Variable regions of a human anti-DNA antibody O-81 possessing lupus nephritis-associated idiotype

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An anti-DNA antibody idiotype (Id), termed O-81 Id, was in the antigen-binding sites of human IgM monoclonal anti-single stranded (ss) DNA antibodies secreted from the O-81 clone, derived from a patient with active lupus nephritis (1, 2). The O-81 Id was specifically detected in circulating immune complex IgG and renal immune deposits of patients with lupus nephritis (3, 4). The paratopes of O-81 were responsible for the idiotype expression of O-81 (unpublished data). These findings suggested that the sequence analysis of O-81 may contribute to understanding the origin of pathogenic autoantibodies in humans.

Poly(A+)RNA was prepared from cells of O-81 clone by using Micro Fast Track (Invitrogen, San Diego, CA) and used to prepare ss-cDNA which was primed with oligo-d(T)12–18 primer. For amplification of the variable regions by polymerase chain reaction (PCR) the following primers were used: VH3 Leader 5'-ATGGAGTTTGGGCTGAGCTGGGTTTTTCTTGTTGCTATTTTAGAAGGTGTCCAGTGTGAGC 3', VK2-Leader 5'-GAAGCTTAGGCTCCCTTGGTCCTC 3', Ck 5'-TCTAGA CTAACACTCTCCTCGTTGAAGCTCCTTTGTTCGCCAGCGCAAG 3'. The products were digested by EcoRI (heavy chain) and HindIII-XbaI (light chain), respectively, and were ligated into the vector pUC118. Sequences were determined by the chain termination method. To avoid misincorporation of TaqI polymerase, DNA amplification was independently performed three times and at least one clone was obtained at each time. The sequences of these clones were identical.

The nucleotide sequences revealed VH3-DHQ52-J4 and VK2-J5. The VH region of O-81 showed the highest homology with the VH3 germ line H11 gene (5), with 86.9% matching (leader-FR3). It was also 86.9% homologous with the FL2-2 gene obtained from cDNA library of fetal liver (6). The VK region was 98.1% homologous with the germ line V6410 gene (7). It was suggested that the VK segment was encoded by the V6410 gene or its relative genes but the VH segment might be originated from an unreported VH3 germ line gene.

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