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Absence of human parvovirus B19 DNA in myoepithelial sialadenitis of primary Sjögren's syndrome

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Sjögren's syndrome (SS) is an autoimmune disease that mainly affects exocrine glands and presents as persistent dryness of the mouth and eyes owing to functional impairment of the salivary and lachrymal glands. The histological hallmark is local infiltration of lymphocytes, which play a major role in tissue damage. Although B cells represent a minority of the lymphoid infiltrates in SS tissue, they may undergo polyclonal activation and oligoclonal/monoclonal expansion, which may, in turn, predispose them to a still unidentified B cell neoplastic transformation. The process of B cell activation and expansion is presumably a consequence of a chronic, although at present unidentified, antigenic stimulus that activates specific subsets of B lymphocytes.[1 2] This process resembles a germinal centre reaction, in which B cells that express the antigen receptor with the highest affinity for the stimulatory antigen are selected, giving rise to the oligoclonal/monoclonal population seen in the advanced phases of the disease.[1 2]

We recently analysed seven monoclonal lymphoproliferations from six patients with primary SS[1] according to the European Criteria of 1993[3]; (one patient with SS showed a different monoclonal B cell population in two subsequent parotid specimens). DNA was extracted from frozen parotid biopsy specimens, and a B cell monoclonal expansion was verified by the VDJ protocol of amplification.[1] The immunoglobulin antigen receptor (IgR) variable region genes and third complementarity determining region segments (CDR3), which mainly contribute to the antigenic specificity of the IgR, were sequenced.[1]

Comparison of the deduced amino acid sequences of the CDR3 region with antibodies of known specificity reported in a database, showed in six cases a high similarity between VH CDR3 and rheumatoid factor (RF) antibodies, presumably autoantibodies produced against an infectious agent(s), and in one case an antibody putatively reactive with parvovirus B19 (table 1).[1] This suggests that RF producing cells have a role in SS pathogenetic events, as recently confirmed by Martin *et al*.[4]

Because human parvovirus B19 is a common DNA virus, present in 30–60% of the population positive to B19 antibodies,[5] which infects not only erythrocytes and erythroblasts but also megakaryocytes, endothelial and epithelial cells[6] and is possibly involved in several autoimmune diseases,[7–9] we searched for B19 genomes in tissues affected by the SS associated lymphoproliferative processes. This was in agreement with the proposed models for the pathogenesis of MALT lymphoma.[10]

A polymerase chain reaction (PCR) amplification using the Ampliquality B19 kit (Ab ANALITICA srl, Padova, Italy) was performed, in accordance with the manufacturer's instructions, to search for the presence of B19 DNA directly in the parotid specimens affected by SS. The PCR products were analysed on 2% agarose gel stained with ethidium bromide. Positive cases must show a fragment of 218 bp, which derives from amplification of the 1390 to 1608 region of the viral genome.

The region encoding for the β-globin gene was also amplified by PCR to confirm the quality of DNA (data not shown).

Despite the high sensitivity of the PCR approach, B19 DNA was not detected in patient 5, who showed a high homology of

Table 1 Deduced amino acid sequences of VH-CDR3 and VK-CDR3 and high similarity with protein sequence with known antigenic specificity

Cases	Most similar VH or VK germline segments	VH-CDR3 or VK-CDR3 deduced amino acid sequences	Protein sequence with known specificity producing a high significant similarity†	E Value*
VH				
1	V4-59; D2-15; J2	DRYCSGGSCFDWYFD	(U85234) rheumatoid factor	6e-08
2	V3-7; D3-22; J3	GDYYDSSDYYIDAFDI	(U03400) rheumatoid factor	0.48
3	V4-59; D2-15; J2	DRYCSGGSCFDWYFD	(U85234) rheumatoid factor	8e-08
4	V3-11; D3-22; J3	GDYYDSSDSFHDVLI	(U85242) rheumatoid factor	0.002
5	V3-7; D1-20; J3	DLTRRPESDAFDI	(AF092498) antibody against parvovirus B19	0.025
6	V1-8; D7-27; J6	APSWATNYFYYGMDV	(AAB58433) rheumatoid factor	6e-04
7	V1-69; D5-18; J4	EGHKDTTMVTPFDY	(L19288) rheumatoid factor	3e-06
VK				
1	V3-20; J1	QQYGSSPRTF	(225028) rheumatoid factor	0.074
2	V3-20; J3	QQYGTSPFT	(L40727) rheumatoid factor	0.073
3	V3-20; J1	QQYGSSPRT	(225028) rheumatoid factor	0.073
4	V3-15; J1	QHYNNWPPWT	(S67061) rheumatoid factor	0.025
5	V3-15; J1	QQYNNWPPWT	(L48242) rheumatoid factor	
6	V3-20; J1	QQYGSSPPYS	(227950) rheumatoid factor	3.3
7	V3-20; J4	QQYGNSPLT	(L19293) rheumatoid factor	0.16

*Smallest sum probability according to BLAST similarity search programme. The E value is inversely correlated with statistical significance. The search in the database was performed adding 3 AA in position 5′ and 7 AA in position 3′ to the CDR3 sequence for the VH gene and 1 AA in position 5′and 4 AA in position 3′ for the VK gene; †the GenBank accession number of the protein sequence is reported in brackets.

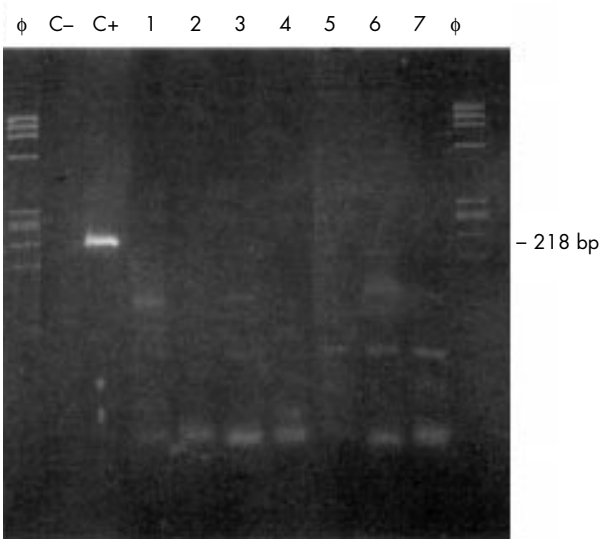

Figure 1 The product of B19 amplification was run through a 2% agarose gel. The positive control (C+), included in the kit, showed a 218 bp band corresponding to the 1390 to 1608 bp region of B19. Patient numbers are reported at the top of the lanes. Lane C– corresponds to a negative control deprived of DNA.

VH CDR3 to anti-B19 antibody; neither was it detected in the other patients (fig 1). These results, according to the proposed model for MALT lymphomagenesis, therefore exclude B19 infection as a local stimulus for parotid MALT lymphoproliferation in SS.

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