ANTIBODIES TO EXTRACTABLE NUCLEAR ANTIGENS IN GRAFT-VS-HOST F_1 MICE AS DETERMINED BY IMMUNOBLOTTING: ABSENCE OF ANTI-SM ANTIBODY

Mikio KIMURA, Hiroshi MOHRI, Kaoru SHIMADA and Toshiwo ANDOH

Department of Internal Medicine, The Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108 and 1Department of Hygienic Chemistry, Meiji College of Pharmacy, Yato, Tanashi, Tokyo 188

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SUMMARY: Antibodies to extractable nuclear antigens (ENA) were determined in autoimmune graft-vs-host F_1 (GVH F_1) mice by the immunoblotting method. Major populations of anti-ENA known in human sera including anti-Sm were not detected. It is thus conceived that different kinds of ENA proteins may be recognized by human autoimmune and GVH F_1 sera.

Some graft-vs-host reactions (GVHR) induced in normal F_1 hybrid mice by injection of parental strain lymphoid cells are excellent models of human autoimmune or collagen-vascular diseases including systemic lupus erythematosus (SLE) (1). These mice produce a variety of anti-nuclear antibodies such as those to double-stranded DNA (2), single-stranded DNA, histones (3), poly(ADP-ribose) and extractable nuclear antigens (ENA) (4). All of these autoantibodies are produced in the serum of patients with SLE.

Anti-ENA antibodies contain many antigen specificities (5), each of which is regarded as a useful tool for the diagnosis of autoimmune or collagen-vascular

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diseases. Among them anti-Sm antibody is considered to be a specific marker for SLE (6). The specificity of anti-ENA has been determined by double immunodiffusion (ID) and counterimmunoelectrophoresis. However, their frequent use has been hampered by the relatively low sensitivity of those tests, non-availability of standard sera and the ambiguity of chemical nature of the antigens used. Recently several groups have developed a highly sensitive immunoblotting method to analyze the chemical nature of antigenic proteins of ENA and to discriminate among several kinds of anti-ENA antibodies (7,8). It is performed on nitrocellulose strips on which electrophoretically separated proteins are adsorbed by the “Western blotting technique” (9). In this study we analyzed by immunoblotting the antigen specificities of anti-ENA antibodies produced in GVH F1 mice, with special emphasis on whether or not anti-Sm is detected.

To obtain GVH F1 mice, (C57BL/6 X DBA/2)F1 mice of 8 to 12 weeks of age were injected intravenously with 50 × 10⁶ DBA/2 spleen cells twice with an interval of 1 week. Sera were obtained by retro-orbital puncture at 2 to 4-week intervals and stored at −20°C until use.

ENA were extracted from rabbit thymus acetone powder (Pel-Freez, Rogers, AR, USA) as described previously (10). According to the method of Laemmli (11), 37 µg of ENA dissolved and boiled in the sample buffer was electrophoresed in 15% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) with a slab gel apparatus at an electric current of 20 to 25 mA. Then the electrophoretically separated proteins were blotted onto nitrocellulose membrane (BA85; Schleicher & Shull, Dassel, FRG) by Western blotting as described by Burnette (9). It was carried out in the buffer containing 25 mM Tris, 192 mM glycine and 20% methanol at 80 V/4 cm for 4 hr with a Trans-Blot Cell (Bio-Rad, Richmond, CA). The nitrocellulose membrane was vertically cut into strips, and some of them were stained in 0.1% amidoblack to visualize molecular weight marker proteins (Pharmacia Fine Chemicals AB, Uppsala, Sweden) and various proteins contained in the ENA preparation. Other strips were incubated in 1% bovine serum albumin in phosphate-buffered saline (BSA-PBS) at room temperature overnight to block residual protein-binding sites. After washing four times with 0.05% Tween 20 in PBS (TW-PBS), they were incubated with 1:200 diluted sera (in BSA-PBS) at room temperature for 2 hr. After washing, the strips were incubated with 5 µg/ml biotinylated anti-mouse IgG (H+L) (in BSA-PBS; Vector Laboratories, Burlingame, CA) at room temperature for 2 hr. After washing again, they were incubated with 1:300 diluted streptavidin-peroxidase conjugate (in BSA-PBS; Amersham International plc, Amersham, UK) at room temperature for 1 hr. The
color development was done, after the final extensive washing, by immersing the
strips in 0.5 mg/ml 4-chloro-1-naphthol (Bio-Rad) supplemented with 0.04%
hydrogen peroxide. The molecular weights of the developed bands were obtained
on a standard curve constructed from those of various marker proteins.

The molecular weights of ENA proteins recognized by GVH F1 sera are
depicted in Table I. Anti-ENA began to appear at week 2 after the GVHR
induction and continued to be present until week 10. However, they were not
detectable at all at week 14. This time course of anti-ENA production is largely
consistent with our recent findings obtained by the ELISA system (4). From the
experience with human autoimmune monospecific sera including our own and the
ones from Centers for Disease Control (Atlanta, GA), anti-RNP antibody showed
the 33K and 31K bands, sometimes together with a 71K band. Anti-Sm showed
the 27K and 15K bands, the major band being the latter. Anti-SS/B showed two
bands at 43K and 38K. Anti-Scl 70 showed two duplets, 75K/72K and 67K/64K.
Monospecific anti-DNA, anti-SS/A and anti-centromere sera showed no
immunoblot band. These molecular weights are largely the same as or are
sometimes slightly different from those obtained in other laboratories. The latter
fact is due to the differences in the source, extraction procedure of ENA or the
conditions of SDS-PAGE.

In comparison with the above description, the immunoblot patterns of anti-
RNP, anti-Sm, anti-SS/B or anti-Scl 70 antibodies were not revealed in GVH F1
sera. Anti-17K of some GVH F1 mice may be regarded as the same as anti-Sm
whose antigen showed the molecular weight of 15K in our assay as already
described. When the GVH F1 and the human anti-Sm sera were determined on the
same blotted nitrocellulose, however, they clearly developed the immunoblot
bands at different sites. Likewise, anti-RNP, anti-SS/B or anti-Scl 70 was not
clarified to be present in GVH F1 sera. Whether these undetermined anti-ENA
specificities revealed in GVH F1 sera belong to the already known minor
populations of anti-ENA antibodies or they are completely new ones remains to be
studied.

Ten GVH F1 sera that showed conspicuous immunoblot bands were
determined for precipitating antibodies by ID with the same ENA at a suitable
concentration, and all were negative (data not shown). Thus, the higher
sensitivity of this immunoblotting than ID was clearly revealed.

Many similarities are known between GVH F1 and the spontaneous lupus-
prone MRL/1 mice such as the prominent production of various anti-nuclear
Table I. Molecular weights of the ENA proteins recognized by sera from GVH F1 mice as determined by immunoblotting

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**a**This means that antibodies were not detected.

**b**Figures represent molecular weights $\times 10^{-3}$.

**c**Not tested.

Antibodies, the severity of renal disease caused by immune-complex deposition, T-cell dependency of autoimmunity, non-existence of sex difference and the capacity of Lyb-3-5- B cells to produce autoantibodies (12). However, anti-Sm reactivity has been revealed in MRL/1 mice (13), while not in GVH F1 mice as shown here. There remains a possibility that the probable anti-Sm antibody was not detected by immunoblotting since the antibody was directed to the conformational protein determinants or the RNA moiety of the Sm antigen both of which could be
destroyed in this condition (14). Studies are in progress to elucidate this possibility.

REFERENCES