LETTER TO THE EDITOR

DETECTION OF ANTI-SKIN ANTIBODIES IN SERA OF NUDE MICE USING SODIUM CHLORIDE TREATED SUBSTRATE

Autoantibodies have rarely been demonstrated in sera of nude mice (11). Anti-cytoplasmic antibodies and anti-nuclear antibodies (ANAs) have been reported by a few investigators (10, 11). Recently, we have found a small number of unique antibodies which affect skin in sera of nude mice by using the indirect immunofluorescence (IF) technique.

Sera were obtained from 29 BALB/c nu/nu mice (nude mice) and 20 BALB/c mice which had been maintained under specific pathogen free conditions. All of these mice were 2 months old and female. Sera were used at a dilution of 1:10. For the indirect IF technique, skin, lip mucosa, esophagus, liver and kidney of nude mice as well as guinea-pig lip mucosa, and cultured fibroblasts (Meloy, USA) were used as substrates. By use of the direct IF technique, it was found that these specimens as substrates showed no remarkable deposition of immunoglobulins and complement components. Unfixed cryostat sections of the substrates were first made to react with diluted serum samples for one hour at room temperature (RT) and then stained with FITC-conjugated anti-mouse immunoglobulins prepared in our laboratory (3, 4) for one hour at RT. ANAs were found in 79.3% (23/29) of nude mice (Fig. 1) and 0% (0/20) of BALB/c mice. The incidence of ANAs was similar, irrespective of the kind of substrates (mouse lip mucosa, guinea-pig lip mucosa and cultured fibroblasts). One nude mouse showed anti-basal cell (BC) antibodies (Fig. 2), and another nude mouse showed IF in the inter-cellular substance of the epidermis (Fig. 3). The anti-BC antibodies were also reactive to the basal cells of esophagus, but not to those of kidney or liver.

It is well known that in human bullous pemphigoid and pemphigus associated with systemic lupus erythematosus (7, 8), pemphigoid and pemphigus antibodies are often masked by a high titer of serum ANAs. Thus, these antibodies have not been demonstrated by routine indirect IF technique (1, 8, 9). Recently, Danno et al. (2) found that 2 M sodium chloride (NaCl) solution-treated substrates, in which nuclear antigens were eluated, uncovered pemphigoid antibodies. Based on their findings, we tried to evaluate the presence of anti-skin antibodies in sera of ANA-positive nude mice. To reduce the reactivity for ANAs before staining, sections of substrates were rinsed in 2 M NaCl solution for 30 min at RT.

Using this procedure, we found that anti-BC antibodies can be detected in 21% (6/29) of nude mice. All 2 M NaCl-treated substrates in our study showed a fluorescence of a slightly weak intensity (Fig. 4). In addition, anti-IC antibodies were not demonstrated in examined sera of nuce mice. None of the anti-skin antibodies in nude mice were blocked by the fresh sera of human pemphigus and bullous pemphigoid.

Our study showed anti-BC and anti-IC antibodies as well as ANAs in sera of
Fig. 1. Indirect immunofluorescence findings of antinuclear antibodies in sera of BALB/c nu/nu mice. Substrate is an unfixed section of guinea-pig lip mucosa. (Original magnification × 200).

Fig. 2. Indirect immunofluorescence findings of anti-basal cell antibodies in sera of BALB/c nu/nu mice. Substrate is an unfixed section of guinea-pig lip mucosa. (Original magnification × 400).
FIG. 3. Indirect immunofluorescence findings of anti-intercellular substance of epidermis in sera of BALB/c nu/nu mice. Substrate is an unfixed section of guinea-pig lip mucosa. (Original magnification × 400).

FIG. 4. Indirect immunofluorescence findings of anti-basal cell antibodies in sera of BALB/c nu/nu mice with positive antinuclear antibodies. Substrate is 2 M NaCl treated section of guinea-pig lip mucosa. (Original magnification × 200).
nude mice by using NaCl-treated substrate. A relatively high incidence of anti-BC antibodies was especially noticeable. Previously, when examining the anti-BC antibodies in sera of autoimmune nude mice, we did not demonstrate these antibodies (5, 6). The appearance of anti-BC antibodies thus seems to be characteristic of nude mice.

It has been reported that anti-BC antibodies were found in patients with burn toxemia, in recipients of bone marrow grafts, and in patients with drug-induced skin eruption from practolol (12). However, the precise mechanism of anti-BC antibody production is still obscure. Recently, we found that nude mice engrafted with syngeneic thymus did not develop these autoantibodies. Nude mice engrafted with allogeneic thymus, however, showed them, eventhough T-cell functions of these mice were fully restored (unpublished data). These results suggest that impairment of T and B cell interaction in chimeric nude mice plays a significant role in the appearance of such autoantibodies.

Our observation of the relatively high incidence of anti-BC antibodies may contribute to the precise interpretation of the meaning of anti-BC antibodies in certain human diseases.

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REFERENCES


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