The Role of *Mycoplasma* Membrane Proteins in the Adsorption of Animal Cells to *Mycoplasma hominis* Colonies

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Colonies of *Mycoplasma pneumoniae* and *Mycoplasma gallisepticum* adsorb many types of animal cell, including erythrocytes of various species, spermatozoa and tissue culture cells (Manchee & Taylor-Robinson, 1969). This adsorption can be prevented by treating the colonies with antiserum to whole mycoplasma organisms. The adsorption-inhibiting activity of the serum can in turn be abolished by treating the serum with haptens isolated from mycoplasmas. We have examined the role of specific mycoplasma antigens, particularly those of the cell membrane, in the adsorption of H-HeLa cells to colonies of a strain of *Mycoplasma hominis*.

METHODS

Mycoplasma hominis, SC4, isolated from the urethra of a man attending a venereal diseases clinic, was used throughout. Antisera to whole organisms, purified membranes and soluble fraction were those prepared by Hollingdale & Lemcke (1969). Antisera to lipid-free and recombined membranes and urea extract of whole membranes were prepared in rabbits according to Hollingdale & Lemcke (1972).

In the test for inhibition of H-HeLa cell adsorption, the culture medium, growth of mycoplasma colonies on agar and origin of H-HeLa cells were as described by Manchee & Taylor-Robinson (1969). Suitable colonies of the mycoplasma, preferably in groups of three or four, were surrounded with a glazed porcelain assay cylinder; about six cylinders were used on each agar plate. Each cylinder was filled with a dilution of the antiserum, prepared by a twofold serial dilution in 20% (v/v) normal guinea-pig serum in phosphate-buffered saline pH 7.2. Control cylinders contained 20% (v/v) normal guinea-pig serum in phosphate-buffered saline alone. After the plates had been incubated at 37° for 3 h, the antiserum was replaced with H-HeLa cell suspension in phosphate-buffered saline (2×10^6 cells/ml) and the plates reincubated at 37° for 2 h. The cylinders were emptied and removed, excess cell suspension removed with a few ml phosphate buffered saline and the colonies examined under a stereo-plate microscope. The adsorption-inhibition titre was taken as the reciprocal of the dilution of antiserum which reduced cell adsorption to 25% compared with untreated control colonies. The method for metabolic inhibition was as described by Hollingdale & Lemcke (1969). The metabolic inhibition titre was the highest dilution of antiserum inhibiting a colour change of 0.5 pH.

RESULTS

The effect of whole organisms and fractions of *Mycoplasma hominis* on the adsorption-inhibition and metabolic-inhibition titres of homologous antiserum is shown in Table 1. Metabolic-inhibition titres were included because the corresponding antigens are located on the membrane (Hollingdale & Lemcke, 1969). Antiserum to whole organisms inhibited cell adsorption, although titres were appreciably lower than those of the metabolic-inhibiting antibody. Whole organisms or membranes, prepared according to Hollingdale & Lemcke (1969), removed most of the adsorption-inhibiting activity as well as metabolic-inhibiting antibody. In contrast, the soluble fraction had little effect on either antibody. Similarly, antiserum against membranes purified on sucrose density gradients (Hollingdale & Lemcke, 1969) also inhibited cell adsorption, whereas antiserum against the soluble fraction did not (Table 1). Thus, antibodies responsible for inhibiting adsorption of H-HeLa cells, like those active in causing metabolic inhibition, are directed against the mycoplasma cell membrane.

With antisera to lipid-free membranes and the same membranes recombined with the lipid extracted by *n*-butanol from whole membranes (prepared according to Rodwell, Razin, Rottem & Argaman, 1967), the adsorption-inhibition titres were very similar, and were of the same order as those of antisera against purified membranes or whole organisms (Table 1).

As proteins rather than lipids are important in the interaction of *Mycoplasma hominis* membranes with H-HeLa cells, two extracts prepared by methods known to bring proteins into solution (namely cold aqueous phenol or 8 M urea: Hollingdale & Lemcke, 1969; 1972) were tested for their ability to adsorb antibody from antiserum to whole organisms. Each extract lowered the titre fourfold in adsorption-inhibition tests and 16 to 32 in the metabolic-inhibition test: in neither test did the extracts remove all activity. Antiserum to the urea extract did not block H-HeLa cell adsorption, although it showed weak metabolic inhibition (Table 1).

Table 1. *Inhibition of H-HeLa cell adsorption, and metabolism by antisera to fractions and extracts of Mycoplasma hominis* SC4

	Inhibition titre for	
	Cell adsorption	Metabolism
Antiserum against		
Whole organisms	80	2560
Purified membranes	40	160
Soluble fraction	< 10	< 10
Lipid-free membranes	40	160
Recombined membranes	40	320
Urea extract	< 10	40
Antiserum, against whole organisms, absorbed by		
Nil	160	2560
Phosphate-buffered saline	80	1280
Whole organisms	< 10	< 10
Crude membranes	< 10	10
Soluble fraction	80	1280
Urea extract	40	160
Phenol extract	40	80

DISCUSSION

These results support the suggestion of Manchee & Taylor-Robinson (1969) that protein receptors in the membrane of *Mycoplasma hominis* are involved in the adsorption of H-HeLa cells. Nevertheless, the proteins extracted with urea or phenol did not absorb all of the adsorption-inhibiting antibody from antiserum to whole organisms, possibly due to the absence of certain antigens, which gel-diffusion tests failed to demonstrate, although they were present in preparations of whole membranes lysed with detergent (M. R. Hollingdale & R. M. Lemcke, unpublished observations). The failure to abolish the metabolic-inhibition reaction completely may have the same cause. The greater sensitivity of the metabolic-inhibition tests probably accounts for the fact that the metabolic-inhibiting antibody, but not adsorption-inhibiting antibody, was detected in antiserum to the urea extract.

That the proteins rather than the lipids of *Mycoplasma hominis* membranes are concerned in H-HeLa cell adsorption contrasts with the results of Šöbeslavský, Prescott & Chanock (1968) with *Mycoplasma pneumoniae*. Here, inhibition by antiserum was blocked by lipids but not by proteins or polysaccharides of the organism. Moreover, the lipid hapten of *M. pneumoniae* was also active in tests for complement-fixation, and precipitin and in blocking metabolic-inhibition and indirect-haemagglutinating antibody, whereas lipid extracts from *M. hominis* were virtually inactive in these tests (Hollingdale & Lemcke, 1969). *Mycoplasma hominis* also differs from *Mycoplasma mycoides* var *mycoides* in that it contains no serologically reactive polysaccharide (Hollingdale & Lemcke, 1969).

Thus, the soluble and membrane antigens determining the serological activity of mycoplasmas differ substantially in their chemical nature from species to species. The same diversity may evidently prove to be true of the receptors associated with cell adsorption.

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