30/30.5 (DP49)        | 12 (4.1)             | D5-12  | JH6b    | ND                            |
| M55     | ND         | VH3       | V3-7 (DP54)              | 24 (8.1)             | D3-22  | JH4b    | ND                            |
| M56     | μ          | VH1       | V1-69 (DP10)             | 7 (2.4)              | D1-14  | JH4b    | <0.4 (11)                     |
| M57     | μ          | VH1       | V1-18 (DP14)             | 14 (4.8)             | NA     | JH6b    | 1.9 (9)                       |
| M58     | μ          | VH3       | V3-53                   | 23 (7.9)             | D3-22  | JH4b    | ND                            |
| M60     | μ          | VH1       | V1-69 (DP10)             | 0 (0.0)              | D2-15  | JH5b    | <0.4 (9)                      |
| M61     | μ          | VH1       | V1-18 (DP14)             | 19(6.5)              | D2-15  | JH4b    | 0.8 (4)                       |

<sup>a</sup>The intraclonal variation is indicated as the mean number of nucleotide differences observed per clone, as compared with the consensus sequence. Numbers in parentheses indicate the number of clones that were sequenced.

<sup>b</sup>IgD was not detected immunohistochemically.

<sup>c</sup>M41 contained distinct subclones (Fig. 1).

<sup>d</sup>M61 had a deletion of the three nucleotides of codon 29.

NA, the germline D gene could not definitely be assigned.

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In M15, lacking ICV in the IgVH gene, an exceptionally high degree of ICV was observed in its IgVk gene (Tables II and III). Interestingly, M41 appeared a somatically diversified, V6-69/D3-22/JH4b-expressing lymphoma, harboring distinct subclones (M41-A, B, C, and D) with 19, 19, 20, and 2 mutations, respectively (Fig. 1).

IgVH-CDR3 amino acid sequences of MALT lymphomas and splenic MZBCL

We compared the IgVH-CDR3 amino acid (aa) sequences of our panel of MALT lymphomas to IgVH-CDR3 aa sequences present on GenBank using the NCBI Protein-BLAST program with the option “search for short nearly exact matches” (BLASTP 2.2.6; reference 37). CDR3 regions consisting of at least 7 aa (all except M20) were analyzed (Table IV). A CDR3 sequence was considered to be homologous to previously reported CDR3 sequences on GenBank (a) if sharing at least 75% aa sequence homology and (b) a length difference between the CDR3 sequences not exceeding 3 aa (maximum gap of 3 aa). Applying these homology criteria, we found that the CDR3 of 4 MALT lymphomas (M23, M46, M56, and M60) displayed homology to different B cell clones analyzed previously in repertoire studies. Interestingly, 4 cases (M5,

Table III. Immunoglobulin variable light chain κ genes of the MALT lymphomas

| Patient | Vκ family | Closest Vκ germline gene | No. of mutations (%) | Jκ gene | Intraclonal variationa |
|---------|-----------|--------------------------|----------------------|---------|------------------------|
| M4      | Vk1       | L9 (Ve+)                 | 58 (20.0)            | Jκ4     | 0.5 (4)                |
| M5      | Vk3       | L2 (kv328, DPK21)        | 9 (3.2)              | Jκ1     | 1.0 (5)                |
| M6’96   | Vk3       | L2 (kv328, DPK21)        | 5 (1.8)              | Jκ1     | 0.4 (5)                |
| M8      | Vk1       | O12/02 (DPK9)            | 27 (9.5)             | Jκ4     | ND                     |
| M9      | Vk3       | A27 (kv325, DPK22)       | 2 (0.69)             | Jκ1     | ND                     |
| M11     | Vk3       | A27 (kv325, DPK22)       | 1 (0.35)             | Jκ1     | 1.5 (6)                |
| M14     | Vk2       | A19/A3 (DPK15)           | 51 (17.0)            | Jκ4     | 1.2 (6)                |
| M15     | Vk4       | B3 (DPK24)               | 17 (5.6)             | Jκ4     | 9.3 (6)                |
| M21     | Vk3       | A27 (kv325, DPK22)       | 13 (4.5)             | Jκ1     | 0.5 (4)                |
| M22     | Vk3       | A27 (kv325, DPK22)       | 6 (2.1)              | Jκ2     | ND                     |
| M23     | Vk1       | O12/02 (DPK9)            | 6 (2.1)              | Jκ1     | 0.8 (8)                |

aThe intraclonal variation is indicated as the mean number of nucleotide differences observed per clone, as compared to the consensus sequence. Numbers in parentheses indicate the number of clones that were sequenced.

Figure 1. Schematic representation of the IgVH clones identified in M41. The lollipop-shaped symbols indicate nucleotide differences as compared with the V1-69 (DP10) germline IgVH gene. Except for the CDR3 region, replacement and silent mutations are indicated with closed and open circles, respectively, with codon numbering according to V-base indicated underneath. 2X, two mutations in the indicated codon. The mutations in codons 76 and 82a are different between M41-A/B and M41-C, respectively. In the CDR3 and in codon 106, interclonal differences are indicated by different filling patterns of the circles. In the CDR3, the deduced aa sequence is depicted in the one-letter code underneath. The CDR3 of M41-A/D, M41-B and M41-C displayed, respectively, 73, 82, and 91% homology to the CDR3 of RF-WOL. M41-D has only two mutations, shared with M41-A, B, and C.
M6, M11, and M41) showed strong homology with CDR3 sequences of other MALT lymphomas, as well as with CDR3 sequences of rheumatoid factors (RF). Salivary gland lymphoma M5 and gastric lymphoma M6, both expressing a V3-7/JH3 rearrangement, were homologous to a plethora of IgVH-CDR3 aa sequences originating from salivary gland MALT lymphomas, gastric MALT lymphomas, HCV-associated lymphomas, B cell clones derived from benign myoepithelial sialadenitis, one DLBCL and two genuine V3-7-encoded RFs (Table S1, available at http://www.jem.org/cgi/content/full/jem.20050068/DC1). Salivary gland lymphoma M11, which also expressed V3-7/JH3, was homologous to five normal B cell clones, one gastric MALT lymphoma and finally to one RF termed RF-WOL (reference 39 and Table S1).

We then, additionally, examined CDR3 of 35 gastric-, 26 salivary gland- and 15 pulmonary-MALT lymphomas as well as 32 splenic MZBCL from literature and/or GenBank (Table V). This revealed that overall 8 out of 45 (18%) gastric MALT lymphomas expressed RF-homologous CDR3 whereas among the splenic MZBCLs, only one case expressed a CDR3 homologous to RF-WOL (reference 39 and Table S1).

Currently, 10 genuine V1-69/JH4-encoded RFs, isolated from rheumatoid arthritis patients or from healthy donors immunized with mismatched red blood cells (HID), one gastric MALT lymphoma and finally to one RF termed RF-WOL (reference 39 and Table S1).

| Patient | V_J rearrangement | CDR3 amino acid sequence* | CDR3 length |
|---------|-------------------|---------------------------|-------------|
| M4      | V3-23 (DP47)/JH4b | CTKAHVPPDFGLPSPNVWGGQ     | 14          |
| M5      | V3-7 (DP54)/D3-3/JH3b | CARQFDWSGDYIDAFDIWGGQ | 14          |
| M6      | V3-7 (DP54)/D3-22/JH3b | CARQDYFSDSGSFIDAFDIWGGQ | 16          |
| M8      | V3-30 (DP49)/D5-24/JH5 | CAGKDSKERFLYWFDSWGGQ | 13          |
| M9      | V1-69 (DP10)/JH4b | CARQDWAGHTERSNLVYWGGQ | 15          |
| M11     | V1-69 (DP10)/D6-13/JH4b | CARQEIAAAVNPFDYWGGQ | 12          |
| M13     | V4-31 (DP65)/D5-24/JH4 | CAGDRGGYNLIDCWCWGQ | 10          |
| M14     | V3-23 (DP47)/JH6 | CAKQOMRLGDTFYGLDVWGGQ | 15          |
| M15     | V1-18 (DP14)/JH4b | CARQSGLLGYDMFWQGG | 11          |
| M19     | V3-53/JH4b | CAGTPSGLTHLYWQGGQ | 10          |
| M20     | V4-30.4 (DP78)/JH3b | CARQDGAFDIWGGQ | 6           |
| M21     | V3-23 (DP47)/D2-2/JH4b | CARQDKLFYVGYCTICGCNTFDYWQGG | 18          |
| M22     | V1-69 (DP88)/D4-17/JH4b | CARQGSDNYDGDSVPVQHPYWWGGQ | 15          |
| M23     | V1-69 (DP88)/JH4b | CARQVSGNSFDYWQGGQ | 9           |
| M30     | V2-5 (VII-5)/JH1 | CAGGRGFRSSGLFYIAFPHPYWQGG | 18          |
| M41-A   | V1-69 (DP10)/D3-22/JH4b | CARQRGTPGYYFYWQGGQ | 11          |
| M41-B   | V1-69 (DP10)/D3-22/JH4b | CARQRGTDGYFYFYQGYGQ | 11          |
| M41-C   | V1-69 (DP10)/D3-22/JH4b | CARQRGDSMYFYFYQGYGQ | 11          |
| M45     | V1-3 (DP25)/D3-10/JH4b | CARQGTPKIRKVPFDYWQGGQ | 14          |
| M46     | V3-30/30.5 (DP49)/D5-12/JH6b | CARQDGSNGYFTMDYWQGGQ | 13          |
| M55     | V3-7 (DP54)/D3-22/JH4b | CARQWYDNESAYFLHYWQGGQ | 12          |
| M56     | V1-69 (DP10)/D1-14/JH4b | CARQDJGNYHFYDYWQGGQ | 9           |
| M57     | V1-18 (DP14)/JH6b | CARQPPQAGDSPYYGMVWQGGQ | 17          |
| M58     | V3-53/D3-22/JH4b | CARQHSYDDNNAYDFWQGGQ | 10          |
| M60     | V1-69 (DP10)/D2-15/JH5b | CARQDPVPDCSGGSCVILWFDPWQGGQ | 17          |
| M61     | V1-18 (DP14)/D2-15/JH4b | CARQDYCSSGIICYGGDYWQGGQ | 13          |

*The FR3 and FR4 are indicated in italics. The assignable nontemplated region-encoded amino acids are underlined.
Table V. Comparison of IgVH-CDR3 amino acid sequences of a panel of mature B-NHL with IgVH-CDR3 amino acid sequences from GenBank

| Type of lymphoma | Mean CDR3 length | N | Overall* | RFa | V1-69 RFa | V3-7 RFa | WOL-RFa |
|-----------------|------------------|---|----------|-----|-----------|----------|----------|
| Gastric MALT    | 13.6             | 45 | 16 (36)  | 8 (18)| 1         | 4        | 2        |
| Salivary gland MALT | 14.5          | 32 | 15 (47)  | 13 (41)| 8         | 4        | 1        |
| Pulmonary MALT  | 12.7             | 19 | 2 (11)   | 0 (0) | 0         | 0        | 0        |
| Other MALT      | Ni               | 4  | 0 (0)    | 0 (0) | 0         | 0        | 0        |
| Splenic MZBCL   | 16.3             | 32 | 8 (25)   | 1 (3) | 0         | 1        | 1        |
| MCL             | 13.3             | 23 | 10 (44)  | 0 (0) | 0         | 0        | 0        |
| B-CLL IgVH unmutated | 17.4         | 165| 73 (44)  | 2 (1) | 0         | 0        | 0        |
| B-CLL IgVH mutated | 13.7          | 143| 24 (17)  | 0 (0) | 0         | 0        | 0        |
| FL              | 12.1             | 48 | 4 (8)    | 0 (0) | 0         | 0        | 0        |
| DLBCL           | 12.2             | 20 | 2 (10)   | 1 (5) | 0         | 1        | 0        |
| BL              | 12.6             | 48 | 5 (10)   | 0 (0) | 0         | 0        | 0        |
| Myeloma         | 13.0             | 31 | 5 (16)   | 0 (0) | 0         | 0        | 0        |

The lymphomas used for the homology analyses are listed in the supplemental legend to this table (available at http://www.jem.org/cgi/content/full/jem.20050068/DC1).

*Indicates the number of lymphomas that show homology at least 75% (a) or more than 90% (b) to at least one IgVH-CDR3 sequence available in GenBank.

| No. of cases with CDR3 homology |
|---------------------------------|
| RFa                             |
| V1-69 RFa                       |
| V3-7 RFa                        |
| WOL-RFa                         |

have been described (42). V1-69/JH4 RFs typically contain a 12–14 aa CDR3 and are combined with an A27(kv325)-encoded IgVκ chain. Among the V1-69-RF-homologous MALT lymphomas, only of salivary gland lymphoma M11 the IgVκ is known, which indeed proved to be the canonical A27(kv325) IgVκ (Table III and Table S2, available at http://www.jem.org/cgi/content/full/jem.20050068/DC1). WOL-RF is also V1-69/JH4-encoded, but with a distinct 13 aa CDR3, again in combination with an A27(kv325)-encoded IgVκ chain. Five V3-7 RFs have been described, isolated from HIDs and an RA patient (42). V3-7-RFs are encoded by V3-7/D3-22/JH3 rearrangements, typically possessing a 16–17 aa CDR3 with the D3-22 in reading-frame 2, and in combination with an L2 (kv328)/jk1-encoded IgVκ chain. The V3-7/JH3-expressing salivary gland lymphomas M5 and SH (43) and gastric MALT lymphoma M6 indeed all co-expressed the canonical L2 (kv328)/jk1-encoded IgVκ chain (Table III and Table S2).

IgVH-CDR3 amino acid sequences of other mature B-non-Hodgkin's lymphomas

For comparison, IgVH-CDR3 analyses were extended to a comprehensive panel of other mature B-NHLs available from literature and/or on GenBank. For most B-NHL entities, the average CDR3 aa length was comparable to that of mature naive B cells being 13.5 aa (44). Only splenic MZB-CLs and the IgVH-unmutated B-CLLs expressed CDR3 of higher mean lengths (Table V). Of 48 FLs, 20 DLBCLs, 48 Burkitt lymphomas (BL) and 31 myelomas, only 16 cases (11%) displayed CDR3 aa homology with other CDR3 sequences present in GenBank. Thirteen of the 16 cases resembled those of normal B cells analyzed in repertoire studies (45–47). As mentioned above, one DLBCL EJ (48) showed homology to several gastric- and salivary gland-MALT lymphomas as well as to V3-7-RFs (Table V).

Analyses of 23 mantle cell lymphomas (MCL), deposited on GenBank, revealed that their CDR3 displayed a high frequency of homology (10 out of 23, 44%), mostly (7 cases) with CDR3 regions of unmutated normal B cells.

We analyzed a panel of 308 B-CLLs, 165 (54%) and 143 (46%) of the IgVH-unmutated and the IgVH-mutated subsets, respectively (Table V). Overall, the CDR3s of 97 out of the 308 B-CLLs (31%) displayed CDR3 homology, 59 of which (19%) with CDR3s of B-CLLs (inter B-CLL homology). Of the group of 97 B-CLLs with any homology, 75% belonged to the IgVH-unmutated subset. This relative overrepresentation was even more outspoken among the group with inter-B-CLL homology in which 50 out of the 59 cases (85%) were unmutated. In fact, applying our criteria for homology, we distinguished eight CDR3-homology groups within 37 of the 97 B-CLL cases (79%).
A significant fraction of B-CLLs derives from poly (auto) reactive B cells (26–30). In vitro RF reactivity has been proven for one representative of homology group 1 (POR) and one of group 2 (AIG) (30). Two B-CLLs, CLL-011 (53) and ID-74 (54) (CLL-011 belonging to homology group 1), showed CDR3 homology to two different, IgVH-unmutated, RFs termed TB-3-D13 (55) and RF-SJ2 (56), respectively (Table V). Poly-reactivity toward different auto-antigens, including IgG, has been demonstrated for homology group 4 member SMI (29). In addition, group 4 members share CDR3 homology with an anti-cardiolipine Ab (AF460965). It is noted that the B-CLLs that displayed in vitro RF reactivity (POR, AIG, and SMI) showed CDR3 homology to two unmutated RFs as well as the MALT lymphoma cases from literature (ML27, Iso-3, and ML16) with V3-7-RF, V1-69-RF, and WOL-RF, respectively, is depicted. The amino acid sequences are depicted by the single letter code. FR3 and FR4, framework region 3 and 4; N, amino acids encoded by the non-templated nucleotides; D, gene segment; JH, gene segment; |, identical amino acids; +, similar amino acids; x, non-matching amino acids; CDR3 length, length of the CDR3 region; Hom, percentage of homologous amino acids; Id, percentage of identical amino acids; Gap, length difference in amino acids of the compared IgVH-CDR3 regions.

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Figure 2. IgVH-CDR3 amino acid sequences of selected cases of MALT lymphoma with homology to IgVH-CDR3 of rheumatoid factors. MALT lymphomas M5/M6, M11, and M41 share homology with V3-7-RF, V1-69-RF, and WOL-RF, respectively. In addition, RF CDR3 homology of three MALT lymphoma cases from literature (ML27, Iso-3, and ML16) with V3-7-RF, V1-69-RF, and WOL-RF, respectively, is depicted. The amino acid sequences are depicted by the single letter code. FR3 and FR4, framework region 3 and 4; N, amino acids encoded by the non-templated nucleotides; D, gene segment; JH, gene segment; |, identical amino acids; +, similar amino acids; x, non-matching amino acids; CDR3 length, length of the CDR3 region; Hom, percentage of homologous amino acids; Id, percentage of identical amino acids; Gap, length difference in amino acids of the compared IgVH-CDR3 regions.

RF activity of recombinant lymphoma-derived IgM antibodies
To prove that MALT lymphomas with RF-CDR3 homology possess IgG-binding activity, lymphoma-idiotype-derived Abs (LIDA) of IgM class were generated of M5, M6, M11, and M41-A, C, and D. Since, due to shortage of DNA, we could not resolve the IgVH and IgVH/H11001 of M41 we combined each of the IgVH variants of the M41-A, C, and D subclones with the RF-canonical IgVH chain of M22. Recombinant IgM LIDAs were also produced of 6 MALT lymphomas (M8, M9, M14, M21, M22, and M23), which are all devoid of RF-CDR3 homology. As additional controls, IgM LIDAs were generated of four follicular lymphomas (FL1, FL6’94, FL8’92, and FL13), one B-CLL, B-CLL26, and two anti-Rhesus(D) Ab producing B cell lines (8D8 and LOS3; reference 57). The IgVH and IgVH sequences of these FLs and
B-CLL have been previously reported by us (18, 58, 59). The lymphomas originally expressed IgM, with exception of M14, M22 (both IgA\textsubscript{H}/H1001), and M23 (IgG\textsubscript{H}/H1001) (Tables I and II). RF-ELISA studies pointed out that the LIDA of M5, M6, and M11 were indeed strongly reactive with human IgG (Fig. 3). The LIDA of M9 and M22, both with a Ig\textsubscript{H}-CDR3 region not completely fulfilling our homology criteria but both with the RF-canonical V1-69/JH4-A27(kv325) combination of Ig\textsubscript{H} and Ig\textsubscript{V}k chains, also displayed strong RF activity (Tables II–IV and Fig. 3). Of note, also sera of patient M22, which contained high concentrations (22 mg/ml and 6.5 mg/ml) of lymphoma-related IgA paraprotein, displayed strong IgA-RF activity in ELISA (Fig. 3). This thus independently confirmed our finding with the M22-LIDA and underscored the validity of our approach of recombinant lymphoma Ab production in the eukaryotic expression system used. Moreover, the LIDA of M21, not even harboring an RF-canonical Ig\textsubscript{H} rearrangement but with an A27(kv325)-encoded Ig\textsubscript{V}k chain, also possessed strong RF activity. In contrast, none of the LIDAs of MALT lymphomas M8, M14, M23, the four FLs, the B-CLL nor the anti-Rhesus(D) Abs, all, except M23, lacking canonical Ig\textsubscript{H} RF rearrangements, bound to IgG (Fig. 3). Finally, LIDA M41-A/M22 (the dominant subclone with 19 mutations) and M41-C/M22 with 20 mutations bound

| Group | Characteristic V\textsubscript{H}-D-J\textsubscript{H} | Mutation status | CDR3 length | No. of cases |
|-------|-------------------------------------------------|----------------|-------------|--------------|
| 1     | (VH1, VH5, VH7)/D6-19 (frame 3)/JH4             | unmutated      | 11–12       | 9            |
| 2     | (V1-69)/V4-34/D2-2 (frame 3)/JH6               | unmutated      | 18–20       | 6            |
| 3     | V4-34/D5-5 (frame 1)/JH6                      | mutated        | 18          | 3            |
| 4     | V1-69/D3-16 (frame 3)/JH3                 | unmutated      | 19          | 3            |
| 5     | (V1-2)/V1-3)/D1-26 (frame 3)/JH6            | unmutated      | 15          | 3            |
| 6     | V4-39/D6-13 (frame 1)/JH5                  | unmutated      | 16–17       | 5            |
| 7     | V1-69/D3-10 (frame 3)/JH6                 | unmutated      | 18          | 5            |
| 8     | (V1-2)/V1-46)/D3-22 (frame 2)/JH4         | unmutated      | 17          | 3            |

The B-CLL cases of the eight Ig\textsubscript{H}-CDR3 homology groups and the resemblance of homology groups 1–7 to earlier described B-CLL homology groups are in the supplemental legend of this table.

Figure 3. RF activity of lymphoma-idiotype-derived antibodies. (a) Titration of IgM LIDAs M5, M6, M9, M11, M21, M22, M41-A, M41-B, FL6/94, B-CLL26, 8D8, and RF control serum in the IgM-RF ELISA. The LIDAs M8, M14, M23, M41-D, FL1, FL8/92, FL13, and LOS3 did not react with human IgG and showed a similar binding curve as that of FL6/94. (b) Binding activity of IgM LIDAs, RF control serum, and anti-Rh(D) control IgM Abs (LOS3 and 8D8) in the IgM-RF ELISA. All samples were tested at a stratified concentration of 500 ng/ml IgM. The ABS 450 nm is plotted without subtraction of the background (BG) ABS 450 nm. (c) IgA-RF ELISA of two serum samples of M22 and as controls an IgA anti-Rh(D) Ab (VT-7G3), an IgA-RF-containing serum, the RF control serum that was also used in the IgM-RF ELISA, and two negative-control serum samples, respectively. All samples were tested at a stratified concentration of 20 \mu g/ml IgA. The net ABS 450 nm is plotted with subtraction of the background ABS 450 nm.
IgG in ELISA whereas the LIDA of M41-D/M22 (a subclone with only two mutations) did not (Figs. 1–3).

We next tested the binding capacities of the RF+ LIDAs of M5, M6, M9, and M11 with recombinant IgG1 and IgG3 preparations. The LIDA of M5, M6, and M9 reacted with IgG1 only. M11 reacted with both IgG1 and IgG3, and may thus be a pan-IgG reacting RF (unpublished data). In chronic gastritis, RF-expressing B cells may theoretically be stimulated by IgG coated on H. pylori or due to existence of cross-reacting epitopes between H. pylori and IgG-Fc. Upon comparison, one H. pylori (strain 26695, GenBank/EMBL/DDBJ accession no. AE000511) peptide, of the gene product “virulence-associated protein homologue VacB” (GenBank/EMBL/DDBJ accession no. AAD08293), was found to share 68% homology with aa 336–354 of the IgG1 Fc at the CH2-CH3 junction. However, none of these peptides, nor did any of these peptides, react with the binding of the RF-LIDAs to IgG. In addition, no binding of any of these LIDAs to H. pylori–infected HM02 epithelial cells (strains 26695 and 1061) was observed. In addition, all LIDAs lacking RF-activity (M8, M14, M23, FL1, FL6’94, FL8’92, FL13, and B-CLL26) showed no antigen-specific antibody (ANA) activity on Hep2 cells either (unpublished data).

To explore whether other BCR ligands are present within the tissue of MALT lymphomas or FLs, we also produced LIDAs of the IgG class of 3 (non-RF-CDR3-homologous) MALT lymphomas (M8, M14, and M15) and of 4 FLs (FL1, FL3’93, FL6’94, and FL63; reference 18). These LIDAs were FITC-labeled and tested immunohistochemically for reactivity on the corresponding lymphoma tissues. In none of these experiments however we detected reactivity with any tissue components (unpublished data).

**DISCUSSION**

We systematically analyzed the immunoglobulin repertoire of a comprehensive panel of mature B-NHLs. Unbiased comparison of IgVH-CDR3–encoded aa sequences of individual B-NHLs with all IgVH-CDR3 presently available in GenBank revealed distinct patterns of the various B-NHL entities. This provided interesting clues concerning their potential ligands, which was functionally confirmed for the group of MALT lymphomas.

FLs, DLBCLs, BLs, and myelomas all exhibited a low degree of overall IgVH-CDR3 sequence homology (Table V). In none of these 147 B-NHLs, recurrent IgVH-CDR3 motifs were found. The majority of the homologous lymphomas expressed CDR3 that resembled those present in normal B cells. A few, however, shared homology with B-CLL and MALT lymphomas. One DLBCL displayed homology with gastric- and salivary gland-MALT lymphomas as well as with V3-7-RFs (Table V). Thus, in general FL, DLBCL, BL, and myelomas, all carrying significantly hypermutated IgV genes, seem to recognize unique epitopes, suggesting that they arise randomly out of the pool of B cells selected for nonself-antigens, most likely during the germinal center reaction. This is in accordance with previous observations that the germline IgVH gene usage of these B-NHLs is similar to that of normal peripheral B cells (18, 60). In contrast, B-CLL and MCL cases showed a high degree of overall CDR3 homology (31 and 44%, respectively) (Table V). Focusing on B-CLL that shared CDR3 homology with at least two other B-CLL (which held for 37 out of the 308 B-CLL analyzed), we distinguished eight CDR3-homology groups (Table VI). These homology groups in part overlap with B-CLL subgroups as reported by others (49–52). Inter-B-CLL homology was largely confined to the IgVH-β-unmutated subset, which shows a strong bias toward V1-69 usage (53): 62 out of 165 (38%) IgVH unmutated B-CLL expressed V1-69, most often (34/62, 55%) combined with JH6. In addition, the previously described poly-autoreactivity of a significant fraction of B-CLL was also clearly reflected in our study: Eighteen B-CLLs shared CDR3 homology with either of five B-CLL for which reactivity with auto-Ags such as IgG (RF), cardiolipin or myoglobulin has been reported (29, 30).

MALT lymphomas were found to express a highly distinctive IgVH repertoire, confirming and extending earlier reports by the groups of Miklos et al. (38) and De Re et al. (63) on salivary gland MALT lymphomas and HCV-associated B cell lymphomas, respectively. Out of a total of 100 MALT lymphomas that we analyzed, 33 cases shared CDR3 aa homology with other, previously published, CDR3. Twenty-one of these 33 MALT lymphomas harbored, according to the criteria chosen, significant homology to RF-related CDR3 and, except for one case, could be classified into either of 3 canonical RF groups; V1-69-RFs, V3-7-RFs and WOL-RFs (Table V). In addition, 5 salivary gland MALT lymphomas were included, reported by Bahler et al. (43, 64) and Miklos et al. (38), which did not completely fulfill our stringent criteria for V1-69-RFs, but did express the typical V1-69/JH4 RF gene rearrangement. The RF-homology group solely involved gastric- and salivary gland-MALT lymphomas. In the vitro binding studies with the recombinant LIDA formally proved that MALT lymphomas with canonical RF IgVH- and IgVk-chain rearrangements and RF-CDR3 homology indeed posses strong RF activity (Fig. 3). Moreover, MALT lymphomas M9, M21, and M22, which did not match our criteria for RF homology but of which M9 and M22 expressed the canonical RF V1-69/JH4 rearrangement, also exhibited strong IgG-binding capacity in vitro (Fig. 3). Thus, the actual proportion of MALT lymphomas with specificity for human IgG is likely to be higher than calculated on basis of our arbitrary homology criteria.

The degree of RF-CDR3 homology found in the 21 MALT lymphomas is strikingly high, taking into account that it concerns heavily mutated IgVH genes and that homology in-
cluded N-region encoded aa residues (Fig. 2). This suggests a distinct pathogenesis and indicates that these lymphomas originate from precursors strongly selected for auto-IgG specificity. The latter notion is well supported by the finding that the LIDA of the major subclone of M41 (M41-A), with 19 somatic mutations, and M41-C, with 20 mutations, exhibited significant intrinsic IgG-binding activity of the expressed IgVH1 chains, whereas this could not be measured of a presumed ancestral subclone, M41-D, with 2 mutations (Figs. 1 and 3).

MALT lymphomas typically evolve in a background of chronic inflammation due to infection or autoimmunity. Evidence exists that the tumor B cells in gastric MALT lymphoma are not H. pylori–specific but largely depend on CD40 stimulation by H. pylori–specific T helper cells (23). It has recently been reported, in a murine model, that RF-expressing B cells can be selectively activated in a T cell independent manner by IgG-chromatin complexes through the synergistic engagement of the BCR and toll-like receptor 9 (TLR9; reference 65). TLR9 is expressed in the endoplasmic compartment and serves as a pathogen sensor that binds unmethylated CpG DNA motifs which are more common in bacterial than in mammalian DNA. In the human system, CpG-DNA was shown to trigger T cell independent proliferation of memory B cells, but not of naive B cells, which correlated with the levels of TLR9 expression of memory and naive B cells, respectively (66, 67). Stimulation of TLR9 may thus, parallel to the CD40/CD40L pathway, operate in lymphoproliferations of MALT. In gastric MALT, RF B cells may receive synergistic signals of the RF-BCR, by IgG–H. pylori complexes and of TLR9 by H. pylori DNA. Also in inflamed salivary gland tissue in Sjögren’s syndrome, as well as in other autoimmune diseases, RF B cells may receive these signals of the BCR and TLR9 by complexes of IgG and DNA released during normal or pathological cell death. This scenario clearly lends support from the fact that virtually all Sjögren’s syndrome patients produce antinuclear antibodies (ANA), including anti-SS-A and SS-B antibodies.

The most frequent genetic alteration found in MALT lymphoma is the t(11;18)(q21;q21) encoding an API2–MALT1 fusion product that constitutively activates the NF-κB pathway (68). The t(11;18) is present in ~40 and ~25% of pulmonary– and gastric-MALT lymphomas respectively whereas it is virtually absent in MALT lymphomas of the salivary gland (~2%; references 69–71). We found that ~40% of the salivary gland– and ~20% of the gastric-MALT lymphomas express RF-like BCRs whereas we did not identify RF-like BCRs in any of the 19 pulmonary MALT lymphomas. In addition, none of the MALT lymphomas with a t(11;18) possessed RF-CDR3 homology (Table VII). Accordingly, the LIDA of the t(11;18)–lung lymphoma M23 did not bind IgG in vitro (Fig. 3). This tentative inverse relation between RF-specificity and the t(11;18) suggests that MALT lymphomas containing t(11;18) do not depend for their expansion on BCR–, CD40–, or TLR9–mediated NF-κB activation (Table VII). The fact that t(11;18)–gastric lymphomas are resistant to H. pylori eradication therapy is in support of this hypothesis (14, 15). By contrast, the t(11;18)–gastric and salivary gland MALT lymphomas with RF BCR may need chronic stimulation by IgG in Ag-Ab complexes in gastric- and salivary gland-MALT lymphomas e.g. as IgG–H. pylori and IgG-ANA complexes, respectively. Finally, the different Ig repertoire of t(11;18)–MALT lymphomas, as compared with MALT lymphomas devoid of this translocation, indicates that this genetic alteration as such provides growth advantage and occurs before the selection process favoring RF-expressing clones.

### MATERIALS AND METHODS

#### Patient material and immunohistochemistry.

Frozen or paraffin-embedded tissue of 23 low-grade and one large cell (M22) MALT lymphomas was obtained from the Westeinde Hospital, The Hague; the Free University Medical Center, Amsterdam; The Netherlands Cancer Institute, Amsterdam and the Academic Medical Center, Amsterdam, The Netherlands. Tumor cell immunophenotypes were determined by immunohistochemical stainings on acetone-fixed cryostat sections and on formalin-fixed paraffin embedded sections using the highly sensitive Powervision− detection system (ImmunoVision Technologies). Monoclonal antibodies used: IgM, κ- and λ-light chains (Becton Dickson), IgG, IgA, CD20 (L26; DakoCytomation), CXCR3 (1C6; BD Biosciences), and αβ7 (Act-1).

M22 was a large cell lymphoma consisting of immunoblasts which had developed in a patient suffering from Sjögren’s syndrome. The original diagnosis MALT lymphoma was not made in our hospital and unfortunately we were not able to recollect material from previous biopsies. This lymphoma most likely developed from a MALT-associated clone given the expression of IgA, CXCR3, the mucosa homing receptor αβ7 as well as the obvious plasmacytoid differentiation with the concurrent lymphoma-related paraproteinemia. These are characteristics highly compatible with extranodal marginal zone lymphomas but extraordinary for diffuse large B cell lymphomas. This study was conducted in accordance with the ethical standards in our institutional medical ethical committee on human experimentation, as well as in agreement with the Helsinki Declaration of 1975, as revised in 1983.

#### DNA and RNA isolation; cDNA synthesis; and IgVH, IgVκ, and t(11;18) RT-PCR.

DNA was isolated from paraffin sections by over night proteinase K digestion. RNA was isolated from frozen sections using the

| t(11;18) | Frequencies of t(11;18) and RF homology among MALT lymphoma cohorts |
|---------|---------------------------------------------------------------|
| n | + | − | n | t(11;18) | RF-CDR3 |
|---|---|---|---|---|---|
| Salivary gland | 6 | 0 (0) | 6 (4) | 114 | 2 (2) | 32 | 13 (41) |
| Gastric | 10 | 5 (0) | 5 (2) | 209 | 50 (24) | 45 | 8 (18) |
| Pulmonary | 4 | 3 (0) | 1 (0) | 113 | 47 (42) | 19 | 0 (0) |
| Other MALT | 4 | 0 (0) | 4 (1) | ND | NA | 4 | 0 (0) |

**Table VII.** Relation between the presence of t(11;18) and RF-CDR3 homology and/or RF activity of MALT lymphoma immunoglobulins

- **c**Numbers in parentheses indicate quantity of cases with RF-CDR3 homology and/or in vitro RF activity.
- **c**The data on t(11;18) and MALT lymphoma localization refer to combined data described previously (69–71).
- **c**Data adapted from Table V. NA, not applicable.
IgM(Invitrogen) and cDNA was synthesized with pd(N) 6 random primers. The IgVH and IgVK genes were amplified using IgVH and IgVK family-specific primer pools combined with the appropriate reverse primer being either JH, Cα, Cκ, Ck, or C′κ. To determine the clonally expressed IgVH gene of the tumor B cells, the CDR3 region was also amplified, directly on cDNA and in nested PCRs on the IgVH family-specific PCR products, using a forward primer specific for the framework region 3 (FR3) in combination with one of the different nested downstream primers specific for JH, Cα, Cκ, or C′κ. The PCR programs and primers sequences were described previously (18, 58). Translocation t(11;18) was determined using the primers and the PCR program as described by Liu et al. (15).

Cloning and sequencing. IgV RT-PCR products of MALT lymphomas were either directly sequenced or cloned into pTOPO-TA-vectors and transformed into TOP10 bacteria (Invitrogen), to generate molecular IgV clones. Sequencing on both strands was performed by an ABI sequencer (Applied Biosystems) using the big dye-terminator cycle-sequencing kit. To identify the IgV germine gene used and the somatic mutations therein, the consensus sequence of each MALT lymphoma was compared with published germline sequences, using the VsBase database (72) and DNAprofile on internet (http://www.mrc-cpe.cam.ac.uk). The IgV sequences of the MALT lymphomas were deposited on GenBank/EMBL/DDBJ (accession nos. AY281324-AY281343, AY466502, AY466503, AY561708 and AY927657-AY927668). The degree of intrachromal variation (ICV) of IgVH genes and IgVK genes was calculated as the mean number of nucleotide differences of each molecular clone as compared with the consensus IgVH or IgVK sequences (18). ICV was considered significant if exceeding 0.4 mutations/clone.

Production of IgM antibodies derived of B-NHL. Recombinant IgMκ antibodies of the lymphomas (lymphoma-idiotype-derived Ab [LIDA]) of patients M5, M6, M8, M9, M11, M14, M21, M22, M23, M41, FL1, FL6/94, FL8/92, and B-CLL26 were produced using the plgH(μ) and plgL(κ) expression vectors as described previously (59). In brief, the IgVH and IgVK sequences of each of these lymphomas, including one EBV B cell clone (SDB), which produces a human monoclonal antibody specific for the erythrocyte Rhesus(D) blood group antigen (57), were each cloned into the plgH(μ) and plgL(κ) vectors respectively. For production of recombinant antibody, 10 μg plgH(μ) and 10 μg plgL(κ) were linearized with PvuI and cotransfected into SP2/0 myeloma cells by electroporation. Subsequently, the transfected cells were selected in genentic-containing medium. The IgVH of M41-A, C, and D were expressed with the IgVκ of M22. An Ig-secreting hybridohybrod of an IgMκ expressing FL FL13 (18), was produced by electroporation with F3B6 (73) as described previously (74). LOS3 and YT-7G3 are an IgMκ and an Igκκ anti-Rhesus(D)-secreting EBV B cell clone, respectively (75). Supernatants were screened for IgMκ or IgMκ using ELISAs as described previously (57). The plgH(μ) and plgL(κ) expression vectors were provided by J. van Es and T. Logtenberg (Utrecht Medical Center, Utrecht, The Netherlands).

LIDA reactivity in rheumatoid factor ELISA and on H. pylori-infected HM02 cells. LIDA reactivity with IgG was determined using the IgM Rheumatoid Factor ELISA kit (Sanquin) according to the manufacturer’s instructions. The plates were developed using TMB as substrate, as described previously (57). For Igλ RF activity, a HRP-labeled rabbit anti-Igλ Ab (DakoCytomation) was used. The “336-354” IgG1 Fc peptide was coated at 4 μg/ml, incubated with LIDAs, followed by mAb anti-IgM-HRP (MH15/1-HRP; Sanquin), and developed as described previously (57). For blocking RF activity, LIDAs were tested in RF-ELISA in the presence of a 50–500 molar excess of the “336–354” IgG1 Fc peptide. To detect LIDA reactivity with H. pylori, ~80% confluent cultures of HM02 cells were incubated with 2×106 CFU H. pylori (strains 1061 and 26695) and cultured for 1 wk. The cells were then fixed with methanol-aceton (1:1), immunocytochemically stained with LIDA, HRP labeled rabbit anti-IgM Ab (DakoCytomation) and developed with AEC as substrate.

Online supplemental material. Table S1 summarizes the IgVH-CDR3 amino acid sequence homology of MALT lymphomas M5, M6, M11, and M41 with other normal and malignant B cells as well as with RF-producing B cells. Table S2 gives an overview of the IgVκ-CDR3 amino acid sequence of the MALT lymphomas of Table III. The supplemental legend to Table V summarizes all lymphomas used in the IgVH-CDR3 homology analysis. The supplemental legend to Table VI depicts the B-CLL cases belonging to the eight IgVH-CDR3 homology groups and their resemblance to homology groups as described previously (49–52). Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.200505068/DC1.

We thank M.E.C.M. Oud, E.J.M. Schilder-Tol, and Dr. M. Spaargaren for performing t(11;18) analyses; J.B.G. Mulder for technical advice and immunohistochemical stainings; Drs. T.A. Out and R.J. van de Stadt for providing sera; Dr. M.H. Delfau-Larue for sharing pathologival information on pulmonary MALT lymphomas; Dr. D. Hamann for help with the RF ELISAs; Drs. K. van Amsterdam and A. van der Ende for providing H. pylori-infected HM02 cells; and C.H. Veltema, M.J.H. Berends, L.J.M. Delahaye, Dr. E.C.M. Onns, and Dr. E.H. Jaspers for providing tissue material.

The authors have no conflicting financial interests.

Submitted: 7 January 2005
Accepted: 3 March 2005

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