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Immunological Studies on Dermatophytes

III. Further Analyses of the Reactivities of Neutral Polysaccharides with Rabbit Antisera to *Microsporum quinckeanum*, *Trichophyton schoenleinii*, *Trichophyton rubrum*, *Trichophyton interdigitale*, and *Trichophyton granulosum*

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The serological reactivities of polysaccharides isolated from five species of dermatophytes, *Microsporum quinckeanum*, *Trichophyton granulosum*, *T. interdigitale*, *T. rubrum*, and *T. schoenleinii*, with rabbit antisera to these species were studied qualitatively by precipitation in gel and quantitatively by complement-fixation analyses. Significant differences in the serological reactivities of the galactomannans I were detected with antisera to *T. schoenleinii* and *T. interdigitale*. The differences appeared to be related to the specificity of these antisera for the galactofuranose residues in the polysaccharides. Antisera to *M. quinckeanum*, *T. granulosum*, or *T. rubrum* did not detect differences between the galactomannans I. The serological reactivities of the galactomannans II were different with each of the five antisera. The reactivities of the glucans could be correlated with the amount of α $1 \rightarrow 6$ linked glucopyranose residues when antisera to *T. schoenleinii* and *M. quinckeanum* were used.

Polysaccharides isolated from five species of dermatophytes, *Microsporum quinckeanum*, *Trichophyton granulosum*, *T. interdigitale*, *T. rubrum*, and *T. schoenleinii* (1-6), have already been shown to react with antiserum produced in the rabbit to autoclaved mycelium of *Microsporum quinckeanum* (7). (*M. quinckeanum*, *T. granulosum*, and *T. interdigitale* are thought by many mycologists to be variants of *T. mentagrophytes*. The latter two species have also been described as the granular and downy form of *T. mentagrophytes*.) By using this antiserum, significant differences in serological reactivities were found among the glucans and galactomannans II, but not among the galactomannans I, isolated from these five species of dermatophytes. To provide more information about the relationship between serological reactivities, chemical structure, and species specificity of these polysaccharides, they have been studied as antigens reacting with rabbit antisera to *T. schoenleinii*, *T. rubrum*, *T. interdigitale*, and *T. granulosum*. The study of the reactivities of these antisera with mannans I and mannans II, prepared by removal of the galactofuranose units from the homologous galactomannans, facilitated the further interpretation of these serological reactivities.

MATERIALS AND METHODS

Antigens. The isolation of the polysaccharides and their gross chemical structures have already been reported (1-6). Mannans were prepared from the galactomannan polysaccharides by mild acid hydrolysis (8).

Antisera. Antisera to autoclaved mycelia of *T. schoenleinii*, *T. rubrum*, *T. interdigitale*, and *T. granulosum* were produced in the rabbit as previously described (7). A single antiserum to each species was used for these analyses.

Immunochemical tests. The methods used have been previously described (7).

RESULTS

Reactions of polysaccharides with antiserum to T. schoenleinii. Each group of polysaccharides, galactomannans I (a mannan was isolated from *T. rubrum*), galactomannans II, and glucans, isolated from *T. schoenleinii*, *M. quinckeanum*, *T. granulosum*, *T. interdigitale*, and *T. rubrum*, reacted with this antiserum.

In gel diffusion, the precipitin bands obtained with each of the galactomannans I appeared to be identical. However, the precipitin band given by the homologous galactomannan I was more intense than the others (Fig. 1). The mannan I prepared from the galactomannan I of *T. schoen-*

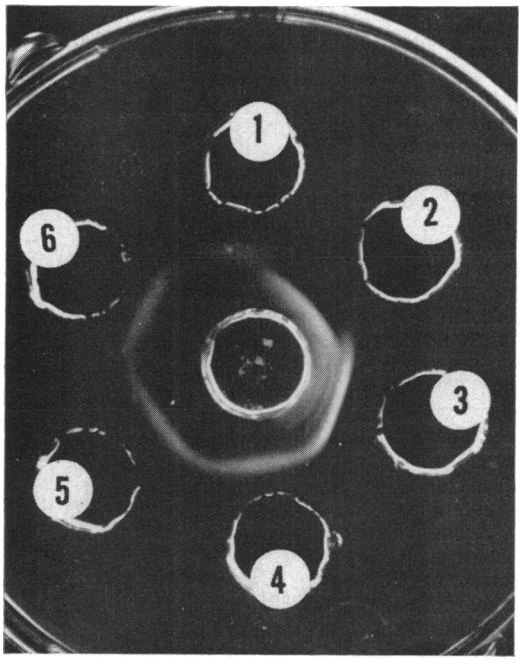

FIG. 1. *Immunodiffusion analysis of galactomannans I with antiserum to T. schoenleinii serum. Center well: anti-T. schoenleinii, undiluted. Peripheral wells: mannan I, T. rubrum* (1); *mannan I, T. schoenleinii* (2; *galactomannans I of T. schoenleinii* (3), *M. quinckeanum* (4), *T. granulosum* (5), *T. interdigitale* (6); *polysaccharide concentration is 1,000 μg/ml.*

leinii by partial hydrolysis showed definite spur formation with the naturally occurring mannan isolated from *T. rubrum* and with its parent polysaccharide, the galactomannan I of *T. schoenleinii.*

Serological reactivities of the galactomannans I were measured quantitatively by complement fixation (Table 1). The homologous galactomannan I was much more reactive than any of the others; the mannan isolated from *T. rubrum* was the least reactive of this group.

Gel diffusion analysis of the galactomannans II with the *T. schoenleinii* antiserum is shown in Fig. 2. The galactomannans II from *T. schoenleinii, T. granulosum,* and *M. quinckeanum* appeared to be identical; those from *T. interdigitale* and *T. rubrum* were much less reactive. The mannan II prepared from galactomannan II of *T. schoenleinii* by partial hydrolysis differed from the parent polysaccharide in this test.

The most reactive galactomannans II with the antiserum to *T. schoenleinii* were those from *M. quinckeanum, T. granulosum,* and *T. schoenleinii* (Table 2).

Reactivities of the glucans with this antiserum are shown in Table 3. The glucan from *M. quinckeanum* was most reactive and the glucan of *T. rubrum* was least reactive.

Reactions of polysaccharides with antiserum to T. rubrum. In gel diffusion, the galactomannans I gave identical precipitin bands with this antiserum. When measured by complement fixation, their reactivities were quite similar (Table 1).

The galactomannans II varied in their reactivities with this antiserum. In gel diffusion, strong precipitin bands were obtained with the galactomannans II from *T. granulosum, T. interdigitale,* and *T. schoenleinii,* but only faint bands were observed with those from *M. quinckeanum* and *T. rubrum.* The galactomannans II from *T. schoenleinii* and *T. granulosum* were the most reactive by complement fixation (Table 2).

The glucans gave no reactions with this antiserum.

Reactions of polysaccharides with antiserum to T. interdigitale. By gel diffusion, only the galactomannans I of *T. interdigitale* and *T. schoenleinii* showed a reaction and gave identical bands. Reactivities of these polysaccharides were meas-

TABLE 1. *Complement-fixation analysis of serological reactivities of galactomannans I*[a]

Source of polysaccharide	Antiserum to:				
	M. quinckeanum[b]	*T. schoenleinii*[c]	*T. rubrum*[c]	*T. interdigitale*[c]	*T. granulosum*[d]
M. quinckeanum	.006	.077	.003	0.400[e]	.007
T. granulosum	.006	.037	.006	0.400[e]	.004
T. interdigitale	.003	.043	.003	0.003	.004
T. rubrum[f]	.002	.110	.003	1.6	.003
T. schoenleinii	.006	.003	.003	0.003	.008

[a] Results expressed as amount of polysaccharide (μg) needed for fixation of 50% of the guinea pig complement in the test system. The difference in optical density, at 541 mμ, between the reaction mixture and the antiserum blank was used as a measure of the amount of guinea pig complement fixed.

[b] Antiserum (0.2 ml) diluted 1:400 was used for analyses (7).

[c] Antiserum (0.2 ml) diluted 1:200 was used for analyses.

[d] Antiserum (0.2 ml) diluted to 1:100 was used for analyses.

[e] *M. quinckeanum* galactomannan I fixed a maximum of 31% and *T. granulosum* galactomannan I fixed a maximum of 43% of the complement in the test system.

[f] A mannan was isolated from *T. rubrum.*

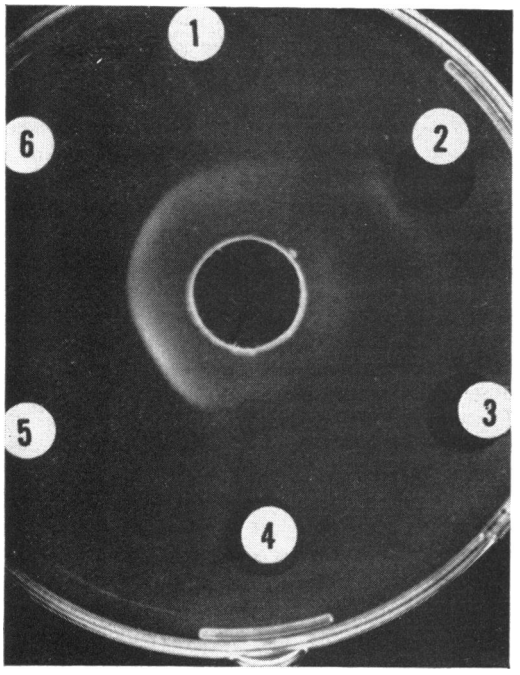

FIG. 2. *Immunodiffusion analysis of galactomannans II with antiserum to T. schoenleinii. Center well: anti-T. schoenleinii, undiluted. Peripheral wells: galactomannan II, T. schoenleinii, 100 μg/ml (1); mannan II, T. schoenleinii, 200 μg/ml (2); galactomannan II, T rubrum, 200 μg/ml (3); galactomannan II, T. interdigitale, 200 μg/ml (4); glalactomannan II, T. granulosum, 100 μg/ml (5); galactomannan II, M. quinckeanum, 100 μg/ml (6).*

ured by quantitative complement fixation (Table 1). The galactomannans I of *T. interdigitale* and *T. schoenleinii* were most reactive, while those from *M. quinckeanum* and *T. granulosum* were least reactive. *T. rubrum* mannan was the least reactive of the polysaccharides of this group.

Of the galactomannans II, only those from *T. granulosum* and *T. schoenleinii* gave strong bands with this antiserum in gel diffusion. When measured by complement fixation, the most reactive galactomannans II were those from *T. granulosum*, *T. schoenleinii*, and *M. quinckeanum* (Table 2).

The glucans gave no reactions with this antiserum.

Reactions of polysaccharides with antiserum to T. granulosum. In gel diffusion, each of the galactomannans I gave identical precipitin bands with this antiserum; their reactivities were similar when measured by quantitative complement fixation (Table 1).

The galactomannans II of *T. granulosum*, *T. schoenleinii*, and *T. interdigitale* gave identical precipitin bands with this antiserum in gel diffusion. Weaker bands were obtained with the galactomannans II from *M. quinckeanum* and *T. rubrum*, but all of the bands joined smoothly.

The galactomannans II differed in reactivities

TABLE 2. *Complement-fixation analysis of serological reactivities of galactomannans II*[a]

Source of polysaccharides	Antiserum to:				
	M. quinckeanum[b]	*T. schoenleinii*[c]	*T. rubrum*[d]	*T. interdigitale*[d]	*T. granulosum*[d]
M. quinckeanum	.005	.004	.100[e]	.005	.115
T. granulosum	.005	.004	.010	.002	.005
T. interdigitale	.050[f]	.042	.050	.085	.018
T. rubrum	.080[f]	.026	.200	.100	.345
T. schoenleinii	.030	.007	.007	.002	.003

[a] Same as Table 1.
[b] Antiserum (0.2 ml) diluted 1:800 was used for analyses (7).
[c] Antiserum (0.2 ml) diluted 1:200 was used for analyses.
[d] Antiserum (0.2 ml) diluted 1:100 was used for analyses.
[e] Maximal complement fixed was 30%.
[f] *T. interdigitale* galactomannan II, 35% complement fixed; *T. rubrum*, 50% maximal complement fixed.

TABLE 3. *Complement-fixation analysis of serological reactivities of glucans*[a]

Source of polysaccharides	Antiserum to:		
	M. quinckeanum[b]	*T. schoenleinii*[c]	*T. granulosum*[c]
M. quinckeanum	.006	.002	0.580
T. granulosum	.048	.006	0.063
T. interdigitale	.035	.003	1.6[d]
T. rubrum	.052	.016	0.950
T. schoenleinii	.025	.007	1.0[d]

[a] Same as Table 1.
[b] Antiserum (0.20 ml) diluted to 1:200 was used for analysis; 1:100 dilution was used for *T. rubrum* and *T. schoenleinii* glucans (7).
[c] Antiserum (0.20 ml) diluted to 1:100 was used for analysis.
[d] Maximal fixation was 30% for *T. interdigitale* and 35% for *T. schoenleinii*.

with this antiserum when measured by complement fixation (Table 2). Those from *T. granulosum* and *T. schoenleinii* were most reactive.

Of the glucans, only that from *T. granulosum* gave a precipitin band in gel diffusion with this antiserum. The glucan from *T. granulosum* was more reactive than the others when reactivities were determined by complement fixation (Table 3).

Reactions of galactomannans I and mannans I with antiserum to the homologous dermatophyte. Analysis of galactomannans I isolated from five species of dermatophytes with antiserum to *M. quinckeanum* showed them to be quite similar in serological reactivities (Table 1). Removal of galactofuranose groups from galactomannans I by partial hydrolysis did not affect the serological reactions with antiserum to *M. quinckeanum* (8). Further analyses revealed differences in serological reactivities among the galactomannans I with antiserum to *T. schoenleinii* and *T. interdigitale* but not with antiserum to *T. rubrum* or *T. granulosum* (Table 1).

To gain more information about the specificity of these antisera, reactions of the galactomannans I and of the mannans I prepared from them with their homologous antisera were studied by immunoelectrophoresis and complement fixation.

A typical immunoelectrophoretic pattern was obtained with a galactomannan I and antiserum to the dermatophyte from which it was isolated (Fig. 3). The galactomannans I of *T. interdigitale* and *T. schoenleinii* gave shorter precipitin arcs than the galactomannans I from the other three organisms. The mannan I of *T. interdigitale* did not show any precipitation with its homologous antiserum. The other mannans I were similar in mobility to their parent galactomannans I when homologous antisera were used in this method of analysis.

The serological reactivities of the mannans I were measured by complement fixation using

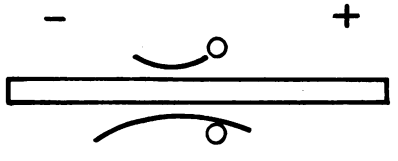

FIG. 3. *Diagramatic representation of immunoelectrophoretic analysis of galactomannans I. Upper well: precipitin arc obtained with galactomannan I of T. interdigitale or T. schoenleinii and homologous antiserum. Lower well: precipitin arc obtained with galactomannan I of M. quinckeanum or T. granulosum, or mannan of T. rubrum, and homologous antiserum. Trough: rabbit antiserum to dermatophyte from which polysaccharide was isolated.*

TABLE 4. *Complement-fixation analysis of reactivities of galactomannans I and II and their derived mannans with antiserum to homologous dermatophyte species*[a]

Antiserum to:[b]	Polysaccharides (μg)			
	Galactomannan I	Mannan I	Galactomannan II	Mannan II
M. quinckeanum....	.001	.001	<.002	>.100[c]
T. granulosum.....	<.001	.002	.005	.005
T. interdigitale.....	.002	>.100[c]	.094	>.200[c]
T. rubrum.....	.003[d]	.003[d]	.300	.750
T. schoenleinii.....	.001	.018	.003	.028

[a] Results expressed as amount of polysaccharide necessary for fixation of 50% of the complement in the test system, measured as the difference between the antiserum control and the reaction mixture.

[b] Antisera (0.2 ml) were used for analysis. They were diluted as follows for galactomannans I and corresponding mannans I, and for galactomannans II and corresponding mannans II: 1:400 and 1:800; 1:200 and 1:100; 1:200 and 1:100; 1:200 and 1:100; and 1:200 and 1:200.

[c] No fixation of complement at concentrations used.

[d] A mannan was isolated from *T. rubrum*.

antiserum to the homologous dermatophyte. The removal of galactofuranose residues decreased the reactivities of the *T. schoenleinii* and *T. interdigitale* polysaccharides but did not affect the reactivities of the *M. quinckeanum* or *T. granulosum* polysaccharides with their homologous antisera (Table 4).

Reactions of galactomannans II and mannans II with antiserum to the homologous dermatophyte. The galactomannans II had been shown to differ in serological reactivities with antiserum to *M. quinckeanum*. (Table 2). Removal of galactofuranose residues from these polysaccharides decreased significantly their reactivities with this antiserum. However, reactivities of the mannans II were still measurable by complement fixation (8).

Immunoelectrophoresis showed that the mannans prepared from the galactomannans II of *M. quinckeanum* and *T. schoenleinii* gave precipitin arcs of greatly decreased intensity with the homologous antiserum. The mannan II prepared from the galactomannan II of *T. granulosum* gave a precipitin arc which was similar both in mobility and intensity to that given by the galactomannan

II with homologous antiserum. The mannans II of *T. interdigitale* and *T. rubrum* could not be studied by this method of analysis because they did not give detectable precipitin bands.

When measured by complement fixation (Table 4), the reactivity of each of the mannans II was less than that of the parent galactomannan II, except for mannan II of *T. granulosum*. This mannan II was similar in reactivity to the galactomannan II from which it was prepared. The reactivities of the polysaccharides from *M. quinckeanum* and *T. interdigitale* were those most affected by the removal of galactose, indicating that the antisera to these dermatophytes had a higher degree of specificity for galactofuranose residues.

DISCUSSION

The galactomannans I showed differences in their serological reactivities with antisera to *T. interdigitale* and *T. schoenleinii* but not with antisera to *M. quinckeanum*, *T. granulosum*, or *T. rubrum*. The galactomannans I isolated from the first two species above have the largest percentages of D-galactofuranose nonreducing end groups, 11.8% and 11.0%, respectively. In contrast, the galactomannans I from *M. quinckeanum*, *T. granulosum*, and *T. rubrum* have only 2.5%, 0.6%, and 0.0%, respectively. It was therefore not surprising that the antisera produced to *T. interdigitale* and *T. schoenleinii* should have a higher degree of specificity for the galactofuranose residues of the galactomannans I. As demonstrated by immunoelectrophoresis and complement fixation, the reactivities of the mannans I of *T. interdigitale* and *T. schoenleinii* were significantly less than those of the parent galactomannans I with homologous antiserum. However, with antisera to *M. quinckeanum* and *T. granulosum*, the reactivities of the homologous mannans I were similar to those of the parent galactomannans I. Thus, these antisera did not appear to have specificity for the galactofuranose residues. The differences in serological cross-reactivities of the galactomannans I, therefore, seem to be related to the specificity of the antisera for galactofuranose residues.

Differences in serological reactivities of the galactomannans II were detected with each of the antisera. It has already been shown (8) that the galactofuranose residues contribute greatly to the serological reactivities of this group of polysaccharides. Each of the mannans II studied showed decreased but different reactivities with antiserum to *M. quinckeanum*, indicating that the mannan chains differ in this group of polysaccharides (8). In the present investigation, it was shown that the antisera varied in their specificity for galactofuranose groups of the galactomannans II. Antisera to *M. quinckeanum*, *T. interdigitale*, and *T. schoenleinii* showed the highest degree of specificity for galactofuranose residues. The antiserum to *T. rubrum* also had specificity for galactofuranose residues but had a higher degree of specificity for the mannan portion of the galactomannans II; differences in cross-reactivities of the galactomannans II with this antiserum might therefore be attributed to differences in either mannose or galactose groups. The antiserum to *T. granulosum* appeared to be specific mainly for the mannan portion of the homologous galactomannan II; variations in cross-reactivities of the galactomannans II with this antiserum may therefore be attributed to differences in linkages in the mannan chains.

Serological reactivities of the glucan polysaccharides appeared to be related to the proportion of α 1 $\rightarrow$ 6-linked D-glucopyranose when antiserum to *M. quinckeanum* was used for analysis (7). With antiserum to *T. schoenleinii*, differences in reactivities among the glucans were not as great as with antiserum to *M. quinckeanum*. The glucan of *M. quinckeanum* was most reactive; the glucan of *T. rubrum*, having the smallest proportion of α 1 $\rightarrow$ 6 linkages, was least reactive. However, with antiserum to *T. granulosum*, the glucans showed much larger differences in reactivities, and these differences could not be correlated with the amount of 1 $\rightarrow$ 6 linkages. None of the other glucans was as reactive as the one from *T granulosum*. This difference in reactivity may be related to the high degree of branching of this polysaccharide as compared to the other glucans.

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