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Medium Components Adsorbed to Mycoplasmal Cells

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Washed cells of *Mycoplasma pneumoniae* mixed with complete Freund's adjuvant were capable of producing lethal anaphylactic shock in mice when injected repeatedly into the foot pad. The causative agent is associated with horse serum contained in the culture medium but cannot be removed from mycoplasmal cells by repeated washing with phosphate buffered saline. Mycoplasmas grown in medium containing rabbit serum had a similar effect. No lethal anaphylaxis occurs when *M. pneumoniae* is injected without adjuvant.

We have been interested in the prevention of potential anaphylaxis in humans which might result from vaccine injections. One such vaccine is that of *Mycoplasma pneumoniae* which is cultivated in the presence of animal sera. Components from the culture medium are known to adsorb to mycoplasmal cells and to interfere with serological reactions [1,2]. In this study we examined the role of adsorbed culture medium components in the production of anaphylaxis. Although the guinea pig is considered the animal of choice for production of anaphylaxis, we chose the mouse since its response is more equatable to the effects seen in humans.

MATERIALS AND METHODS

Female mice of the ICR strain, six weeks of age, were used. *Mycoplasma pneumoniae,* FH strain, was grown in Chanock's liquid medium, supplemented with either horse or rabbit serum, at 36°C ± 1° for three to four days. The preparations used for injections of mice consisted of (*a*) culture fluid of *M. pneumoniae* (CF); (*b*) 20 percent horse serum in saline (HS); (*c*) *M. pneumoniae* cells (2×10^7 colony forming units washed three times with phosphate buffered saline, pH 7.6) (MPC); (*d*) *M. pneumoniae* cells and horse serum (MPCHS); (*e*) *M. pneumoniae* cells emulsified with complete Freund's adjuvant (MPCFA); (*f*) 20 percent rabbit serum in saline (RS); (*g*) 25 percent fresh yeast extract (FYE); (*h*) 2.1 percent PPLO broth (PPB); (*i*) penicillin G, 100 IU per ml; (*j*) 0.2 percent bovine serum albumin, five times recrystallized. Sensitization was achieved by three injections of 0.1 ml amounts of preparations into the foot pad at various time intervals (Tables 1 and 2). The fourth injection served as the challenge dose for development of anaphylaxis. The mortality due to shock was assessed one hour following challenge. An immunosuppressive control, achieved by administration of cyclophosphamide (40 mg per kg, daily during the three-week sensitization period), was included.

RESULTS

Table 1 presents the schedules of injections and the mortality rates of mice treated with various preparations. Mice sensitized with *M. pneumoniae* in Freund's adju-

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Table 1
Causative Agents of Lethal Anaphylactic Shock in Mice

Injection into Foot Pad							No. of Deaths	No. of Mice	% Mortality
1	2	3	4 (Weeks)	5	6	7			
MPCFA[a]	•	MPCFA	•	MPCFA	•	HS	14	15	93.3
•	•	•	CF[b]	CF	CF	CF	10	12	83.3
•	•	•	HS[c]	HS	HS	HS	9	10	90
•	•	•	HS[d]	HS	HS	HS	0	15	0
•	•	•	MPC[e]	MPC	MPC	MPC	0	15	0
•	•	•	MPCHS[f]	MPCHS	MPCHS	MPCHS	19	20	90.5
•	•	•	BSA[g]	BSA	BSA	BSA	12	14	85.5
•	•	•	RS[h]	RS	RS	RS	8	10	80
•	•	•	HS	HS	HS	RS	0	10	0
•	•	•	RS	RS	RS	HS	0	10	0

[a] *M. pneumoniae* cell emulsion with Freund's adjuvant
[b] Culture fluid of *M. pneumoniae*
[c] 20 percent horse serum
[d] Cyclophosphamide (40 mg/kg/day) injection during three weeks of sensitization
[e] *M. pneumoniae* cells (2×10^7 CFU washed three times with PBS)
[f] *M. pneumoniae* cells and HS
[g] 0.2 percent bovine serum albumin
[h] 20 percent rabbit serum

TABLE 2
Assay System for Lethal Anaphylaxis Caused by Medium Components Attached to *Mycoplasma pneumoniae* Cells

Injection into Foot Pad							No of Deaths	No. of Mice	% Mortality
1	2	3	4 (Weeks)	5	6	7			
•	•	•	CF[a]	CF	CF	HS[b]	9	10	90
•	•	•	CF	CF	CF	FYE[c]	0	20	0
•	•	•	CF	CF	CF	PPB[d]	0	20	0
•	•	•	CF	CF	CF	PCG[e]	0	20	0
•	•	•	CF[f]	CF	CF	RS[g]	8	10	80
MPCFA[h]	•	MPCFA	•	MPCFA	•	HS	14	15	93.3
MPCFA	•	MPCFA	•	MPCFA	•	FYE	0	20	0
MPCFA	•	MPCFA	•	MPCFA	•	PPB	0	20	0
MPCFA	•	MPCFA	•	MPCFA	•	PCG	0	20	0
MPCFA[i]	•	MPCFA	•	MPCFA	•	RS	13	15	86.6

[a] Culture fluid of *M. pneumoniae*
[b] 20 percent horse serum
[c] 25 percent fresh yeast extract
[d] 2 percent PPLO broth
[e] 100 IU/ml penicillin G solution
[f] Same as [a] but horse serum was replaced by rabbit serum in culture medium
[g] 20 percent rabbit serum
[h] *M. pneumoniae* cell emulsion with Freund's adjuvant
[i] Same as [h] but horse serum was replaced by rabbit serum in culture medium

vant and challenged with horse serum exhibited a mortality rate of 93.3 percent; those sensitized with culture fluid and challenged with the same culture fluid showed a mortality rate of 83.3 percent. Control groups sensitized and challenged with mammalian sera behaved as expected, i.e., high mortality. Immunosuppressed animals, mice challenged with heterologous serum, and mice treated with *M. pneumoniae* in the absence of Freund's adjuvant survived. These results suggest that anaphylaxis resulting from injection of *M. pneumoniae* is due to proteins from horse serum tightly adsorbed onto the mycoplasmal cells. The usual washing procedure is inadequate to remove these adsorbed proteins.

A protocol was devised to confirm that the serum component of the culture medium was the cause of anaphylaxis (Table 2). Only horse serum or rabbit serum caused lethal anaphylaxis in mice sensitized with either culture fluid or *M. pneumoniae* emulsified in complete Freund's adjuvant.

DISCUSSION

The ICR strain of mice, known to be a high responder group, proved to be useful in the study of anaphylaxis under the conditions of sensitization and challenge employed in this study. Our results demonstrate that serum proteins from the culture medium become irreversibly adsorbed onto *M. pneumoniae* cells and that immunization with these mycoplasmas can sensitize mice to these proteins. Although reports exist demonstrating that animal serum proteins attached to mycoplasmal cells interfere with serological reactions [1,2], this study is the primary demonstration of the development of sensitivity to lethal anaphylaxis. The method employed in this study could apply to quality control of vaccines. Additional studies are required to identify the specific proteins in serum which become adsorbed to *M. pneumoniae* and which are responsible for the anaphylaxis.

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