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大腸桿菌莢膜多醣類菌株外毒素之病原性之研究

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摘 要

以生化特性相似的大腸桿菌，探討外毒素在莢膜多醣類菌株（ Capsular polysaccharide-synthesizing strains；CPS）之病原性角色。結果：

(1) CPS株及NON-CPS株之差在於細菌懸浮液（ LD_{50} ：3.2mg／kg 及 70.4mg/kg ）及培養上清液（ LD_{50} :2.8mg/kg及 44.0mg/kg ）對小白鼠腹腔注射之致死毒性，亦表現於腹腔細胞數，腹腔細胞外細菌數，血清之殺菌活性及對內毒素的去毒作用。但兩者之內毒素對小白鼠則具相似的毒性。

(2)但當病原性CPS株經Mitomycin-C 處理及加熱後，其對小白鼠之毒性（ LD_{50} ：260mg/kg）則類似NON-CPS株（ LD_{50}：280mg/kg），其未經Mitomycin-C處理僅加熱之CPS株則具較強之毒性（ LD_{50} ：140mg/kg）。

(3)微量純化外毒素會加強CPS株對小白鼠之感染能力（ LD_{50} 由 2.51mg /kg減少到 0.08 mg/kg）。

(4) CPS株及NON-CPS 株之細菌懸浮液及培養上清液等免疫小白鼠對病原性株感染及外毒素注射之免疫能力都具非常明顯的差異。

(5)若以Mitomycin-C，spectinomycin，polymyxin-B，Colistin 處理去外毒素之CPS 株，其免疫小白鼠對病原性株之感染具較佳之防禦能力，但對外毒素攻擊之抵抗能力則大大的下降。

故不論細菌之感染能力或對小白鼠之免疫能力，細菌之病原性與外毒素之產生能力息息相關。即外毒素不但是病原性大腸桿菌之最重要病原性因子，亦是小白鼠獲得免疫能力之主要抗原物質之一。

緒 言

前曾報告大腸桿菌在Proteose peptone glycerine salt 培養基，可分爲產生粘稠菌落之莢膜多醣類菌株（ Capsular polysaccharide-synthesizing strains；CPS）及NON-CPS株[1-6]。並由 CPS 株分離到小白鼠致死毒性之外毒素，粗毒素經 40％ Saturated ammonium sulfate 沈澱及膠層過濾，可得琥珀色混濁之純化外毒素[7-8]。此種外毒素會引起大白鼠之白血球及血小板的減少，紅血球脆性的增加，血管通透性的改變及低血壓等[9]。故

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推測它可能是宿主被革蘭氏陰性桿菌感染後引起繼發性休克（Secondary shock）之毒性物質。

本文擬以另一角度來探討此種可釋放於培養基及純化之外毒素，是否是病原性大腸桿菌之重要病原性因子，及其在宿主與細菌感染間可能扮演的角色。

材料與方法

菌種：使用之大腸桿菌都來自臨床患者之糞便檢體。其中具病原性之 E－4 株是在PGS培養基產生粘稠菌落之菠膜多醣類產生株（CPS）[1-6]，而非病原性 E－2 株則僅產生光滑型菌落之 NON－CPS 株，兩者之生化特性及生物活性如表 1 所示。

外毒素之製備：依照前報的方法[7-8]。係以40 % Saturated ammonium sulfate 沈澱，沈澱物再經 Sephadex G－150（Pharmacia Fine chemicals，Sweden）column在 void volume 附近獲得琥珀色混濁層液峯是謂純化外毒素。

內毒素之製備：根據*GALANOS* 氏等[10]之方法，將乾燥細菌置於抽取液〔石炭酸（90 gm之結晶石炭酸加 11ml 蒸餾水），氯仿（Chloroform）和 Petroleum ether ；其比例是 2：5：8〕，混合液經 30 分鐘離心（5,000 xg，4 ℃）後，取含脂多醣的上清液通過濾紙（Whatman No. 2），細菌再連續抽取 3 次。抽出液中的 Petroleum ether 和氯仿以 rotary evaporator 在 30 ℃— 40 ℃ 處理完全去除後，已形成結晶的石炭酸加入足量的蒸餾水溶解之。並繼續滴入蒸餾水直到脂多醣形成沈澱物。經高速離心 10,000 x g，30 min，4 ℃）後倒掉上清液，以濾紙去除殘餘水份，再以少量的 80 % 石炭酸洗 2 — 3 次。最後沈澱物以乙醚（ether）洗 3 次，去除殘留的石炭酸，去除乙醚後之脂多醣體再加蒸餾水後經高速離心（10,000 x g，1 hr，4 ℃

Table 1. Biochemical Characteristic of CPS and NON-CPS Strains

Property	CPS*	NON-CPS	Property	CPS	NON-CPS
Hydrogen sulfide	—	—	Inositol	—	—
ONPG	+	+	Rhamnose	+	+
IPA	—	—	Sorbitol	+	+
VP	—	—	Maltose	—	—
Indole	+	+	Sucrose	—	—
Citrate ultilization	—	—	Nitrate reduction	+	+
Lysine decarboxylase	—	—	Mucoid colony on PGS medium	+	—
Ornithine decarboxylase	+	+			
Arginine dihydrolylase	—	—	CPE (Adrenal cells)	+	+
Urease	—	—	Hemagglutination (guinea pig)	+	—
Malonate	—	—			
Glucose	+	+	Mice lethality (i.p.)	+	—
Mannitol	+	+	Antigenic structure	086a@ :K61	086a :K61
Adonitol	+	+			
Arabinose	+	+			

*CPS: Capsular polysaccharide-synthesizing Escherichia coli. Strain which developed mucoid colony on PGS medium.

@ Weak reaction by slide agglutination.

）後將此脂多醣體再溶於蒸餾水中，並冷凍乾燥之。

抗生素預處理細菌懸浮液（Antibiotics-treated bacterial suspension）的製備：經37℃靜置培養16小時後，其菌體先以0.02 M Tris-(hydroxymethyl)-amino-methane加0.9% NaCl的緩衝液（pH 8.0）清洗3次，清洗之菌體再平均分置於含各種抗生素的0.15 M Tris-Chloride pH 6.6的緩衝液，並混合之。使用之抗生素包括Mitomycin-C（Kyowa, Japan；1 μg/ml），Spectinomycin（Upjohn；200 μg/ml），Polymyxin-B（Meiji；1,000 μg/ml）及Colistin-M（Meiji；500 μg/ml）經37℃，30分鐘處理後，以高速離心機離心15分鐘（1,000 x g）。處理細菌再分別加入含10倍量同類抗生素緩衝液繼續處理30分鐘，最後以0.02 M Tris-Chloride緩衝液（pH 8.0）清洗3次，泡成適當的細菌懸浮液，立刻置於100℃處理15分鐘[11-12]。

腹腔細胞之研究：以CPS株及NON-CPS株懸浮液（1 mg/mouse）或其培養上清液（0.5 ml/mouse）注射ICR株小白鼠後，定時將5 ml無菌PBS注入已麻醉或全採血之小白鼠腹腔，充分混合後採出腹腔洗液，以血球計算器計算腹腔細胞數及做白血球分類。腹腔洗液以800 x g離心10分鐘後，取0.1 ml上清液於100 ml的PBS加以稀釋，再取0.1 ml稀釋液於液態的Heart-infusion（HI）agar並混合均勻之，經37℃，48小時培養後計算其菌落。沈澱做抹片後觀察腹腔細胞之吞食作用。結果之計算以每組5隻之平均值表示之[13]。

血清之殺菌活性（Bactericidal activity）及內毒素之去毒作用（Endotoxin detoxification activity）：

(1)血清之採集：將CPS株或NON-CPS株感染或其培養上清液作用6小時後之小白鼠，由頸部採全血，血清分離後置於－80℃備用。

(2)血清殺菌活性之試驗：取10倍稀釋之18小時*Staphlococcus aureus* 209 p培養液0.1 ml於1 ml血清及HI broth混合液中，經37℃水浴槽培養4小時後，分別以Mannitol salt agar計算其菌落。以細菌增加率表示血清之殺菌活性，其計算方法是處理後之菌數減原菌數再除之並乘100%。

(3)細菌內毒素之去毒作用：把0.1 ml血清及5 μg內毒素（*Escherichia coli* 0127：B8；Lipopolysaccharide；Difco）混合液置於37℃水浴槽4小時後，以PBS把5 μg內毒素稀釋成0.04 μg/ml，然後和等量Actinomycin-D（32 μg/ml；Sigma）混合之，取此混合液1 ml/mouse注射於ICR株小白鼠腹腔，48小時內觀察其死亡率[14]。

Candida killing：各取0.2 ml的7 X 10^6 cell/ml的Peritoneal leukocytes（於Medium 199），Fetal calf serum，Phosphate buffer及7 X 10^6 cell/ml的24小時*Candida albicans*培養液等於37℃水浴槽中處理1小時，每15分鐘搖動1次。將試管取出置於冰塊中後加入0.2 ml的Na deoxycholate（2.5% in saline；pH 8.7）來溶解白血球，5分鐘後每支試管加4 ml的0.01% Methylene blue，以1,500 x g離心5分鐘，取沈澱物以血球計算器計算200個*Candida albicans*，記錄*Candida albicans*的死亡率[15]。

結　果

對小白鼠致死毒性之差異：以具類似生化特性及抗原性之CPS株及NON-CPS株，做爲病原性大腸桿菌病原性因子之檢討。雖然在18小時HI broth靜置培養中獲得類似之菌體濕重（1.13 g/l及1.24 g/l），但對小白鼠之病原性却具非常明顯的差異，其中CPS株不論是細菌感染（LD_{50}：3.2 mg/kg）或其培養上清液（LD_{50}：2.8 mg/kg），及純化外毒素（LD_{50}：0.3 mg/kg）[8]都較NON-

Table 2. Acute Toxicity of Mitomycin-C Pretreated Bacterial Suspension to Mice

Strains	Treated with Mitomycin-C	LD_{50} (mg/kg)
CPS (E-4)	Yes	260
	None	140
NON-CPS (E-2)	None	280

CPS 株強。但兩者之菌體內毒素則具相似之小白鼠急性毒性（死亡率皆 42.9％）。如表 2 所示Mitomycin-C 處理之加熱 CPS 株，則對小白鼠之致死毒性（LD_{50}：260.0 mg/kg）較未經 Mitomycin-C 處理之同一株細菌之急性毒性減弱將近 1 倍（LD_{50}：140.0 mg/kg），其結果類似 NON－CPS 株（LD_{50}：280.0 mg/kg）。證明 CPS 株與 NON－CPS 株引起小白鼠死亡之主要原因是會受 Mitomycin-C 去毒作用之毒性物質，其眞正機轉則尚未明瞭。

對小白鼠腹腔細胞之影響（表 3）：以 0.5 ml 之 CPS 株細菌懸浮液（2 mg/ml）感染小白鼠 2 小時後，其腹腔細胞數由對照組之 38 X 10^5 cell/mouse 快速增加到 91 X 10^5 cell/mouse 然後維持在 73 X 10^5 cell/mouse 之間，白血球之噬菌作用亦由 77.3 ± 5.4％增加到 87.3 ± 3.2％及 86.2 ± 3.8％，而細胞外細菌數則一直維持相當多數（> X 10^7 cfu/mouse）並無減少的傾向。反觀 NON－CPS 株注射之小白鼠之腹腔細胞數慢慢增加，由 2 小時之 39 X 10^5 cell/mouse 到 6 小時的 73 X 10^5 cell/mouse。而噬菌作用則以感染後 4 小時達最高峯 95.3 ± 2.2％，腹腔內細胞外菌數 6 小時降到 76 X 10^4 cfu/mouse，故 CPS 株比 NON－CPS 株對小白鼠腹腔細胞具較強的趨化性，抗噬菌作用及本身繁殖能力。以 CPS 株 18 小時 HI Broth 培養上清液注射小白鼠後，對小白鼠腹腔細胞之影響與 NON－CPS 株間仍有差異（表 4）。不論 CPS 株或 NON－CPS 株對小白鼠腹腔細胞多少有抑制作用，較 HI broth 注射

Table 3. Changes of Total Numbers of Intraperitoneal Leukocytes, Phagocytic Activity and Extracellular Bacteria in Mice after Bacterial Infection

Challenger	After treatment (hr)	No. of leukocytes (x 10^5 cell/mouse)	Phagocytosis	No. of extracellular bacteria (x 10^4 cfu /mouse)
CPS (E-4)	2	91±22#*	77.3±.543*	1,000
	4	73±12***	87.3±3.23**	1,000
	6	73±7	86.2±3.75	1,000
NON-CPS (E-2)	2	29±7	93.7±2.83	224±129
	4	57±7	95.3±2.20	189±109
	6	73±9	83.3±2.91	76±44
Control	0	38±3	–	–

Each group of 5 mice was used.
Mean±standard error.
By student t test (vs NON-CPS): *$P < 0.001$, **$P < 0.01$ and ***$P < 0.05$.

Table 4. Total Number of Exudate Leukocytes in Mice after Treatment with Bacterial Culture Supernatant

Challenger	After treatment (hr)	No. of leukocytes (x 10^5 cell/mouse)	Differential count (%)		
			PMN	Macrophage	Lymphocytes
CPS (E-4)	2	35±12#	82.7±2.7	11.3±1.4	6.0±0.4
	4	39±9**	77.0±7.2	13.0±5.8	10.0±2.7
	6	57±4**	85.0±0.9*	10.0±4.9	5.0±4.1
NON-CPS (E-2)	2	32±2	80.3±4.1	11.3±0.9	8.3±3.3
	4	57±3	80.7±3.3	10.0±1.3	9.0±2.5
	6	72±9	87.0±1.5	10.0±0.6	3.0±1.0
HI broth only (control)	0	30±8	79.0±1.2	7.0±2.5	14.0±1.5
	2	60±12	86.0±2.9	5.3±1.8	8.7±2.8
	4	90±16	76.7±4.4	8.7±1.8	11.3±2.4
	6	87±19	89.7±3.5	6.0±1.5	4.3±2.4

0.5 ml/mouse of culture supernatant was used for challenger.
Mean±standard error. By student t test (vs NON-CPS): *$P < 0.05$ and **$P < 0.01$.

Table 5. Serum Bactericidal Activity of Mice After *Escherichia coli* Infection or Supernatant Challenge Against *Staphylococcus aureus* 209p.

Strain	Challenger	
	Bacterial suspension (%)	Supernatant (%)
CPS (E-4)	> 100.0*	74.5
NON-CPS (E-2)	32.2	37.5
Control	31.7	

Blood specimens were collection from 6 hr after the mice were challenged i.p. with 0.5 ml/mouse of CPS strain (2mg/ml) and 24 hr culture supernatant, respectively.
*Percentage of bacteria increase=

$$\frac{\text{No. tested-Initial No.}}{\text{Initial No.}} \times 100$$

之腹腔細胞少。其中 CPS 株 4 小時後則具較強之抑制作用。

接種CPS 株及 NON-CPS 株及培養上清液小白鼠之血清，對殺菌活性及內毒素去毒作用之影響：

(1)對*Staphylococcus aureus* 209 p 之殺菌能力（表 5 ）：CPS 株感染及其外毒素注射 6 小時小白鼠之血清，其細菌增加率分別是> 100.0 % 及 74.5 %，較NON-CPS 株之 32.2 % 及 37.5 % 相差 3.0 倍及 2.0 倍以上，而 NON-CPS 株則類似對照組小白鼠血清之殺菌活性，故 CPS 株及其外毒素注射小白鼠，其血清殺菌能力已大受影響。

(2)對大腸桿菌（0127：B8 ）內毒素之去毒作用（表 6 ）：CPS株感染 6 小時後小白鼠血清之內毒素去毒作用比 NON-CPS 株及正

Table 6. Detoxification of Bacterial Endotoxin by Bacteria-Infected Mouse Serum

Detoxifying agent	Deaths in assay mice
CPS infected serum	6/8 (75.0)*
NON-CPS infected serum	4/8 (50.0)
Normal serum	3/8 (37.5)
Endotoxin+Actinomycin-D	5/8 (62.5)

*Dead mice/tested mice, figures in parenthesis show percentage of mortality.

Table 7. Potentiation of Pathogenicity with Exotoxin

Challenger*	Exotoxin added (LD_{50}; mg/kg)		Relative potency
	Yes (5 μg/ml)	None (0)	
CPS (E-4)	0.08	2.51	31.3
NON-CPS (E-2)	32.31	70.44	2.2

*Challenger: A 18 hr heart-infusion broth culture was washed with normal saline three times, then made into suspensions of different bacterial concentration either with normal saline containing exotoxin 10 μg/ml or with normal saline without exotoxin addition. 0.5 ml of each suspension was injected intraperitoneally into a mouse.

常對照組顯著下降（死亡率 75.0％，vs 50.0％及 35.0％）。故 CPS 株感染小白鼠血清之去毒能力亦受明顯的破壞。

CPS 株純化外毒素可增強細菌對小白鼠之感染能力（表 7）：以 5 μg/mouse（約 0.25 mg/kg）純化外毒素注射小白鼠，並不引起明顯的不適症狀或死亡。但若與 CPS 株共同注射時會增強 CPS 株對小白鼠之病原性約 31.3 倍，而此種微量外毒素亦可提高 NON－CPS 株之感染力約 2.2 倍。

CPS 株及 NON－CPS 株對小白鼠免疫力之差異：CPS 株或其培養上清液 2 次免疫後小白鼠分別對 CPS 株之感染及其外毒素注射都呈現相當良好的防禦作用（表 8），但NON－CPS株或其培養上清液免疫小白鼠對 CPS 株或外毒素注射則缺少防禦能力，其中僅NON－CPS株免疫原免疫之小白鼠部份具保護作用而已。免疫小白鼠之免疫血清以 Cellulose acetate membrane 血清蛋白電泳分析之結果，免疫小白鼠所增加之 γ－globulin 量與其對細菌感染或外毒素注射之防禦能力之間並無平行的關係。

若以經各種抗生素分別處理之 CPS 株免疫原免疫小白鼠，則該免疫小白鼠對 CPS 株

Table 8. Protection of Mice Immunized with CPS and NON-CPS Strains Against Bacteria or Exotoxin of CPS Strain Challenge

Strains	Immunogen*	Challenger (E-4)		γ-globulin** (mg%)
		Bacterial suspension (2mg/mouse)	Exotoxin (0.5mg/mouse)	
CPS (E-4)	Bacterial suspension	0/8 (0) #	1/8 (12.5)	0.49
	Supernatant	0/8 (0)	0/8 (0)	0.26
NON-CPS (E-2)	Bacterial suspension	4/8 (50.0)	6/8 (75.0)	0.42
	Supernatant	8/8 (100.0)	8/8 (100.0)	0.20
Non-immunized mice		8/8 (100.0)	8/8 (100.0)	0.20

* Immunization: Mice were injected intraperitoneally with 0.5 ml of heat-inactivated (100°C for 15 min) bacterial suspension (MacFarland No. 3) or culture supernatant of CPS and NON-CPS strains respectively. The immunization was boosted once one week after the injection. Another week after that, the immunized mice were challenged with bacterial suspension or exotoxin.

** Electrophoretic analysis of mice serum on cellulose acetate membrane.

No. of death/No. of test; figures in parenthesis show the percentage.

Table 9. Protection of Mice Immunized with Antibiotic-Pretreated Bacteria Against Bacterial Suspension or Exotoxin Challenges

Pre-treated with	LD_{50} of bacterial challenge (mg/kg)	Relative @ resistance	Exotoxin challenge (0.5mg/mouse)	γ-globulin* (mg%)
Mitomycin-C	23.5	9.4	6/8 (75.0) #	0.80
Spectinomycin	16.9	6.8	7/8 (87.5)	0.94
Polymyxin-B	35.9	14.4	3/8 (37.5)	0.82
Colistin-M	D**	—	4/8 (50.0)	0.78
Without treatment	23.2	9.3	0/8 (0)	0.48
Non-immunized	2.5	1.0	8/8 (100.0)	0.20

* Electrophoretic analysis of mice serum on cellulose acetate membrane.
\# No. of death/No. of test; figures in parenthesis show the percentage. For the method of immunizing mice see table 8.
** Not determined.
@ Tested group/Non-immunized group.

Table 10. Candida Killing Activity of Peritoneal Leukocytes in Bacteria-Immunized Mice

Strain (n)	No. of leukocytes (10^7 cell/mouse)	Candidacidal index#	Differential count (%)		
			PMN	Macrophage	Lymphocytes
CPS (E-4) (5)	2.72±0.24*	21.4±1.50	56.8±4.10	26.0±2.70	17.2±3.40
MMC-CPS@ (E-4) (5)	3.06±0.23	19.2±0.97	60.0±2.00	24.8±1.90	14.6±2.80
NON-CPS (E-2) (5)	2.94±0.30	21.4±1.50	60.8±1.90	23.4±1.60	15.8±2.40
Control (5)	1.84±0.31	20.6±1.30	41.7±1.40	40.0±0.50	18.3±1.40

\# Candidacidal index: Percentage of dead candida (blue color) in test − percentage of dead candida in control.
* Mean±standard error.
@ Mitomycin-CPS.

之感染及其外毒素注射呈現不同之防禦能力（表2及9）。免疫小白鼠對同株（CPS株）細菌之感染具非常良好的防禦效果。如Mitomycin-C，Spectinomycin及Polymyxin-B等分別處理之CPS株免疫小白鼠較未免疫對照組之LD_{50}劑量提高9.4倍，6.8倍及14.4倍，但較未經抗生素處理之同株細菌單劑免疫小白鼠之免疫效果，除Polymyxin-B略好外，則無明顯的差異。這些免疫小白鼠之血清γ-globulin含量在0.78mg%—0.94mg%之間，較之對照組（0.20mg%）或CPS株單劑免疫之小白鼠（0.48mg%）等高出很多

，雖然經抗生素處理免疫原免疫之小白鼠對細菌感染具很好的防禦能力，但對 CPS 株外毒素注射之防禦能力，較之未經抗生素處理之同株免疫小白鼠則遜色很多。

CPS株，NON-CPS株及MMC-CPS株等細胞免疫小白鼠後，其腹腔細胞數及其 Candida killing能力如表10所示，以腹腔內細胞數，Candidacidal index 及白血球分類等來看，3者之間差異並不明顯，其中 MMC-CPS株免疫群之腹腔細胞數稍爲增加（3.06 X 10^7 cell/mouse）。

討　論

大腸桿菌之病原性鑑定，除腸毒素產生株外，其他腸外病原性株則以對小白鼠之致死毒性（LD_{50}）較被廣用[16-19]。病原性株之病原性除受宿主條件之影響外，尚受細菌本身的侵犯能力及細胞外物質產生之影響。本實驗以小白鼠之致死毒性，小白鼠腹腔細胞數之變化，血清對內毒素之去毒作用及殺菌活性及免疫能力等來觀察大腸桿菌之病原性。結果發現細菌之外毒素產生能力是最具密切關係的病原性因子之一。

CPS株及NON-CPS株除在 PGS 培養基產生粘稠菌落[5]及對天竺鼠紅血球具凝集能力外[4]，其主要差異亦可見於對小白鼠之感染能力，及其培養上清液對小白鼠之毒性及兩者之免疫能力。而培養上清液對小白鼠之致死毒性與細菌感染力之間具相當一致之關係。CPS 株之微量純化外毒素不但增強 CPS 株且及於 NON-CPS 株對小白鼠之感染能力。故若以對小白鼠之急性毒性來看，CPS 株是具病原性之菌株而 NON-CPS 株則否，而其所以具有病原性之主要物質，可能是其外毒素。因根據*CALSNOS* 氏等[10]方法所得之內毒素或以Mitomycin-C 處理之 CPS 株等，對小白鼠之致死毒性來分析，則 CPS 株及 NON-CPS 株並無差異。故大腸桿菌若欠缺外毒素之產生能力則無所謂病原性及非病原性之分。

不論是以細菌內毒素注射或菌血症患者，常會發現補體的減少，故補體之存在與繼發性休克具密切的關係[20-23]。*HOHNSON* 及 *WARD* 氏等[14]認爲血清對內毒素之去毒作用須 C_5 及 C_6 補體的存在。當小白鼠受CPS 株感染或其外毒素注射後，其血清對 *Staphylococcus aureus* 209 p 之殺菌活性則受相當明顯的影響，較 NON-CPS 株感染及其培養上清液注射，或來自正常對照組小白鼠血清之抗菌效果差，對內毒素之去毒作用亦有明顯的下降。證明 CPS 株外毒素是導致血清殺菌活性及內毒素去毒作用下降之最重要物質之一。

由宿主獲得免疫力加以分析，CPS 株或其培養上清液免疫小白鼠，對 CPS 株或其外毒素注射都獲得良好之防禦效果（活存率87.5％－100.0％），而NON-CPS 株則否，而且以各種抗生素處理之 CPS 株，其免疫小白鼠雖然可以獲得對 CPS 株感染之防禦能力，如Mitomycin-C 處理株免疫小白鼠較未免疫小白鼠之 LD_{50} 提高9.4倍，但對外毒素注射之保護效果則較單劑未處理 CPS 株免疫者（死亡率0.0％）顯著下降。並由 CPS 株及 NON-CPS 株內毒素免疫小白鼠，對 CPS 株感染之防禦效果相似（死亡率分別是57.1％及75.0％）。故小白鼠獲得免疫力主要來自外毒素之免疫能力。

由以往之研究得知，大腸桿菌 CPS 株是一群荷有腸毒素及小白鼠致死毒性等產生能力，及對抗生素耐性或多劑耐性之細菌群，它們不但是腸外感染株亦可能是腸病原性株[5-9,16]，而 NON-CPS 株則否。而且由其對小白鼠之病原性及免疫能力等。證明 CPS 株之病原性因子主要是釋放於培養基之外毒素，亦是宿主獲得免疫力之最主要物質。

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Studies on Pathogenicity of Capsular Polysaccharide-Synthesizing Escherichia Coli Exotoxin

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SUMMARY

Two strains of Escherichia coli having similar biochemical characteristic, one capsular polysaccharide synthesizing (CPS) strain and the other non-synthesizing (NON-CPS) strain, were used to investigate and compare their pathogenicity and immunizing capacity to mice. The results were as follows: (1) The pathogenicity and toxicity to mice of the bacterial suspension and the culture supernatant of the CPS strain were stronger than those of the NON-CPS strain. (i.p. LD_{50}: 3.2 mg/kg vs 70.4 mg/kg for bacterial suspension, 2.8 mg/kg vs $>$ 44.0 mg/kg for culture supernatant). But both strains possessed the similar toxicity of endotoxin to mice (mortality: 42.9% vs 42.9%).

(2) When the CPS strain was pretreated with mitomycin-C and heated, its bacterial suspension showed nearly the same toxicity to mice as the NON-CPS strain (LD_{50}: 260 mg/kg vs 280 mg/kg). However, the mitomycin-C untreated CPS strain showed stronger toxicity (LD_{50}: 140 mg/kg).

(3) CPS and NON-CPS strains showed significant difference each other in the change of the peritoneal leukocyte number, phagocytic activity and extracellular bacteria number after CPS or NON-CPS strain infection or their culture supernatant challenges in mice, respectively. This phenomenon was also observed in the bactericidal activity of infected mice serum against Staphylococcus aureus 209p and detoxificational ability of the serum against bacterial endotoxin.

(4) A very small quantity of purified exotoxin could intensify the infectiousness of CPS strain to mice.

(5) Defensive activity of the CPS and NON-CPS immunized mice against CPS strain infection and its exotoxin challenge showed significant difference. The survival rates of the CPS strain immunized were 100.0% and 87.5%, and those of the NON-CPS strain immunized mice were 50.0% and 0.0% after CPS strain infection or exotoxin challenge, respectively. In the cases of the culture supernatant immunized mice the survival rates were CPS 100.0% and NON-CPS 0.0% against CPS strain infection, and 100.0% and 0.0% against exotoxin challenge.

(6) The mice immunized with the CPS strain which was pretreated with various antibiotics mitomycin-C, spectinomycin, polymyxin-B or colistin-M respectively, obtained rather good protection against bacteria infection of the CPS strain. However, their protection against the purified exotoxin challenges was remarkably lower than the mice immunized with the antibiotics untreated CPS strain.

(7) The mice immunized with the CPS and NON-CPS strains showed similar responses in their peritoneal leukocytes number and candidacidal activity.

Therefore, both of the infectiousness of bacteria themselves and the defense ability of the immunized mice had intimate relation with the presence of exotoxin. Namely, the exotoxin is not only the decisive factor of pathogenic E. coli, but also one of the essential substances for immunization of mice.

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