urea, provides a unique opportunity to analyze the spectrotypes of antibodies in immune complexes in vivo. We have analyzed the clonotypes of anti-DNA antibodies expressing specific idiotypes in the circulating immune complexes of patients with active lupus nephritis. Serum anti-ss and anti-ds DNA antibodies showed heterogeneous spectrotypes. The antibodies isolated from circulating immune complexes (CIC) had a restricted clonotype and a neutral charge. CIC-derived antibodies were directed mainly to ssDNA and, to a lesser extent, to dsDNA. Importantly, anti-DNA antibodies expressing specific idiotypes, termed 0-81 or NE-1 were detected only in the immune complexes of patients with active lupus nephritis. Anti-DNA clonotypes, including 0-81 and NE-1 idiotypes, were also found in the eluates of renal glomeruli of lupus patients. These results indicate that the subpopulations of anti-DNA antibodies in circulating immune complexes are limited, and may play an important role in the pathogenesis of lupus nephritis (6). We then showed that the incidence of 0-81 Id in circulating immune complexes (CIC) in lupus patients was closely associated with diffuse proliferative lupus nephritis, whereas most cases with negative results for 0-81 Id-CIC showed minimal mesangial alteration or

membranous lupus nephritis.

Thus, detection of 0-81 Id in circulating immune complexes may serve as a useful tool for predicting renal histological changes in lupus patients.

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3. Molecular Cloning, Expression and Epitope Mapping of Autoantigens

Kazuhiko YAMAMOTO

Department of Medicine and Physical Therapy, Faculty of Medicine, University of Tokyo, Tokyo

Autoantibody is one of the major characteristic phenomena in autoimmune diseases. It is known that there is a good correlation between symptoms or diseases and the types of autoantibodies in these patients. Therefore, the study of the mechanisms of autoantibody production is thought to be important in the elucidation of the pathogenesis of autoimmune diseases. The conventional methods to detect autoantibodies have been useful thus far in clinical studies. However, these methods are not sufficient for more precise investigations. Thus, we have applied the recently developed molecular

biology to this field. cDNA which encode autoantigens have been cloned and their protein products were expressed as recombinant fusion proteins in *E. coli*. By this procedure, we could establish simple and reproducible methods to detect autoantibodies. Furthermore, epitope mappings of the autoantigens could be performed using deletion mutants generated by enzymatic manipulations of the cDNAs. These epitope studies will facilitate a more precise understanding of the mechanisms of autoantibody production. Analyses of B and T cells which react with these epitopes would then become

possible. Here a discussion of molecular cloning, the use of ELISA to detect autoantibodies and epitope mappings of the U1snRNP proteins and the SS-B/La protein is presented.

Molecular cloning of autoantigens

In order to obtain cDNAs which encode autoantigens, λ gt11 expression cDNA libraries from human fibroblasts were screened in the sera of patients with a high autoantibody titer. To characterize positive cDNA clones, it was thought that sufficient amounts of proteins encoded by these cDNAs should be obtained. Thus, we immediately recloned the positive cDNA inserts into a plasmid expression vector, pEX-2. This pEX-2 vector produced, after temperature inactivation of the host repressor, a cro- β -galactosidase fusion protein with a polypeptide encoded by the inserted cDNA. This fusion protein was then semi-purified and conjugated to a Sepharose 4B column. For a negative control, vector producing pEX protein (cro- β -galactosidase) was also conjugated to Sepharose 4B. Using these antigen columns, antibodies were purified from patient's sera or rabbit sera obtained from hyperimmunization of these proteins. The purified antibodies were used to immunochemically determine the true protein molecules encoded by the cloned cDNAs in human cells.

A representative case was as follows. In the case of immunofluorescent staining of HeLa cells a purified antibody with a fusion protein exhibited a typical speckled pattern in the interphase nuclei. In immunoblot analysis, this antibody recognized the 22kDa protein. Using immunoprecipitation of the cell lysate and a subsequent RNA analysis, this antibody was shown to precipitate U1snRNA only. From these studies it was found that this cDNA encodes the C protein of U1snRNP (1).

To obtain full length cDNAs, several series of screening using the initially cloned cDNAs and a human fibroblast cDNA library in Okayama-Berg vector were performed. Final full length cDNAs were further subcloned into pEX vectors and positive clones were again selected by patients' sera. Even though there are several ways to produce foreign proteins in *E. coli*, we have used this fusion protein method, because one of our aims is to obtain several different recombinant autoantigens in the

same condition.

Enzyme-linked immunosorbent assay (ELISA) using recombinant autoantigens

Foreign proteins produced in large amounts in *E. coli* have a tendency to form inclusion bodies. In order to purify fusion proteins in inclusion bodies, we have taken advantage of this insolubility of the inclusion bodies. *E. coli*, after induction of a fusion protein, was lysed in a lysozyme solution and the bacterial DNA was solubilized by DNase treatment. Inclusion bodies were then separated by centrifugation and washed with a Triton X-100 solution. Finally the fusion proteins in inclusion bodies were solubilized in 8M urea. By this procedure, fusion proteins of more than 90% purity could be obtained.

For ELISA, these fusion proteins were coated on 96-well multi-titer plates and usual ELISA procedures were performed using peroxidase-conjugated protein A or protein G as the second reagent. To exclude false-positive reactions to bacterial components or to cro- β -galactosidase, sample sera were preincubated with the bacterial lysate produced from *E. coli* carrying the pEX vector. For the negative control, the pEX protein (cro- β -galactosidase), purified using the same method as the fusion proteins, was coated on the other plate. The real datum of each serum was calculated by subtraction of the value against the pEX protein from the value against the fusion protein.

In the results, the reactivities of various auto-immune sera to the recombinant autoantigens were correlated well with anti-RNP or anti-SS-B/La activities determined by the conventional methods. Furthermore, when the reactivities to each component of RNP antigens were analyzed, it was found that the reactivities to the 68K protein and the C protein of U1snRNP were not correlated, indicating that the mechanisms to produce autoantibodies to these two RNP-related antigens might be different.

Epitope mapping of the autoantigens

For the studies of more precise mechanisms of autoantibody production, epitope mapping of the autoantigens was performed. In our method, many deletion mutants are produced by exonuclease III treatment at the 3' ends of the cloned cDNAs in the expression vector, pEX. This treatment does not change the proper reading frame of the cDNA and the resultant in-frame deletion proteins can be

Fig. 1. Schematic diagram of a full-length cDNA encoding the SS-B/La protein and the deletion mutants used for epitope mapping.

directly analyzed for the reactivities of many patients' sera.

An example of deletion mutants of the SS-B/La cDNA for epitope mapping is shown in Fig. 1. FSBF is the full length cDNA. Deletion mutants, FD1-FD7 were produced from this FSBF. In immunoblot analysis, all sera reacted with the products of FD1-FD4. FD5 showed weak reactivities and FD6 was negative with all the sera tested. The difference in length of the DNA between FD4 and FD6 was 43 base pairs (14 amino acids). Therefore, one of the major epitopes should exist in the area between the C-terminals of FD4 and FD6. Moreover, since the difference between FD5 and FD4 is only four amino acids, this epitope (Ept-1) seems to reside in the vicinity of the C-terminal of the FD5 product. Next, in order to exclude the influence of Ept-1 antigenicity, the FSBF cDNA was cut at the Bgl II recognition site and the 3' part was subcloned into the pEX vector (FSBB). Deletion mutants (BD1-BD7) were again constructed from FSBB. From the study of these mutants, a large region (between BD1-BD5, 55 amino acids, Ept-2) was

found to be recognized by all the positive sera. However, there was no distinct single epitope in this region that was recognized by all the sera. There seemed to be several epitopes in this region and each positive serum reacted with some of these epitopes. In addition, another epitope (between BD6 and BD7, Ept-3) was found to be reactive with some patients' sera.

Furthermore, computer analyses of the sequences of the determined epitopes revealed that the most distinct autoepitope (Ept-1) had a striking homology to a retroviral gag polyprotein. These findings indicate that exogenous or endogenous retroviruses may play a role in the initiation of the anti-SS-B/La autoimmunity (2).

Thus far we have studied the epitopes of the 68K, C and A proteins of U1snRNP as well as the SS-B/La protein. These studies have suggested that, in general, universal epitopes exist in these autoantigen molecules. These epitopes were recognized by all the positive sera. Moreover, there were some patients' sera that additionally recognized other minor epitopes. Thus, the mode of recognition of autoantigens seems to be different from that of normal immunogens. There seems to be epitope progression in autoantibody production. More precise studies of the differences of the reactivities between normal immunogens and autoantigens would enable us to understand more detailed mechanisms of autoantibody production.

In conclusion, molecular cloning of autoantigens is an important method for the clinical and basic studies of autoimmune diseases. Recombinant proteins will serve as useful tools for a wide range of immunological studies of the diseases.

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