Biochemical and Biophysical Research Communications **271,** 240–243 (2000)
doi:10.1006/bbrc.2000.2604, available online at http://www.idealibrary.com on IDEAL®

Light Chain Determines the Binding Property of Human Anti-dsDNA IgG Autoantibodies

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Received March 6, 2000

We have previously prepared human anti-double-stranded (ds) DNA IgG Fab clones using phage-display technology. Nucleotide sequence analysis of genes of immunoglobulin (Ig) heavy and light chain variable regions in these Fab clones suggested that the DNA-binding activity of the clones depended on light chain usage. To confirm the role of the light chain in antibody binding to DNA, we constructed in the present study's new recombined Fab clones by heavy and light chain shuffling between the original anti-dsDNA Fab clones. Clones constructed by pairing Fdγ fragments with the light chain from a high DNA-binding clone showed high DNA-binding activities, whereas other constructed clones using light chains from low DNA-binding clones showed low DNA-binding activities. Our results indicate that light chains in anti-dsDNA antibodies can determine the DNA-binding activity of the antibodies. Ig chain shuffling of phage-display antibodies may be useful for investigating the molecular mechanisms for antigen–antibody binding of human autoantibodies. © 2000 Academic Press

Key Words: **lupus nephritis; human monoclonal anti-DNA autoantibody; DNA-binding activity; phage-display technology.**

Systemic lupus erythematosus (SLE) is a typical autoimmune disease but its pathogenic mechanisms remain unknown. The presence of anti-double stranded (ds) DNA antibodies in the serum is a hallmark of the disease (1). In fact, anti-DNA IgG antibodies are detected in the kidney of patients with lupus nephritis (2, 3) and of murine models of lupus (4). However, anti-DNA antibodies are also found in some healthy individuals (5, 6). Murine studies have indicated that non-autoimmune mice develop nephritis when certain forms of anti-DNA antibodies are transferred (7, 8). These findings suggest that not all anti-DNA antibodies are pathogenic in SLE.

To elucidate the molecular basis of pathological effects of anti-DNA antibodies in SLE, we have previously prepared human combinatorial anti-dsDNA IgG Fab clones using phage-display technology (9). These Fab clones were divided into two groups based on the binding activity to dsDNA; clones with a high DNA-binding activity and clones with a low DNA-binding activity. Nucleotide sequence analysis of the immunoglobulin (Ig) heavy chain variable region (VH) genes of these clones revealed that all clones were derived from VH26 germline gene. In contrast, the light chain variable region (VL) genes were diverse. Hence, we postulated that the binding of anti-DNA Fab antibodies might be determined by the VL gene usage.

Recent studies have shown the role of both VH and VL genes in the antibodies (10, 11). VH26 germline gene is used in many antibodies (12, 13) and its characteristics have been elucidated; however, few studies of the paired VL genes have been performed (14). In this study, we investigated the contribution of VL genes to DNA-binding activity using newly constructed Fab clones by Ig chain shuffling.

MATERIALS AND METHODS

Preparation of DNA. dsDNA was prepared from calf thymus DNA (Sigma Chemical Co., St. Louis, MO) by S1 nuclease digestion. ssDNA was prepared by heating dsDNA at 95°C for 5 min followed by cooling on ice.

Anti-DNA Fab clones. Anti-DNA Fab clones 7-11, 1-3, and 5-1 were obtained by phage-display technology as described previously (9). In brief, total mRNA was isolated from peripheral blood lymphocytes of a patient with lupus nephritis who had a high titer of anti-dsDNA antibodies. cDNA was synthesized and Ig sequences were PCR-amplified using the primer sets described previously (15). Amplified DNA fragments of IgG Fd (Fdγ) and light chain were inserted into a phagemid vector (pComb3-H), which can express Fab molecules on the surface of M13 phage. The Fab phage-antibody libraries were panned against solid-phase dsDNA to select DNA-binding phages. Phagemids derived from the selected phages were used to produce soluble anti-DNA Fabs. Nucleotide sequences of the

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0006-291X/00 $35.00

individual Fab clones were determined by an ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, CA).

Shuffling of Ig heavy and light chains in anti-DNA Fab clones. A high DNA-binding clone 7-11 and low DNA-binding clones 1-3 and 5-1 (9) were used for the shuffling of Ig chains (Fig. 1). pComb3-H phagemid vectors containing these anti-DNA Fab genes were digested with *Sac*I and *Xba*I to release the light chain genes. The latter genes and the vectors containing the Fdγ gene were separated on agarose gels and purified. Each light chain gene was relegated with another pComb3-H vector containing the Fdγ gene; we recombined Fdγ with VL between clones 7-11 and 1-3 (Fig. 1A), and between clones 7-11 and 1-3 (Fig. 1B). Figure 1C shows amino acid sequences of VL CDRs of these original Fab clones 7-11, 1-3, and 5-1.

Preparation of soluble Fab. The phagemids constructed by heavy and light chain shuffling were electroporated into XL-1 blue cells and phagemid-bearing cells were cultured in SB medium with 50 μg/ml of carbenicillin, 10 μg/ml of tetracycline, and 20 mM of $MgCl_2$ for 5 h. Fab protein synthesis was induced with 1 mM of isopropyl-thiogalactopyranoside (Sigma) by overnight culture at 30°C. The cells were then pelleted and resuspended in PBS. The suspension was sonicated, centrifuged, and the supernatant containing the soluble Fab was stored until use. The concentration of soluble Fab was determined by ELISA as described previously (9).

ELISA analysis of soluble Fab binding. To examine the DNA-binding activity of soluble Fab, 96-well microtiter plates were coated with 1 μg/well of dsDNA. Other antigens, such as ovalbumin, thyroglobulin, recombinant SS-A, and RNP, were also examined. Recombinant SS-A and RNP proteins were kindly provided by MBL Laboratories (Gifu, Japan) (16). Wells were blocked with 1% BSA in PBS for 1 h. Fab supernatants diluted at 100 ng/ml were added to the wells and incubated for 2 h at room temperature. The wells were washed five times with 0.05% Tween in PBS, and reacted 1 hour with 1/1000 diluted alkaline phosphatase-labeled anti-human IgG Fab antibodies (Pierce, Rockford, IL). The wells were washed and 5 mg/ml of p-nitrophenyl phosphate (Sigma) was added. Absorbance was measured at 405 nm.

RESULTS AND DISCUSSION

Four new clones rH7L1, rH1L7, rH7L5, and rH5L7 were constructed by heavy and light chain shuffling (Fig. 1). Two clones, rH7L1 and rH1L7, were constructed by recombining the Fdγ with light chain between clones 7-11 and 1-3 (Fig. 1A). Other clones rH7L5 and rH5L7 were constructed by recombining the Fdγ with light chain between clones 7-11 and 5-1 (Fig. 1B).

Figure 2 shows binding activities of these clones to dsDNA. The clones of rH1L7 and rH5L7, using a light chain of a high DNA-binding clone 7-11, exhibited a high binding activity, whereas clones rH7L1 and rH7L5, using light chains of low DNA-binding clones 1-3 and 5-1, respectively, exhibited low binding activity. These results indicate that DNA-binding activities of these clones are determined by the light chain usage.

We next examined the binding specificity of Fab clones to DNA. Figure 3 shows the cross-reactivities of these clones to several antigens. The clones did not react with other antigens, such as SS-A, RNP, ovalbumin, and thyroglobulin, as did the original clones 1-3, 5-1, and 7-11 (Fig. 3). These results indicate that the binding of the shuffled clones is also DNA-specific.

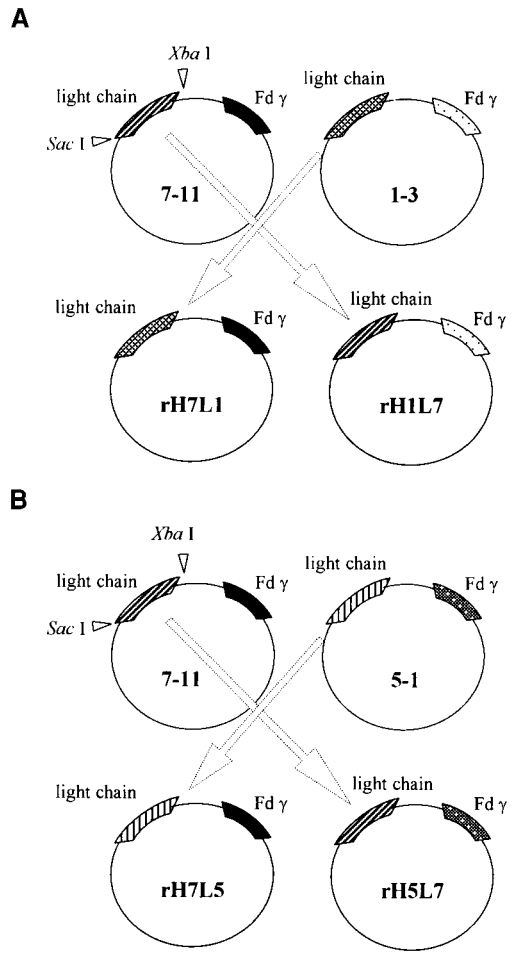

FIG. 1. Schematic diagram of the Ig chain shuffling between anti-dsDNA clones (A, B). VL genes were released with *Sac*I and *Xba*I from pComb3-H vectors containing anti-dsDNA Fab genes. The VL gene was inserted into another pComb3-H containing the Fdγ gene. (A) Recombination of the Fdγ with VL between clones 7-11 and 1-3. (B) Recombination of the Fdγ with VL between clones 7-11 and 5-1. (C) Amino acid sequences of VL CDRs of original Fab clones 7-11, 1-3, and 5-1. The sequences are compared to their most homologous germline genes. Dashes indicate amino acid identities to the germline sequence.

A high level of anti-dsDNA antibodies is a hallmark of SLE. To examine the pathogenicity of anti-DNA autoantibodies, we have previously reported the generation of eight human anti-DNA Fab clones by the phage-display technology (9). Three of these clones showed a higher DNA-binding activity than the other five clones. Nucleotide sequence analysis of these

clones revealed a great diversity of VL genes between the clones in contrast to the very similar VH usage. Thus, we postulated that the difference in DNA-binding activities of these antibodies might be determined by the VL gene usage. In fact, murine studies have shown that light chains influence the binding activity of anti-DNA antibodies (17, 18). Furthermore, the fate of B cells, which produce anti-dsDNA antibodies, can be determined by light chain usage (19, 20).

Our previous study suggested that the lower DNA-binding activity of clones 1-3 and 5-1 might be due to the negatively charged amino acid, aspartic acid in VL CDR1 (9); position 8 in the carboxy-terminal half of CDR1 was substituted with an aspartic acid in both clones. A murine study of anti-DNA antibodies showed that light chains with one or more negatively charged aspartic acid residues in VL CDR1, especially in the carboxy-terminal half, strongly inhibited DNA binding activity (17). This interference of the DNA-binding of the antibodies may be caused by the charge repulsion between DNA and aspartic acid in CDR1. Clones rH7L1 and rH7L5 with light chains derived from low DNA-binding clones 1-3 and 5-1, respectively, reduced DNA binding activities even though they paired Fdγ derived from a high binding clone 7-11. A negatively charged amino acid (aspartic acid) of VL CDR1 in clones 1-3 and 5-1 (Fig. 1C) may reduce the binding activity to DNA. Further experiments on DNA binding using clones bearing aspartic acid replacement of residues in the CDR1 region should enhance our understanding of the DNA-binding activity of anti-dsDNA autoantibodies.

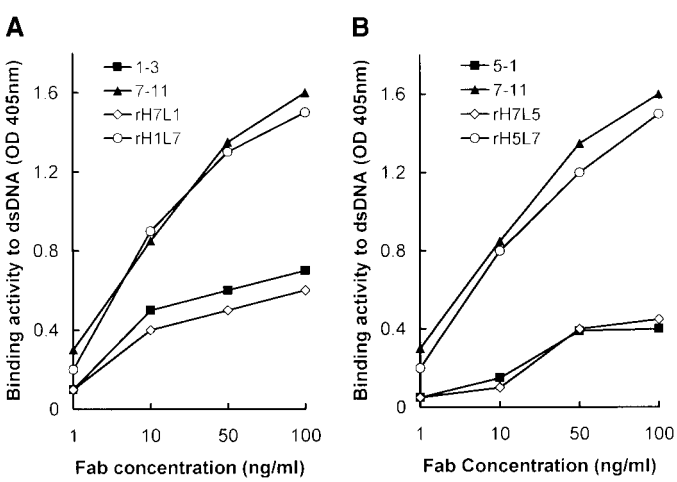

FIG. 2. Binding activities of Fab clones to dsDNA. (A) A low DNA-binding clone 1-3, a high DNA-binding clone 7-11, and their Ig chain shuffled clones rH7L1 and rH1L7. (B) A low DNA-binding clone 5-1, a high DNA-binding clone 7-11, and their Ig chain shuffled clones rH7L5 and rH5L7. Fab concentrations of each clone were determined by ELISA from a standard curve created by human IgG Fab (Sigma) of known concentration. These data are means of two different experiments, each one by duplicate.

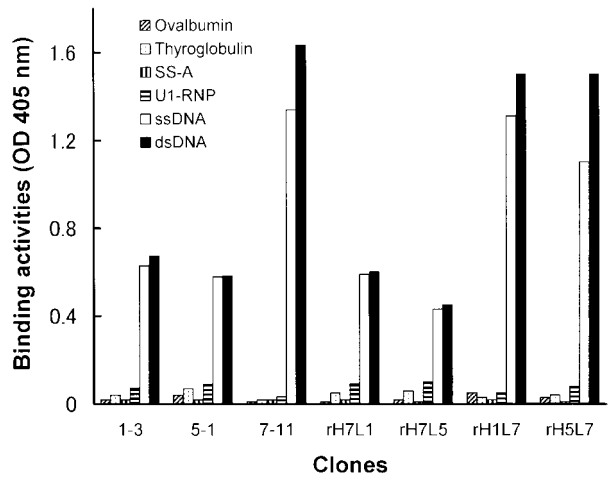

FIG. 3. Cross-reactivities of Fab clones to a panel of solid-phase antigens tested by ELISA. Fabs at 100 ng/ml were added to antigen-coated wells. These data are means of two different experiments, each one by duplicate.

In summary, we generated human anti-dsDNA IgG Fab fragments by phage-display technology. Using these antibodies, we demonstrated that the light chains in anti-dsDNA antibodies determined the DNA-binding activities of the autoantibodies. Furthermore, reconstruction of phage-antibody libraries by Ig chain shuffling may be useful for investigating the molecular mechanisms of antigen-antibody binding of specific human autoantibodies.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan, and a grant from the University of Tsukuba Research Project.

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