SALIVARY GLAND LYMPHOMAS IN PATIENTS WITH SJÖGREN’S SYNDROME MAY FREQUENTLY DEVELOP FROM RHEUMATOID FACTOR B CELLS

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Objective. Patients with Sjögren’s syndrome (SS) have an increased risk of developing monoclonal B cell non-Hodgkin’s lymphomas (MNHL), which frequently occur in the salivary glands (SG). The transition from the benign lymphocyte infiltrate of the gland that characterizes SS to MNHL is not well understood. Previous sequence analyses of the expressed variable (V) region genes have supported the theory that the surface Ig (sIg) plays an important role in the initial expansion of nonmalignant B cell clones and in lymphomagenesis. However, the antigenic specificities of these B cells were unknown. We describe the specificities of the Ig expressed by 2 cases of MNHL that developed in the SG of 2 patients with SS.

Methods. The expressed V genes were amplified by polymerase chain reaction from biopsy specimens, sequenced, and subcloned into eukaryotic expression vectors. The constructs were transfected into P3X63-Ag8.653 cells to obtain 2 monoclonal cell lines, each secreting 1 of the sIg expressed by the MNHL. These IgM were tested by enzyme-linked immunosorbent assay and immunofluorescence against a panel of antigens potentially implicated in SS.

Results. Our main finding was that the Ig products of the neoplastic B cells were rheumatoid factors (RF). Contrary to expectations, they did not react with nuclear or cytoplasmic antigens, double-stranded DNA, self antigens commonly bound by natural autoantibodies, or SG tissue.

Conclusion. Previous analyses of V gene use have provided indirect evidence that SG MNHL may frequently express RF. We demonstrate that this hypothesis is true in the 2 patients we studied. Large-scale studies will be needed to establish the exact frequency of RF specificity among SS-associated MNHL.

Primary Sjögren’s syndrome (SS) is an organ-specific autoimmune disease characterized by a lymphocytic infiltration of salivary and lacrimal glands resulting in xerostomia and keratoconjunctivitis sicca. This infiltrate, which consists of T and B lymphocytes, is usually termed myoepithelial sialadenitis (MESA) (1,2). Primary SS is considered to be a benign autoimmune disease; however, this is tempered by the high frequency of non-Hodgkin’s B cell lymphomas in primary SS patients, estimated to be 44 times greater than that observed in a comparable normal population (3,4). These lymphomas preferentially occur in extranodal sites and are most often identified in the major salivary glands (2,5).

The relationship between MESA and lymphomas is generally considered to represent a multistep process (2,6–8). Early, low-grade lymphomas can be difficult to distinguish from MESA using histologic criteria, and monoclonal B cell populations have been detected recurrently in salivary gland biopsies of patients with primary SS (8–11). Although these B cell clones could be very early lymphomas, recent data indicate that they represent nonmalignant B cell expansions (8,9). The transition from the early clusters of lymphocytes often organized in germinal center (GC)–like structures (9) to MESA and, ultimately, to lymphoma is still not well understood. It has been speculated that chronic stimu-
lation by exoantigens or, more likely, by autoantigens plays an important role by driving the proliferation of specific B cells and thus increasing the risk of occurrence of an oncogenic event. This model is supported by data showing that 1) the variable (V) region gene repertoires expressed by these different B cell populations are highly nonrandom (8,9,12), 2) the same V genes are overexpressed in the initial GC-like structures, in MESA, and in the overt lymphomas (9,12), and 3) these V genes are frequently somatically mutated (8,9,12).

It has been speculated that MESA- and lymphoma-associated clones may frequently express autoantibodies and, in particular, rheumatoid factors (RFs) for two main reasons. First, autoantibodies, including RF, anti-Ro/SSA, and anti-La/SSB antibodies, are frequently detected in the saliva of patients with primary SS (13). Second, the V gene repertoires used by MESA- and salivary gland lymphoma–associated clones are dominated by V genes previously shown to be frequently involved in the production of autoantibodies and, in particular, of RF (8,9,12). For instance, Bahler et al reported results of a study of 5 salivary gland lymphomas, 3 of which used the 51p1 V\textsubscript{H}I and Kv325 V\textsubscript{III} genes that encode the G6 and 17.109 idiotypes, respectively (12). However, with rare exceptions, it is now clear that the reactivity of an antibody cannot be inferred from the analysis of the coding V genes, and that so-called autoantibody-associated V genes are frequently used by antibodies against pathogens (14–16).

For example, although the EVI-15 anticytomegalovirus antibody was shown to use the above-mentioned 51p1 V\textsubscript{H}I and Kv325 V\textsubscript{III} genes, it had no RF activity (17).

We report the molecular study of 2 cases of salivary gland lymphomas and provide direct proof that the Ig expressed by the lymphoma cells are RF. These findings illustrate the links among primary SS, autoantibody-producing cells, and lymphoma.

**METHODS**

**Patients and lymphoma cells.** Lymphoma cells were obtained at biopsy from 2 patients—patient FRI, an 81-year-old man, and patient OPP, a 67-year-old woman.

**Flow cytometry analysis of lymphoma cells.** In both cases of salivary gland lymphoma, a fragment of the biopsy material was mechanically disrupted and the suspended cells were frozen. For 1- and 2-color flow cytometry, 4 $\times$ 10^6 cells were incubated in 96-well plates (Becton Dickinson, Le Pont de Clai, France) for 15 minutes at 4°C with monoclonal antibodies (mAb) at the appropriate concentration, or with control isotype-matched irrelevant antibodies at the same concentration. After washing, 10^4 events were analyzed with a fluorescence-activated cell sorter (FACScan; Becton Dickinson).

son) using CellQuest software (Becton Dickinson). Fluorescein isothiocyanate (FITC)–conjugated anti-human CD5 mAb (clone BL1a) and phycoerythrin (PE)–conjugated anti-CD19 mAb (clone J4.119) were purchased from ImmunoTech (Marcelles, France). PE-conjugated anti-human $\mu$ chain antibody and FITC-conjugated anti-human $\kappa$ chain were purchased from Jackson ImmunoResearch (West Grove, PA) and Caltag (South San Francisco, CA), respectively.

**Construction of the pK-Zeo cassette expression vector.** The detailed construction of this vector will be described elsewhere. Briefly, pK-Zeo is derived from the pZeoSV eukaryotic expression vector (Invitrogen, Groningen, The Netherlands) in which we have cloned the complete complementary DNA (cDNA) of the $\kappa$ chain expressed by the leukemic cells of a patient with chronic lymphocytic leukemia (18). A unique Pvu II site was introduced as a silent mutation in the $\kappa$ constant region exon at amino acid position 17 to allow the shuffling of the upstream V coding sequence as described (15).

**Molecular analysis of the Ig V genes expressed by the lymphoma cells.** Fifty thousand lymphoma cells originating from the biopsies were thawed to prepare total RNA as described (18).

The cDNA was prepared by using synthetic oligonucleotides hybridizing either with the 3′ end of the human $\kappa$ constant region (TG202: 5′-CCTCTAACACTCTCCCCTGTTG) or with the $\mu$ constant region (18). For $\kappa$ chain analysis, 2 sets of seminested polymerase chain reactions (PCR) were performed in a GeneAmp PCR system (Perkin-Elmer Cetus, Norwalk, CT). For the first set of 4 PCRs, we used TG202 and a mixture of 4 leader V family–specific primers (1 $\mu$M) which contained Hind III restriction sites (underlined) at their 5′ ends (V\textsubscript{E}: 5′-GCAAGCTTGAGGGAACCATGGTGTTGCAGA; V\textsubscript{II}: 5′-GCAAGCTTGGAGGGAAACCATGAAGG; V\textsubscript{II}: 5′-GCCAGCTTGGAGGGAAACCATGAGG); V\textsubscript{III}: 5′-GCCAGCTTGGAGGGAAACCATGAGG).

One unit of Expand High Fidelity Taq polymerase (Boehringer Mannheim, Meylan, France), Taq buffer, and 100 $\mu$M dNTP were added to a final volume of 50 $\mu$L PCR conditions were 20 cycles of 1 minute at 94°C, 1 minute at 45°C, and 1 minute at 72°C. Five microliters of each PCR reaction mixture was used to perform a second set by adding 1 of the same leader V\textsubscript{E}, family–specific primers and an oligonucleotide hybridizing to the human C\textsubscript{\kappa} region to allow subsequent cloning into pK-Zeo (UD52: 5′-GCCAGCTTGGAGGGAAACCATGAGG).

**Cloning and sequencing.** The $\kappa$ chain PCR products were digested by Hind III and Pvu II and ligated into pK-Zeo. After transformation into XL1-Blue (Stratagene, La Jolla, CA), several colonies were randomly picked, and plasmid DNA was purified and sequenced with the Thermo Sequenase cycle sequencing kit (Amersham, Les Ulis, France) using the external PCR primers. The H chain PCR products were cloned into pRTM1 expression vector and sequenced as previously described (15,16).

**Transfectomas.** The pRTM1 and pK-Zeo containing, respectively, the H and light (L) chain rearranged V gene
complexes expressed by each lymphoma were cotransfected by electroporation into P3X63-Ag8.653 cells as described (15,16). The composition of the culture medium, which allowed the selection of cells successfully transfected by both vectors, was RPMI 1640 supplemented with 10% fetal calf serum, 1 mg/ml G418 (Gibco BRL Life Technologies, Paisley, UK), and 1 mg/ml Zeocin (Invitrogen). Cell lines secreting sufficient amounts of human IgM were selected for the study.

Analysis of the specificities of the IgM expressed by the lymphoma cells. The IgM were precipitated from culture supernatants in 45% ammonium sulfate as described (16). The IgM RF paraproteins HUL and ALT were isolated from patients with type II cryoglobulinemia (19,20). The reactivities with self-antigens and the affinities ($K_d$) of the IgM RF SMI and HUL were previously characterized (16,19). The concentrations of IgM and the binding to human IgG, actin, thyroglobulin, myoglobin, and DNA were estimated by enzyme-linked immunoabsorbent assay (ELISA) as previously described (16). The polyreactive RF SMI was used as a positive control. The binding to single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) was evaluated by ELISA as described (21), with the following modification: after the transfectoma IgM was incubated for 2 hours at 37°C on microtiter plates coated with either ssDNA or dsDNA, the plates were washed, and bound IgM was identified with peroxidase-conjugated goat antibody specific for human IgM chain (Cappel, Durham, NC) as described (16).

Inhibition studies of RF binding to IgG in solid phase by soluble aggregated IgG were performed as described (16). Each assay was done in triplicate. Indirect immunofluorescence on HEp-2 cells and on normal salivary gland was performed by incubating each IgM at 1–5 μg/ml at room temperature for 30 minutes. Bound IgM was identified with FITC-labeled anti-human μ chain goat antibody (1 μg/ml, 30 minutes, room temperature; Jackson ImmunoResearch). The IgM SMI that reacts with DNA was used as the positive control. The salivary gland tissue originated from surgical material obtained from patients with parotid tumor. Normal salivary gland tissue adjacent to the tumor was used for the immunofluorescence studies.

RESULTS

Patients. Patient FRI was an 81-year-old man when the diagnosis of primary SS was made in 1987, based on the presence of xerostomia, keratoconjunctivitis sicca, low serum titers of RF, antinuclear antibodies (ANA; 1:160 serum dilution), and positive minor salivary gland biopsy findings (lymphocytic focus score 3). In 1989, the patient was again admitted to our clinic because of a painless, large swelling of the right parotid gland.

A right parotid gland biopsy revealed a diffuse lymphoid infiltrate including a majority of small lymphocytes with round nuclei and rare centrocyte-like cells consistent with a small lymphocytic lymphoma or, less likely, a low-grade mucosa-associated lymphoid tissue (MALT) lymphoma (revised European-American lym-
phoma [REAL] classification; see ref. 22). Cytofluorometric analysis of thawed lymphoma cells showed that they expressed a surface IgM\(_k\), CD19, and CD5. The expression of CD5 is consistent with the classification of the lymphoma as a small lymphocytic type (Figure 1).

Patient OPP was a 67-year-old woman who had had primary SS for 3 years when she was admitted to our clinic. The primary SS was diagnosed on the basis of xerostomia, xerophthalmia, the presence of ANA (1:320 serum dilution), including anti-SSA and anti-SSB antibodies, a positive latex test result, and positive labial biopsy findings (focus score 2). She had recently developed a large tumor of the right submaxillary gland, and the tumor was biopsied. The histologic pattern showed diffuse proliferation of lymphoplasmacytoid lymphoma cells, which infiltrated the epithelium, consistent with a somewhat aggressive MALT-type lymphoma (22). Immunophenotyping of the lymphoma cells showed that they expressed a surface IgM\(_k\) and CD19, but were CD5 negative (not shown).

In addition, both patients shared certain features at the time the lymphomas were diagnosed. Bone marrow examinations (bone marrow aspirates and trephine biopsies) did not show evidence of lymphoma infiltration by histologic criteria. Serodiagnoses for hepatitis C infection were negative. Neither cryoglobulin nor monoclonal Ig was detected in the sera.

Molecular analysis of lymphoma V genes. The rearranged V genes were amplified from cDNA prepared from the lymphoma specimens using 5' \(V_H\) or \(V_k\) family-specific leader primers in conjunction with 3' \(J_H\) consensus or \(C_k\) primers. For both patients, single clonal

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Figure 3. Sequences and intraclonal diversity of the V regions of the heavy chains (A and C) and light chains (B and D) expressed by the lymphoma cells of patients OPP (A and B) and FRI (C and D). The molecular analysis of the lymphoma V genes is discussed in the Results. The sequences are designated with the patients' three-letter code names. Numbers in parentheses represent the numbers of 100% identical sequences analyzed (sequences obtained from independent recombinant plasmids). Identities with the germline sequences are indicated by dashes, replacement mutations are shown by upper case letters, and silent mutations are shown by lower case letters.
bands of the expected size were detected after electrophoresis of the V\textsubscript{H} and V\textsubscript{\kappa} PCR products (Figure 2). The amplified rearranged V\textsubscript{H} and V\textsubscript{\kappa} genes were cloned into pRTM1 and pK-Zeo plasmids, respectively, and sequenced. For each tumor, several V\textsubscript{H} and V\textsubscript{\kappa} sequences were analyzed to study the intraclonal heterogeneity. For each patient, the V\textsubscript{H} gene rearrangement used the same V\textsubscript{H} gene and shared the same H chain third complementarity-determining region (CDR3), confirming the monoclonality of the tumors. The sequences were compared with those in VBase and GenBank databases and aligned to the most closely related germ-line genes (Figure 3).

The OPP lymphoma expressed a V\textsubscript{H} gene which had 95% homology with an allelic variant of V3-7 (V3-7*01, DP54) and was rearranged to D21-9 and JH3b. The L chain was most probably derived from the V\textsubscript{\kappa}III gene Humv328h5 (L2, DPK21) (23) (98.2% homology) rearranged to J\textsubscript{k}. The sequence analysis indicated that the FRI lymphoma expressed a mutated copy of the V\textsubscript{H}6 gene V6-1*02 (DP74) (96.4% homology) rearranged to DK1 and JH5a, while the V\textsubscript{\kappa} gene was 100% homologous to the V\textsubscript{\kappa}III gene V\textsubscript{g} (L6). There were additional amino acids (Gly and/or Pro) between the V\textsubscript{\kappa} and J\textsubscript{k} exons, as was frequently described for RF (24).

**Mutation analysis.** The analysis of the somatic mutations provides the following two kinds of information: 1) their presence usually indicates that the lymphoma arose from a GC or post-GC B cell, and 2) their distribution may show evidence of antigen selection. With the exception of the FRI L chain, all the sequences of the rearranged V genes contained several point mutations with respect to the closest germline sequences. These nucleotide differences were most likely due to somatic mutations, since we used a high-fidelity Taq polymerase (error rate \(\sim 8.5 \times 10^{-6}\)), and since most, if not all, of the human V\textsubscript{H} and V\textsubscript{\kappa} genes and their allelic variants have been identified and are now included in VBase. Most of the mutations were seen in all the clonally related sequences, while other, rare mutations were only present in the clonal OPP V\textsubscript{\kappa} sequences. There was no significant clustering of replacement (R) mutations in the CDR above the expected values (binomial model–derived probabilities [25]) that would indicate positive antigen selection.

**Reactivities of IgM\kappa from the lymphomas.** The rearranged V genes of both lymphomas were cloned into eukaryotic expression vectors and electroporated in a nonsecreting murine myeloma cell line. After limiting dilutions, we obtained 2 cell lines, each producing the corresponding IgM\kappa (designated IgM-FRI and IgM-OPP). These IgM\kappa were tested against a panel of autoantigens potentially implicated in primary SS.

The most interesting finding was that both IgM\kappa had significant RF activity in ELISA. Figure 4 also shows the relative affinities of these RF compared with those of a natural RF, SMI (previously evaluated \(K_d \sim 10^{-6} M\) [16]), and of 2 pathogenic high-affinity RF, ALT and HUL (\(K_d \sim 4 \times 10^{-8} M\)) (19). The IgM-FRI had a relative affinity for human IgG similar to that of SMI, while IgM-OPP affinity seemed higher without reaching that of the pathogenetic monoclonal RF. RF activities and relative affinities were checked by inhibition studies using aggregated IgG in soluble phase (data not shown). In contrast, no reactivity could be detected with the following: 1) nuclear or cytoplasmic antigens (by indirect immunofluorescence with HEp-2 cells), suggesting in particular that the IgM\kappa did not react with SSA or SSB antigens (checked by ELISA) (not shown); 2) dsDNA (by ELISA); 3) human salivary gland tissue (indirect immunofluorescence was preferred to Western blot analysis because we were looking for cell membrane–expressed antigens) (not shown); or 4) self-antigens commonly bound by natural autoantibodies.

**Figure 4.** Reactivities of IgM from lymphomas of patients OPP and FRI. Rheumatoid factor (RF) activity of each transfectoma protein was compared with that of the IgM RF paraproteins HUL and ALT and with that of a natural RF, SMI. SMI\textsubscript{10100VH,N} is a negative control IgM with no RF activity (18). The optical density (OD) at 492 nm of the enzyme-linked immunosorbent assay for RF activity measured on plates coated with total human IgG is plotted against the IgM concentration (ng/ml) of the assayed sample. Symbols representing titration of each sample are indicated.
including actin, thyroglobulin, myoglobin, and ssDNA (by ELISA) (Figure 5).

**DISCUSSION**

Primary SS offers a relevant pathologic condition in which to study the relationships between organ-specific autoimmune disease, chronic stimulation of autoantigen-specific B cells, and lymphomagenesis. Previous sequence analyses of the V genes expressed by the GC-like clusters, the MESA-associated B cell clones, and the salivary gland lymphomas supported the theory that the surface Ig was playing an important role in their development and subsequent expansion (8,9,12). The supposition is that the chronic stimulation of specific B cells could favor the occurrence of additional oncogenic events. In addition, the high Ig V gene restriction, along with similar amino acid sequence motifs in the H chain CDR3, suggested to several investigators that MESA clones might be selected for clonal expansion by a unique antigen (8,9).

RF activity was suspected for the following reasons: 1) the repertoire is strongly biased toward V genes previously shown to be involved in RF production, particularly the production of cryoglobulin monoclonal RF (8,9,12); 2) striking similarities in the pattern of somatic mutations exist among MESA clones, salivary gland lymphomas, RF induced after immunization of healthy donors, and hepatitis C virus (HCV)–associated cryoglobulin RF and immunocytomas (see below); and 3) primary SS is frequently characterized by the presence of RF and cryoglobulins. However, prior to this study, there was no evidence directly linking the V region sequences expressed by primary SS lymphomas to the binding specificity of the antibodies they encoded. A definitive conclusion could not be arrived at because 1) these V genes had also been shown to participate in the generation of many other kinds of antibodies, including autoantibodies (14), and 2) RF activity was highly dependent on the somatically generated H chain CDR3 (15,16).

In this study, we demonstrate clearly that the above hypothesis is correct in 2 cases of lymphomas representative of the 2 most frequent types of lymphomas associated with primary SS (5,12). Regarding V gene use, the H chain expressed by the OPP lymphoma is derived from V3-7 rearranged to D21-9 and JH3b. It is highly interesting to note that Bahler et al found the same combination of VH, D, and J H genes expressed by 1 in 5 cases of salivary gland MALT lymphoma and by several MESA-associated B cell clones isolated from different patients with primary SS (8,12). In addition, the lymphoma reported by these authors used Humkv328 (L16), a germline gene highly homologous to Humkv328h5 (L2). The L chains expressed by the MESA clones were not indicated. In the case of our patient FRI, it is noteworthy that although the lymphoma cells use V6-1, a VH gene not particularly known to be associated with RF generation, the expressed IgM has RF activity without crossreacting with self antigens commonly bound by natural autoantibodies. Our data, taken together with previous sequence analyses, suggest that salivary gland lymphomas in patients with primary SS frequently develop from RF B cells.

In keeping with previous studies, with the exception of FRI κ chain, the V genes expressed by the lymphomas from our 2 patients are mutated from germ-line. There is no evidence of intraclonal heterogeneity in the FRI lymphoma, which is consistent with its small lymphocytic type. The low level of intraclonal diversity in the OPP lymphoma is less expected, since ongoing Ig gene hypermutation may be a frequent feature of MALT.
lymphomas. However, the levels of intraclonal diversity in salivary gland MALT lymphomas seem quite variable, and previous cases with poor or no intraclonal diversification in the V region coding sequences have been reported (12).

High R:silent (S) mutation ratios in the CDR are usually interpreted as an imprint of antigen-driven selection, while R:S ratios are usually lower than expected by chance in the frameworks, due to structural constraints. The number of R mutations and the R:S ratios are rather low in the CDR of our sequences. Interestingly, similar features were found in other salivary gland lymphomas as well as in many MESA- and GC-like associated clones. This suggested to several groups of investigators that there might be a selection against R mutation in the antigen-binding sites (8,9,12). The most straightforward explanation is that clonal expansion depends on the preservation of the specificity of the antigen-binding site, but that a mechanism prevents the expansion of B cells with high affinities. Such a mechanism, which seems to operate when the stimulating antigen is present at a high concentration during the GC reaction (26), may play an important role in preventing the occurrence of high-affinity RF in normal individuals. Indeed, low R:S ratios in the CDR seem to be a feature of mutated RF from healthy donors (27).

The finding that the lymphomas from our 2 patients did express RF is consistent with the above data. Correspondingly, it is also noteworthy that a similar pattern of somatic mutations was reported in a 51p1/Humkv325-encoded monoclonal RF from a patient with type II cryoglobulinemia (28) and in HCV-associated immunocytomas (29). In these latter cases, the binding specificities of the Ig from the lymphomas were unknown, but the frequent use of the 51p1/Humkv325 combination suggested to the authors that HCV-associated immunocytoma represented the malignant counterpart of type II mixed cryoglobulinemia.

Why do RF B cells expand and, in some cases, undergo transformation in the salivary glands of patients with primary SS? RF B cells occur frequently in the normal repertoire (for review, see ref. 30) and can present any antigen complexed to IgG to specific helper T lymphocytes, which in turn, activate them (30,31). However, the importance of these mechanisms during normal immune responses remains undetermined. In healthy individuals, the activation of RF B cells is transient and strongly controlled in the periphery, since isotype switching is rare and the affinities of the RF remain low even if somatic mutations do occur (27,30). Regarding primary SS, RF B cells may expand in salivary glands for several reasons: 1) IgG is the autoantigen inducing the MESA, 2) RF crossreactivity or molecular mimicry, or 3) there is abnormal control of RF B cell activation in ectopic GC.

The first possibility is highly unlikely for the same reasons that IgG is not the autoantigen responsible for rheumatoid synovitis, namely, 1) both MESA and synovitis most probably implicate specific T cells (32,33), and 2) anti-Fcγ-specific T cells have never been detected in either disease (30).

The second hypothesis is more attractive, since crossreactivity may be a frequent property of RF (30,34,35). RF may react with a pathogen or with another self antigen present in the salivary gland. This last possibility is less likely, since we did not detect any reactivity of the IgM-FRI and -OPP with salivary gland tissue, although we used relatively high IgM concentrations. The possibility that RF B cells may bind a
pathogen (such as a virus) offers an alternative to simple chronic stimulation as the first step of lymphomagenesis. Indeed, a virus that could specifically infect RF B cells might have oncogenic properties (mechanism 2 in Figure 6). Alternatively, this pathogen complexed to IgG might be acquired nonspecifically by RF B cells (mechanism 3 in Figure 6).

The third hypothesis involves the pathophysiology of the ectopic GC in which RF B cell expansion seems to occur. During common immune responses, antigen-specific B cells are stimulated to proliferate and are selected for higher affinity within GC that normally arise in primary follicles in the lymph nodes or in the spleen. GC in nonlymphoid tissues are uncommon but have been described in other autoimmune or inflammatory conditions (36–38). In normal GC, several mechanisms operate to prevent the expansion and maturation of autoreactive B cells. For most autoreactive B cells, the main mechanism probably involves tolerance at the T cell level. However, RF B cells are distinctive because they can receive help from T cells, and the mechanisms that control their maturation are less known. It is possible that the microenvironment of these ectopic GC might hamper these mechanisms. The increased affinity of the IgM-OPP for IgG compared with that of natural IgM-RF is consistent with this hypothesis. This, together with the large amounts of IgG produced in the salivary gland during primary SS, could result in an uncontrolled, chronic stimulation of RF B cells (mechanism 1 in Figure 6). In this view, it is worth noting that abnormal RF production and maturation frequently occur during diseases in which such ectopic GC-like structures have been reported.

The continual cycling of RF B cells may increase their chance of malignant transformation. However, RF B cells are probably not the only activated B cells in the salivary gland in patients with primary SS, since other autoantibodies are produced, including anti-Ro/La Ig. It is therefore surprising that most MESA-associated B cell clones and the subsequent lymphomas may express RF. One explanation is that RF B cells may have some intrinsic sensitivity to transformation, possibly because most of them belong to the B1 population, a group of long-lived B cells that have self-renewing capabilities (39). In support of this is the well-known fact that RF B cells are frequently involved in many types of monoclonal B cell lymphoproliferations (40). Primary SS–associated lymphomas are the latest additions to this already-long list. However, a similar study in a larger number of patients will be needed to establish the actual prevalence of RF specificity in primary SS–associated lymphomas. Such a study, which could also determine the range of affinities of the expressed RF, might help in understanding the mechanism behind RF expression in lymphomas.

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