Analysis of Serotype-Specific Antibodies to *Trichosporon cutaneum* Types I and II in Patients with Summer-Type Hypersensitivity Pneumonitis with Monoclonal Antibodies to Serotype-Related Polysaccharide Antigens

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Summer-type hypersensitivity pneumonitis is the most prevalent type of hypersensitivity pneumonitis in Japan. We constructed a sandwich enzyme-linked immunosorbent assay system for diagnosis of summer-type hypersensitivity pneumonitis in which monoclonal antibodies were used to bind serotype-related polysaccharides to plastic plates, and this system was proven to have sufficient sensitivity and specificity.

Summer-type hypersensitivity pneumonitis (SHP) is a unique and prevalent type of hypersensitivity pneumonitis in Japan (2, 3, 11). We have reported that the causative antigen or antigens are present in a high-molecular-weight (high-MW) polysaccharide-rich fraction of culture filtrate of *Trichosporon cutaneum* (high-MW antigen) prepared by gel filtration (16, 20, 21). Two strains of *T. cutaneum*, TIMM1513 (serotype I) and TIMM1318 (serotype II), are known to cause SHP in a serotype-specific manner in most cases (20, 21).

Although it is important in diagnosing SHP to identify anti-*T. cutaneum* antibodies in patients' sera (17), determination of serotype-specific anti-*T. cutaneum* antibodies by conventional immunosorbent assay has been hampered because the high-MW fraction contained two or more of the antigenic molecules of polysaccharide, and they were poorly adsorbed on the immunoassay plates. To overcome these difficulties, we prepared serotype-specific anti-*T. cutaneum* monoclonal antibodies (MAbs), 11-7 and D-8 for serotypes I and II, respectively, and used them to coat the immunoassay plates with serotype-specific polysaccharide antigens. BALB/c mice were immunized with high-MW antigen (100 µg per mouse) of each serotype, and then spleen cells (2×10^8 cells) from these mice were fused with murine myeloma cell line PA-1 (1×10^7 cells) by using polyethylene glycol. Hybridomas secreting serotype-specific antibody were cloned by limiting dilution and enzyme-linked immunosorbent assay (ELISA). The serotype specificity of 11-7 and D-8 were confirmed by immunofluorescence staining of respective *T. cutaneum* strains and ELISA. These MAbs did not stain *Cryptococcus neoformans* B, C, D, and A-D strains by indirect immunofluorescence staining. By adsorbing these MAbs to plastic plates, serotype-specific antigens could be bound to the plates.

The sandwich ELISA was carried out as follows. Polystyrene plates were first coated with MAbs (10 µg/ml, 100 µl per well), at 37°C for 4 h, and then were incubated with high-MW antigens (10 µg/ml, 100 µl per well) at 37°C for 1 h. The plates were then incubated with sample sera (100 µl per well) at 37°C for 1 h, and the antibodies bound were assayed with

alkaline phosphatase-conjugated anti-human immunoglobulin (Ig) class-specific goat antibodies [F(ab')₂] (mu, gamma, or alpha chain specific; MBL, Nagoya, Japan) (500-fold diluted) by incubation at 37°C for 1 h and by measurement of the bound enzyme activity by incubation with *p*-nitrophenyl phosphate (1 mg/ml, 100 µl per well) at 37°C for 30 min. Antibody levels were defined as the optical density at 405 nm of the test sera at the serum dilution at which IgA antibodies in the standard serum give an optical density at 405 nm of 0.5. The antibody levels were compared statistically by Student's *t* test for uncoupled data, and *P* values <0.05 were considered significant.

We examined the specificity and the sensitivity of this system with serum samples obtained from 13 SHP patients with the typical history and clinical findings of SHP (4 male and 9 female; mean age, 45 years; age range, 15 to 75 years), serum samples from 5 patients with farmer's lung (3 male and 2 female; mean age, 42 years; age range, 30 to 52 years), and serum samples from 16 patients with other respiratory diseases (5 with pulmonary tuberculosis, 4 with pulmonary fibrosis, 3 with lung cancer, 2 with bronchial asthma, 1 with bronchiectasis, and 1 with aspergillosis) who had no history of hypersensitivity pneumonitis (6 male and 10 female; mean age, 58 years; age range, 18 to 84 years). Serum samples from 10 healthy volunteers with no history of allergic diseases (4 male and 6 female; mean age, 40 years; age range, 20 to 60 years) were used as controls. The standard serum used was one obtained from a patient which contained antibodies against both antigens of serotypes I and II as tested by ELISA, double immunodiffusion, and immunofluorescence.

Figure 1 shows the levels of serotype-specific IgM, IgG, and IgA antibodies in sera from patients with SHP or other respiratory diseases and healthy volunteers. Levels of anti-serotype I antibodies of all three classes in the sera from SHP patients were significantly higher than those in control sera (Fig. 1A). The antibody levels of IgM and IgA classes in the sera of patients with other respiratory diseases or healthy individuals were negligible. Levels of anti-serotype II antibodies of all three classes in the sera from SHP patients were also significantly higher than those from patients with other respiratory diseases or healthy control subjects (Fig. 1B).

Furthermore, antibody levels of all classes in patients with

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FIG. 1. Anti-serotype I (A) or anti-serotype II (B) antibodies of IgM, IgG, and IgA classes in SHP patients' sera. Levels of antibodies were determined as the optical density at 405 nm (OD_{405}) of sample sera at the same dilution at which standard serum gives an optical density at 405 nm of 0.5. Sample sera were obtained from SHP patients (a), patients with other respiratory diseases (b), and healthy volunteers (c). Bars indicate mean $\pm$ standard deviation.

farmer's lung were significantly lower than those of SHP patients.

The IgG antibody level of the control subjects seemed to be somewhat higher than the IgM and IgA levels. Nevertheless, the differences between the IgG antibody levels of SHP patients and those of the control subjects were highly significant, as shown in Fig. 1. In control experiments for serotype specificity, the plates coated with 11-7 or D-8 and incubated with high-MW antigen of the opposite serotype did not show a positive reaction with standard serum or patients' sera. These results suggest that 11-7 and D-8 antigenic epitopes on serotype-related antigen or antigens of serotype I and II *T. cutaneum* were highly specific for their respective strains and that any other potential epitopes present on these antigen molecules were not cross-reactive with other ubiquitous bacteria or fungi.

In our previous study, there were patients who showed positive reactions in the provocation test to both serotypes of *T. cutaneum*, and both serotypes were found in their houses (4). In the present study, patients were found who were responsive to provocation with serotype I or II *T. cutaneum* and who had not only high levels of antibodies against serotype I or II antigens but also relatively high levels of antibodies against opposite serotypes. On the basis of the specificity of our assay system, it is suggested that these patients were sensitized with both serotypes of *T. cutaneum* which had been growing in their houses.

Many kinds of polysaccharides from fungi or bacteria

other than *T. cutaneum* have been analyzed (1, 5–10, 12–15, 18, 19). In general, these polysaccharides have the repetitive structure of a mannose-containing backbone with a few kinds of side chains which often contribute to the species- or serotype specificity. Although the structural bases of the serotype-related antigens of *T. cutaneum* have not yet been clarified, the antigens may also have a repetitive structure with serotype-related epitopes. Indeed, our recent study on serotype II-specific antigen (D-8 antigen) shows that it contains a repetitive structure unique to *T. cutaneum* (12a). Structural study of 11-7 serotype I antigen is currently under way.

In conclusion, it is possible to detect serotype-specific antibodies in sera from patients with SHP by the sandwich ELISA system we developed. Use of this system will be of great help in the serodiagnosis of SHP.

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