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□ 원 저 □

Cell Surface Antigenic Relationship of Pathogenic Mycobacteria

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=국문 초록=

병원성 Mycobacteria의 세포표면항원간의 항원적 상관 관계

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김 상 재

석탄산으로 사멸한 균으로 감작시켜 얻은 토끼 항혈청으로 주요 병원성 mycobacteria의 세포표면항원간의 항원적 상관관계를 효소결합면역분석법으로 관찰하였다. *M. avium-intracellulare* (MAI) 항혈청을 이용한 동종 및 이종반응 분석결과를 보면 동종내 항원적 상관관계는 매우 가깝고 *M. scrofulaceum*(MG)을 포함하면 MAIS군내 종간 항원적 관계도 가까웠다. MAI 혈청이 *M. chelonei*(MC), *fortuitum*(MF), *nonchromogenicum*(MNC), *terrae*(MTR) 및 *triviale* (MTV)와는 반응하지 않거나 미약하게 반응하지만 *M. kansasii*(MK)와 결핵균과는 강하게 반응했다. 그러나 후자의 두균종으로 흡수해도 MAI혈청의 동종반응은 감소되지 않았다. *M. avium*(MA) (K40004) 항혈청은 *M. intracellulare*(MI) 균주를 제외한 다른 균들과는 미약하게 반응했고 항원적 상관관계가 가까운 MI와 MG로 흡수하면 동종반응의 50~89%가 감소했다. MI 혈청의 종내반응이 종간반응보다 물론 더 강하지만 공유항원량의 크기에 따라 달랐다. MI균주 중 N-260D, N-260R, N-260T 및 K41014간의 항원적 상관관계가 N-242D, N257T, N281D 및 N275T와의 관계보다 더 가깝게 나타났다.

MNC 항혈청은 MC와 MTV를 제외하고는 강한 교차반응을 보여주었다. 종내 항원적 상관관계 다음으로는 MTR와 MG(K30003)과 가까웠다. NC-3 혈청은 MA, MC 및 MF와 상당한 반응력을 나타내지만 NC-11은 그렇지 않았다. MTR 항혈청은 MI와 강하게 반응하지만 MI로 흡수한다고 해서 그들의 동종반응이 감소하지 않는 것으로 보아 MTR 표면에 공유항원이 많지 않음을 보여주었다. MTR과 MNC, MC 및 MF 사이에도 상당량의 공유항원이 있음이 알려졌다. MTR 가운데 T-8 혈청과는 달리 T-13 항혈청은 MA, MG, MK 및 MTB와 강하게 교차반응하였다.

이상의 실험으로 밝혀진 mycobacteria의 항원적 상관관계가 각종 생물학적 및 생화학적 방법으로 갈라놓은 분류군과 잘 일치하고 있었다.

Key Words: Pathogenic mycobacteria, Antigenic relationship

INTRODUCTION

Most of mycobacterial taxons have been described by the computer-assisted numerical analysis of the various biological and biochemical characteristics that is still considered imperfect to delineate the precise phylogenetic relationships of mycobacteria[1,2]. Recent development of genomic DNA hybridization technique was found to be very useful to confirm hypothetical phylogeny of the phenetic clusterings based on the various morphological and biochemical characteristics[2~4]. In addition, the various immunological procedures also have been studied to delineate the phenetic differences of the species, subspecies, biobars or serobars, some of which had been found useful[2,5~7,9~10].

A numerous studies on soluble antigenic relationship between mycobacteria have been made by many investigators[6,7,10]. Relatioship of cell surface antigen also has been studied by seroagglutination technique that was found very useful for serotyping of medically important mycobacteria[9]. However this technique is based on agglutinating antibody directed to the carbohydrate epitopes of the cell surface glycopeptidolipids[8] and better adapted for smooth colony forming strains[8,9]. All these procedures have advantages and disadvantages depending on the circumstances.

This study aimed to investigate cell surface antigenic relationships between medically important mycobacteria by enzyme immunoassay using antisera raised in rabbits.

MATERIALS AND METHODS

1. Mycobacterial Strains

The following species and strains were used in this study

Mycobacterium tuberculosis H37Rv
M. kansasii K20001
M. scrofulaceum K30001 & K30003
M. avium K40004
M. intracellulare N-238, N-242D, N-245, N-257R, N-257T, N-260T, N-260D, N-260R, N-275R, N-275T, N-279T, N-281D, $F_0$3193T, K41014
M. nonchromogenicum NC-3, NC-6 & NC-11
M. terrae T-7, T-8 & T-13
M. triviale V-4 & V-12
M. chelonei R-20
M. fortuitum F-19
M. smegmatis K64002

2. Culture and Immunogen Preparation

All the strains were cultured on Sauton's broth medium at 37 °C in a stationary phase by transferring bacterial pellicle formed on the surface of Sauton's potato medium. Few strains that usually does not form pellicle were cultured on a gyrotory shaking incubator at 110 rpm. Cells were killed by treating with 2% phenol for 3 days at 37 °C and dispersed by a tissue homogenizer and washed 5 times with a sterile 0.067 M phosphate buffered saline (pH 7.0) by centrifugation.

3. Immunization

White New-Zealand rabbits weighing 3~4 kg were intravenously immunized with phenol killed bacilli according to the following schedule: 1 mg at lst day, 2 mg at 3 rd day, 3 mg at 7th day, 4 mg at 10th day and 5 mg at 14th day. Further immunization with 5 mg of the phenol-killed cells was followed weekly. Rabbits were bled prior to immunization and after 14 days of immunization. If antibody titer of the last serum was same with those of one or two previous sera, animals were subjected to the total bleeding and serum separated was divided into small aliquotes before freezing at −40 °C. Some strains, for example K40004 and N-260T, killed animals usually at 14th day intravenous immunization, so that they were immunized subcutaneously every week until

maximum antibody titer was obtained.

4. Enzyme Immunoassay

In order to determine optimum cell concentration for coating, two fold dilutions of 16 mg/ml cell suspension (to 0.5 mg/ml) were reacted with varying serum dilutions and selected optimum dilution of cell suspension that showed a highest reaction. Most of strains showed a highest reaction with 2 mg/ml of bacterial suspension for coating. Serum dilution that showed a optical density (OD) of 1.0 to 1.3 at 492 nm was used for assay.

Heterologous cell removable antibodies reacting with homologous organisms (HARD) were measured according to the following formula. Large removal of homologous antibodies with

$$\text{HARD (\%)} = \frac{\text{OD of homologous reaction with unabsorbed serum}}{\text{OD of homologous reaction with unabsorbed serum}} - \frac{\text{OD of homologous reaction with heterologous cell absorbed serum}}{\text{OD of homologous reaction with homologous cell absorbed serum}} \times 100$$

cells from OD heterologous organisms indicates that the homologous cells possess a large amount of cross reacting antigenic epitopes. And homologous cell removable antibodies reacting with heterologous cells (OARH) were also measured according to the following formula in order to estimate relative amoung of cross reactive and specific antigenic determinants.

$$\text{OARH (\%)} = \frac{\text{OD of heterologous reaction with unabsorbed serum}}{\text{OD of heterologous reaction with unabsorbed serum}} - \frac{\text{OD of heterologous reaction with homologous cell absorbed serum}}{\text{OD of heterologous reaction with heterologous cell absorbed serum}} \times 100$$

RESULTS

Cross reactivity of *M. terrae* antisera (T-8 & T-13) with other mycobacteria was shown in Table 1. Both *M. terrae* antisera cross-reacted with a wide range of myobacteria. They showed a strong cross-reaction with strains of *M. avium, intracellulare,* and *terrae* as their ROD were found to be 1.00 or nearly so (>0.70). More than 80% of these cross-reacting antibody were removed by the homologous absorption, indicating an abundance of shared antigenic determinants on *M. terrae* cell surface. Homologous absorption of T-8 and T-13 antisera removed a largest amount of antibody cross-reacting with strains of *M. terrae, M. avium* (K40004), and certain strains of *M. intracellulare* (N-260T, N-2600, N-275T, $F_0$3193T, K41014), but removed a least amount of antibody reacting with N-257R, N260R, and N-281D. Both strains of *M. terrae* may possess less ammount of antigenic derterminants shared with the latter strains of *M. intracellulare*. Ratios of the heterologous reactions to *M. tuberculosis, kansasii, scrofulaceum*, and *fortuitum* over the homologous reaction of T-13 antiserum were 1.00 or nearly so, whereas those of T-8 antiserum were less than 0.60. Cross-reacting antibody to the strains of *M. chelonei, nonchromogenicum, smegmatis*, and *triviale* were present in a small amount in both sera. It was interest that T-13 antiserum possessed much less amount of antibody to NC-11 than that to NC-3 and both sera also showed a weaker reaction to V-12 than to V-4.

T-8 homologous absorption removed most of cross-reactiong antibody to the strains of *M. chelonei, nonchromogenicum, scrofulaceum, smegmatis* and *triviale*. Cross-reacting antibody to *M. fortuitum, kansasii* and *tuberculosis* could be removed relatively less by the homologous absorption, showing 67.9% to 76.0% reduction of the corresponding heterologous reaction. Nearly none of T-8 homolgous antibody by the heterologous absorption except

Table 1. Reactivity of Rabbits Anti-*M. terrae* Sera (T-8 & T-13) with Other Mycobacteria

Mycobacteria	T-8			T-13		
	ROD	OARH (%)	HARD (%)	ROD	OARH (%)	HARD (%)
M. tuberculosis H37Rv	0.59	67.9	0.0	1.03	38.6	43.2
M. kansasii K20001	0.54	68.6	0.0	0.89	61.8	37.0
M. scrofulaceum K30001	0.36	92.0	0.0*	0.77	100.0*	27.7
M. scrofulaceum K30003	0.55	81.7	0.0	1.20	73.5	48.8
M. avium K40004	1.32	85.8	0.0	1.11	100.0	35.6
M. intracellulare N-238	0.87	75.7	0.0*	0.92	75.0	0.0
M. intracellulare N-242D	0.89	76.5	0.0*	0.94	73.5	0.0*
M. intracellulare N-245	0.84	80.6	0.0*	0.90	75.3	0.0*
M. intracellulare N-257T	1.28	75.8	0.0*	1.24	69.5	0.0*
M. intracellulare N-257R	0.90	69.5	0.0*	0.97	67.5	0.0*
M. intracellulare N-260T	1.25	82.8	0.0	0.92	97.9	0.0
M. intracellulare N-260D	1.28	84.3	0.0*	1.33	80.9	0.0*
M. intracellulare N-260R	1.41	70.4	0.0*	1.68	63.8	0.0*
M. intracellulare N-275T	1.12	83.3	0.0*	1.34	79.9	0.0*
M. intracellulare N-275R	1.19	75.0	0.0*	1.40	71.4	0.0*
M. intracellulare N-279T	1.33	75.7	0.0*	2.15	79.1	0.0*
M. intracellulare N-281D	0.77	72.6	0.0*	0.98	67.9	0.0*
M. intracellulare Fo3193T	0.71	88.3	0.0*	0.77	84.6	0.0*
M. intracellulare K41014	1.41	80.4	0.0	0.95	98.7	0.0
M. nonchromogenicum NC-3	0.43	96.6	69.1	0.54	100.0*	48.1
M. nonchromogenicum NC-11	0.39	100.0	43.2	0.18	100.0*	16.8
M. terrae T-7	0.94	83.0	68.9	0.91	78.2	69.8
M. terrae T-8				0.27	100.0*	17.5
M. terrae T-13	0.80	89.4	37.9			
M. triviale V-4	0.58	100.0*	0.0*	0.60	100.0*	0.0*
M. triviale V-12	0.10	100.0	0.0*	0.09	100.0	0.0*
M. chelonei R-20	0.34	100.0	10.6	0.58	101.7	40.5
M. fortuitum F-19	0.58	76.0	25.1	1.26	72.7	66.4
M. smegmatis K64002	0.41	84.8	0.0*	0.56	88.5	4.6

ROD=ratio of OD of heterologous over homologous reaction.
Explanation for HARD and OARH, see the text.
*Homologous or heterologous absorption did not reduce the heterologous or homologous reaction at all but it increased, instead.

M. chelonei, fortuitum, nonchromogenicum (NC-3, NC-11), and *M. terrae*, NC-3 and T-7 removed a largest amount of T-8 homologous antibody, indicating presence of a considerable amount of shared antigenic determinants on them. *M. chelonei* and *fortuitum* removed only 10.6% and 25.1% of the homologous reaction. T-13 antiserum was different from T-8 antiserum because the former showed a stronger reaction to *M. fortuitum, kansasii, scrofulaceum* and *tuberculosis* than the latter and T-13 homologous absorption removed less amount of antibody cross-reacting with *M. tuberculosis*. The striking differance of T-8 and T-13 antisera was a removal of homologous antibody by the heter-

Table 2. Reactivity of Rabbits Anti-*M. nonchromogenicum* Sera (NC-3 and NC-11) with Other Mycobacteria

Mycobacteria	NC-3			NC-11		
	ROD	OARH (%)	HARD (%)	ROD	OARH (%)	HARD (%)
M. tuberculosis H37Rv	0.78	49.3	0.0*	0.80	89.4	0.0*
M. kansasii K20001	0.76	63.9	0.0*	0.78	84.6	0.0*
M. scrofulaceum K30001	0.42	100.0*	0.0*	0.75	93.6	0.0*
M. scrofulaceum K30003	0.83	75.6	13.3	0.94	81.2	14.7
M. avium K40004	1.09	74.0	18.6	1.07	95.6	0.0*
M. intracellulare N-238	1.05	75.2	0.0	0.87	95.5	0.0
M. intracellulare N-242D	0.67	86.1	0.0	0.76	93.4	0.0*
M. intracellulare N-245	0.88	82.3	2.8	0.95	79.9	0.0
M. intracellulare N-257T	1.15	72.8	0.0	0.94	93.6	0.0*
M. intracellulare N-257R	0.89	84.7	6.3	0.86	86.3	0.0
M. intracellulare N-260T	1.01	92.8	0.0	0.82	95.5	0.0*
M. intracellulare N-260D	1.11	88.9	6.0*	1.05	89.8	0.0
M. intracellulare N-260R	1.18	67.8	5.6	1.12	81.1	0.0
M. intracellulare N-275T	0.89	87.7	0.0	0.84	85.3	0.0*
M. intracellulare N-275R	1.03	87.5	0.0	1.01	84.2	0.0
M. intracellulare N-279T	1.15	78.0	5.7	1.03	87.9	0.0
M. intracellulare N-281D	0.76	70.7	7.5	0.65	87.5	0.0
M. intracellulare Fo3193T	0.76	71.5	0.3	0.69	85.9	0.0
M. intracellulare K41014	1.10	87.3	0.0	0.88	96.7	0.0
M. nonchromogenicum NC-3				0.95	89.6	71.4
M. nonchromogenicum NC-6	0.78	86.0	86.4	1.07	84.1	86.5
M. nonchromogenicum NC-11	0.58	100.0*	66.4			
M. terrae T-7	0.71	87.5	15.8	0.87	81.5	14.5
M. terrae T-13	0.82	83.6	25.2	1.01	81.8	28.7
M. triviale V-4	0.62	93.3	0.0	0.54	88.2	0.0
M. triviale V-12	0.21	100.0	0.0	0	0	0.0
M. chelonei R-20	0.48	95.4	20.8	0.58	95.3	0.0*
M. fortuitum F-19	0.93	77.3	31.8	0.90	74.0	0.0*
M. smegmatis K64002	0.49	69.8	0.0*	1.43	100.0*	0.0*

ROD=ratio of OD of heterologous over homologous reaction.
Explanation for HARD and OARH, see the text.

*Homologous or heterologous absorption did not reduce the heterologous or homologous reaction at all but it increased, instead.

ologous absorption with *M. avium, kansasii, scrofulaceum*, and *tuberculosis*. Reduction of the homologous reaction of T-13 antiserum by *M. chelonei* and *fortuitum* absorption was also greater than that of T-8 antiserum. The strains of *M. intracellulare* and *triviale* did not reduce homologous reaction of both antisera. Rabbits immunized with both strains (T-8, T-13) of *M. terrae* produced a considerable amount of antibody cross-reacting with *M. nonchromogenicum* NC-3 though nearly all were absorbed out by homologous organisms T-8 or T-13. And NC-3 reduced 48.1% or 69.1% of the homologous reaction of T-13 or T-8 antiserum, indicating that NC-3 possessed a large amount of shared

Table 3. Reactivity of Rabbits Anti-*M. intracellulare* Sera (N-260T, N-260D, N-260R & K41014) with Other Mycobacteria

Mycobacteria	N-260T			N-260D			N-260R			K41014		
	ROD	OARH (%)	HARD (%)	ROD	OARH (%)	HARD (%)	ROD	OARH (%)	HARD (%)	ROD	OARH (%)	HARD (%)
M. tuberculosis H37Rv	0.24	100.0	0.0*	0.56	96.6	0.0	0.55	99.9	0.0	0.58	95.9	5.2
M. kansasii K20001	0.31	97.7	0.0*	0.44	96.9	0.0	0.75	98.2	0.0	0.56	100.0	3.3
M. scrofulaceum K30001	0	0	1.2	0.27	100.0*	7.1	0.38	100.0*	1.6	0.46	100.0	10.4
M. scrofulaceum K30003	0.34	100.0	13.0	0.66	96.0	18.1	0.65	98.8	31.5	0.83	99.1	24.3
M. avium K40004	0.33	100.0*	29.8	0.95	100.0	21.3	0.96	100.0	17.6	1.02	100.0	33.2
M. intracellulare N-238	0.80	97.4	24.6	0.84	94.5	25.0	0.80	99.5	59.2	0.85	98.4	58.9
M. intracellulare N-242D	0.46	100.0	13.1	0.58	100.0	13.1	0.54	100.0	32.3	0.60	100.0	31.4
M. intracellulare N-245	0.74	100.0	34.9	0.81	96.6	36.2	0.87	99.1	43.6	0.89	98.2	44.7
M. intracellulare N-257T	0.55	100.0	10.9	0.66	100.6	11.2	0.55	100.0	30.5	0.65	100.0	29.5
M. intracellulare N-257R	0.66	100.0	24.4	0.78	100.0	25.5	0.77	100.0	55.6	0.86	95.8	54.6
M. intracellulare N-260T				1.11	94.8	100.0	1.00	98.3	98.6	0.96	100.6	48.9
M. intracellulare N-260D	0.90	100.0*	86.2				1.16	99.2	97.9	1.14	100.0	96.3
M. intracellulare N-260R	1.04	98.0	92.9	0.98	93.7	83.8				1.15	96.3	100.0
M. intracellulare N-275T	0.56	100.0	16.9	0.62	100.0	15.2	0.65	97.0	48.0	0.68	94.8	47.4
M. intracellulare N-275R	0.75	100.0	17.0	0.79	100.0	16.2	0.76	97.3	41.2	0.80	94.4	40.2
M. intracellulare N-279T	0.69	100.0	32.1	0.73	100.0	30.7	0.70	100.0	45.9	0.91	92.8	45.5
M. intracellulare N-281D	0.68	88.0	23.1	0.52	100.0	20.9	0.51	100.0	48.7	0.52	99.0	48.4
M. intracellulare Fo3193T	0.57	100.0	41.2	0.66	100.0	40.8	0.58	100.0	47.7	0.57	100.0	47.0
M. intracellulare K41014	0.83	100.0	57.9	0.90	96.0	82.1	0.91	100.0	99.8			
M. nonchromogenicum NC-3	0	0	8.3	0.09	100.0*	4.5	0.01	100.0	10.2	0.34	100.0	2.6
M. nonchromogenicum NC-11	0	0	0.4	0	0	4.8	0	0	0.0	0.17	100.0	5.7
M. terrae T-7	0.08	100.0*	0.0	0.11	100.0*	0.0	0.10	100.0*	0.0*	0.37	100.0	0.0
M. terrae T-13	0.16	100.0*	18.2	0.11	100.0*	5.8	0.27	92.7	17.7	0.46	96.4	13.7
M. triviale V-4	0.02	100.0	2.4	0.09	100.0	0.0	0.17	100.0*	0.0*	0.18	100.0*	0.0*
M. triviale V-12	0	0	5.4	0	0	6.2	0	0	0.0*	0	0	0.0*
M. chelonei R-20	0	0	11.9	0.11	100.0*	8.3	0.01	0	22.2	0.16	100.0*	9.6
M. fortuitum F-19	0.17	100.0*	0.0*	0.07	100.0*	0.0	0.20	100.0*	4.9	0.51	100.0	6.0
M. smegmatis K64002	0.09	100.0*	0.0*	0.15	100.0	0.0	0.17	100.0*	0.0	0.40	100.0	4.2

ROD=ratio of OD of heterologous over homologous reaction.

Explanation for HARD and OARH, see the text.

*Homologous or heterologous absorption did not reduce the heterologous or homologous reaction at all but it increased, instead.

antigenic determinants. Unlike T-8, T-13 did not raise much of antibody cross-reacting with NC-11 in rabbits and NC-11 reduced only 16.8% of homologous reaction.

There were of course a considerable amount of shared antigens between the strains of *M. terrae*. T-8 antiserum reacted strongly with T-7 (ROD, 0.94) and T-13 (ROD, 0.80) which of 83.0% and 89.4% were removed by the homologous (T-8) absorption. T-7 reduced 68.9% of the homologous reaction of T-8 antiserum, while T-13 reduced merely 37.9%, indicating that T-7 cell surface possessed larger amount of shared antigens than T-13. T-13 antiserum also showed a strong reaction with T-7 which of

78.2% were removed by the homologous absorption and T-7 absorption of T-13 antiserum reduced 69.8% of homologous reaction. In contrast with T-8, T-13 produced a small amount of antibody reacting with T-8 in rabbits and T-8 was able to absorb out merely 17.5% of homologous reaction. This finding sugeses that antigenic relationship of T-7 is equally close with T-8 and T-13 and T-8 cell surface possess a measurable amount of antigens shared with T-13 but not much of shared antigen on T-13 cell surface.

Rabbits antisera raised with *M. nonchromogenicum* NC-3 and NC-11 contained a large amount of cross-reacting antibody against other mycobacteria except *M. triviale*. NC-3 antiserum unlike NC-11 serum showed a relatively weak reaction to *M. chelonei, scrofulaceum*(K30001) and *smegmatis*. Nearly all of these cross-reacting antibody (> 90%) to K30001, R-20, V-4 and V-12 were removed by the homologous absorption. More than 80% of the heterologous reaction of NC-3 antiserum with *M. intracellulare* strains (N-242D, N-245, N257R, N-260T, N-260D, N-275T, N-275R, K41014), and *M. terrae* were removed by the homologous reaction and 70~79% removal with *M. avium, fortuitum*, the rest of *intracellulare* strains, *scrofulaceum*(K30003), and *smegmatis*. Homologous absorption, however, removed 49.3% and 63.9% of antibody cross-reacting with *M. tuberculosis* and *kansasii* respectively. Homologous reaction was not reduced much by the heterologous absorption except for *M. avium, chelonei, fortuitum, scrofulaceum*(K30003), and *terrae*. *M. chelonei* and *fortuitum* reduced 20.8% and 31.8% of homologous reaction. *M. terrae* T-7 and T-13 reduced 15.8% and 25.2% respectively, *M. avium*, 18.6% and *M. scrofulaceum*(K30003), 13.3%. More than 80% of NC-11 antiserum reaction with other mycobacteria except for *M. triviale* V-12 and *fortuitum* were removable by the homologous absorption. NC-11 antiserum did not contain a measurable amount of antibody reacting with *M. triviale* V-12. Homologous absorption of NC-11 antiserum reduced 74.0% of heterologous reaction with *M. fortuitum*. Heterologous absorption of NC-11 antiserum with the various mycobacteria except *M. scrofulaceum* K30003 and *terrae* did not reduced homologous reaction, on the contrary, it increased in many occasions,

Table 4. Reactivity of Rabbits Anti-*M. avium* Sera (K40004) with Other Mycobacteria

Mycobacteria	K40004 ROD	OARH (%)	HARD (%)
M. tuberculosis H37Rv	0.48	87.5	9.2
M. kansasii K20001	0.29	100.0	13.6
M. scrofulaceum K30001	0.14	100.0	37.5
M. scrofulaceum K30003	0.46	78.2	69.3
M. intracellulare N-238	0.36	99.3	88.8
M. intracellulare N-242D	0.14	100.0*	63.7
M. intracellulare N-245	0.38	89.5	70.6
M. intracellulare N-257T	0.19	100.0*	63.0
M. intracellulare N-257R	0.51	97.8	87.5
M. intracellulare N-260T	0.49	100.0*	61.8
M. intracellulare N-260D	0.71	100.0	75.8
M. intracellulare N-260R	0.44	100.0	50.6
M. intracellulare N-275T	0.30	89.6	63.4
M. intracellulare N-275R	0.38	100.0	58.8
M. intracellulare N-279T	0.41	100.0	67.9
M. intracellulare N-281D	0.25	100.0	66.2
M. intracellulare Fo3193T	0.39	81.4	84.4
M. intracellulare K41014	0.72	95.1	75.0
M. nonchromogenicum NC-3	0.06	53.1	55.1
M. nonchromogenicum NC-11	0.05	93.2	0.0
M. terrae T-7	0.02	0	0.0
M. terrae T-13	0.28	89.7	39.7
M. triviale V-4	0.04	0	1.8
M. triviale V-12	0.11	100.0*	43.9
M. chelomei R-20	0.04	0	60.8
M. fortuitum F-19	0.14	100.0	18.6
M. smegmatis K64002	0.18	100.0*	6.9

ROD=ratio of OD of heterologous over homologous reaction.
Explanation for HARD and OARH, see the text.

*Homologous or heterologous absorption did not reduce the heterologous or homologous reaction at all but it increased, instead.

suggesting that some homologous antibody might have cross-linked heterologous antigens bearing shared antigenic dorminants, on which additional antibodies were bound, to the coated homologous antigens. As it was in NC-3 antiserum, *M. scrofulaceum* K30003, *terrae* T-7 and T-13 reduced 14.7%, 14.5% and 28.7% of the homologous reaction respectively.

Although NC-3 antiserum showed a little less strong reaction to NC-11 and heterologous absorption with NC-11 reduced homologous reaction less when compared with those of NC-6, close antigenic relationship was observed between the strains of *M. nonchromogenicum* because their antiserum cross-reacted strongly and a great reduction of homologous or heterologous reaction was occurred by the heterologous or homologous absorption.

Rabbits immunized with *M. intracellulare* N-260T did not produce a measurable antibody reacting with *M. chelonei, nonchromogenicum* NC-3 & NC-11, *scrofulaceum* K30001, *smegmatis, terrae* T-7, and *triviale* V-4 & V-11 and produced very small amount of cross-reacting antibody to *M. fortuitum, terrae* T-13, and *tuberculosis*, all of which were easily removable by the homologous absorption. N-260T antiserum also reacted weakly with *M. scrofulaceum* K30003 and *avium* K40004 and the homologous absorption removed all of these antibody. However K30003 or K40004 absorption reduced merely 13.0% or 29.8% of the homologous reaction. N-260D and N-260R antisera also did not react at all or weakly reacted with *M. chelonei, fortuitum, nonchromogenicum, smegmatis, terrae* and *triviale*. K41014 antiserum did not react or weakly reacted with *M. chelonei, nonchromogenicum* NC-11, and *triviale*. All the antisera except N-260T antiserum had a considerable amount of antibody to *M. tuberculosis* H37Rv which of more than 95% were absorbed by the homologous absorption, whereas H37Rv absorption did not reduce the homologous reaction. Relatively small amount of antibody reacting with *M. kansasii* was present homologous reaction. Relatively small amount of antibody reacting with *M. kansasii* was present in N-260T and N-260D antisera and a considerable amount in N-260R and K41014 antisera, all of which could be removed by the homologous absorption. *M. kansasii* absorption did not reduce the homologous reaction of all of *M. intracellulare* antisera. *M. scrofulaceum* K30001 antibody was present in a relatively small amount in N-260D and N-260R antisera and a considerable amount in K41014 antiserum and the homologous absorption removed all these cross-reacting antibody but K30001 absorption did not reduce the homologous reaction except K41014 serum whose homologous reaction was reduced 10.4% by this strain (K30001). The other strain (K30003) of *M. scrofulaceum* seemed to show a closer antigenic relationship with *M. intracellulare* because all the *M. intracellulare* antisera except N-260T antiserum showed a 0.65 or higher RODs to this species and K30003 absorption reduced 18.1%, 24.3% or 31.5% of the homologous reaction of N-260D, K41014 or N-260R antiserum respectively. Except N-260T antiserum, all the other antisera contained a large amount of antibody to *M. avium* K40004 and all these antibody were removed by the homologous absorption. *M. avium* absorption reduced 17.6%, 21.3% or 33.2% of the homologous reaction of N-260R, N260D or K41014 antiserum respectively. A close antigenic relationship was of course observed between the strains of *M. intracellulare, M. avium* and *scofulaceum* K30003 also showed a close antigenic relationship with the strains of *M. intracellulare* used for antiserum production. N-260T antiserum showed a relatively weak reaction against N-242D, N-257T, N-275T, and Fo3193T and a moderately strong reaction against N-245, N-257R, N275R, N279T, and N-281D. Relatively strong reactions (RODs, >0.80) were observed with N-238, N260D, N-260R and K41014. Homologous absorption removed 100% or nearly so of these cross-reacting

antibody. Antigenic relationship between N-260T, N260D, and N-260R was so close that their homologous or heterologous absorption rendered almost complete reduction of homologous or heterologous reaction. A close antigenic relationship was also evident between N-260T, N260D, and K41014. However antigenic relationship between N-260T and K41014 was somewhat less close because their heterologous absorption reduced merely 48.9% or 57.9% of the homologous reaction even if their antisera cross-reacted strongly to each other and homologous absorption removed all of these cross-reacting antibody. In other words, shared antigens between N-260T and K41014 were present on their cell surface in smaller amount than those between the other strains. The heterologous absorption of N-260T antiserum with N-257T, N242D, N-275T, and N-275R, reduced less than 20% of the homologous reaction and with the other strains, 23~41%. Serological reactivity of N-260D antiserum with the other strains, N-260T, N-260R, and K41014, was very similar with N-260T antiserum. N-260D antiserum reacted weakly with N-242D, N-257T, N-281D, Fo3193T, and N-275T, that absorbed out approximately 30~49% of the homologous reaction. N-238 and N-275R showed somewhat close antigenic relationship with N-260R next to N-260T, N-260D, and K41014. K41014 antiserum showed a relatively weak reaction with N-281D, Fo3193T, N-242D, N-257T, and N-275T, that absorbed out approximately 29 ~49% of the homologous reaction. This strain also seemed to have somewhat close antigenic relationship with N-238 and N-275R next to the intimate strains N-260T, N260D, and N-260R.

M. avium K40004 antiserum unlike other antisera reacted with the other mycobacteria. Of the strains of taxonomically and antigenically close species *M. intracellulare* and *scrofulaceum*, N-260D and K41014 showed most strong reaction (RODs, 0.71 and 0.72) with *M. avium* antiserum and RODs of *M. intracellulare* N-238, N-245, N275T, N275R, N281D, and Fo3193T were 0.20~0.39. *M. avium* antiserum reacted very weakly with all the other mycobacteria. Most of these cross-reacting antibody were removed by the homologous absorption. RODs of *M. tuberculosis* H37Rv and *kansasii* K20001 reaction over *M. avium* homologous reaction were 0.48 and 0.29 respectively and these species reduced 9.2% and 13.6% of the homologous reaction. All the strains of *M. intracellulare* reduced more than 50.0% of *M. avium* homologous reaction indicating an abundance of shared antigens on them. *M. scrofulaceum* also considerably reduced homologous reaction and a greater reduction was obtained with K30003 than with K30001. *M. avium* antiserum did not react with *M. terrae* T-7 but weakly with T-13 that absorbed out 39.7% of the homologous reaction. Very weak or no reaction was also observed with *M. chelonei, fortuitum, nonchromogenicum* NC-3 & NC-11, *smegmatis*, and *triviale* V-4 & V-12.

DISCUSSION

It has been long understood there are considerable amount of the antigenic cellular constituents or metabolites shared by a wide range of mycobacterial species, which might be closely related to their phylogeny. Though the phenetic configuration of antigenic structure should be useful to delineate the phylogenetic relationship between different mycobecterial taxons, most of the recent immunological technology have not been proved to make it possible mainly due to the variation in antigenic expression of individual strains depending on the cultural conditions and to the heterogeneity in immunogenecity of individual antigens and in immune response of individual hosts. Nevertheless several immunological procedures have been successfully used for the classification and identification of certain mycobacterial species, subspecies or serobars. Shaeffer's seroagglutination tests opened a way of serotaxonomic scheme of mycobacteria and

it has been found very useful for the taxonomy of *M. avium-intracellulare-scrofulaceum*(MAIS) complex and some other mycobacteria[9]. This procedure, however, is not applicable to the mycobacterial species or strains whose colony is rough or dry. Immunodiffusion and immunoelectrophoresis have been also found useful to elucidate the antigenic relationships of mycobacteria although they have some drawbacks too because the antigens and antisera used in these procedures have not been standardized so that each laboratory uses its own reference reagents[10]. Rabbits immunized with *M. terrae* T-8 or T-13 produced a large amount of antibody cross-reacting with *M. avium* and *intracellulare* strains and the homologous absorption removed most of these cross-reacting antibody to the majority of strains but less to certain strains such as N-257R, N-260R, and N-281D that may possess shared antigens more than *M. terrae, M. intracellulare* strains bear a considerable amount of antigens shared with *M. terrae* that does not possess much as they did not reduced homologous reaction. *M. avium* did not reduce homologous reaction of T-8 antiserum at all but 35.6% of T-13 antiserum indicating the presence of a considerable amount of shared antigens on T-13 cell surface. It was obvious that amounts of shared antigens on the different strains of *M. intracellulare* varied as the removal of cross-reacting antibody by the homologous absorption was not same between the strains.

In contrast with *M. terrae* antisera, *M. avium* and *intracellulare* antisera except K41014 showed a very weak or no reaction to *M. terrae* and their homologous reaction was not reduced much by *M. terrae* absorption with an exception of *M. avium* antiserum whose homologous reaction was reduced 39.1% by *M. terrae* T-13 absorption.

M. terrae antisera reacted weakly with *M. nonchromogenicum*, however the latter species absorbed out a considerable amount of homologous reaction probably due to comparative abundance of shared antigens on both species. Antigenic sharing between T-13 and NC-11 was apparently less remarkable than between T-13 and NC-3. *M. nonchromogenicum* antisera showed a strong reaction indicating a close antigenic relationship. *M. terrae* seemed not to have a close antigenic relationship with *M. triviale* because antiserum of the former species did not or weakly react with the strains of the latter species that did not reduce their homologous reaction. *M. fortuitum* showed a considerably close antigenic relationship with *M. terrae* especially T-13. T-13 antiserum recognized *M. fortuitum* even closer than T-8 with which it reacted weakly and it's homologous reaction was reduced much less by T-8 absorption than by F-19.

T-8 and T-13 were found to be antigenically close with T-7 but less close to each other as though T-13 possessed some more shared antigens than T-8 because reaction to T-13 was stronger and reduction of T-8 homologous reaction was larger.

Reaction with *M. kansasii, scrofulaceum*, and *tuberculosis* was strikingly different between T-8 and T-13 antisera of which the former serum reacted weakly with them and it's homologous reaction was not reduced by the heterologous absorption with them, whereas the latter serum showed a strong reaction with them and their absorption reduced homologous reaction considerably. *M. kansasii* or *tuberculosis* cross-reacting antibody in *M. terrae* antiserum were removed less by the homologous absorption when compared with others, suggesting that shared antigenic determinants distributed in a considerable amount on *M. kansasii* or *tuberculosis*, on the contrary they are very rare on T-8 and relatively small on T-13.

M. scrofulaceum K30003 seemed to be antigenically closer to *M. terrae* than K30001.

M. nonchromogenicum antisera cross-reacted strongly with the other mycobacteria except few species. Homologous absorption removed most of those cross-reacting antibody with and exception

that reaction of NC-3 antiserum to *M. tuberculosis* was reduced merely 49.3% by the homologous absorption. Close antigenic relationship was obvious only between them next to *M. terrae* and *scrofulaceum* K30003. *M. avium, chelonei*, and *fortuitum* reduced homologous reaction of NC-3 antiserum considerably, indicating a good amount of antigenic sharing. The findings that NC-3 antiserum showed a weaker reaction with NC-11 than with NC-6 and the homologous reaction was reduced more by NC-6 absorption than by NC-11 indicated that NC-3 was antigenically closer with NC-6 than with NC-11. Analysis of NC-11 antiserum reaction also confirmed such relationship.

M.terrae antisera reacted weakly with *M. nonchromogenicum* but there were considerable amount of shared antigens on both organisms. It was interesting to note that *M. nonchromogenicum* antisera reacted strongly with *M. avium* and *intracellulare* while antiserum of the latter species poorly or did not react with *M. nonchromogenicum*.

M. intracellulare antiserum assay revealed that strains of this species seemed to have no or very little antigenic sharing with *M. chelonei, fortuitum, nonchromogenicum, scrofulaceum* K30001, *smegmatis*, and *triviale*. *M. chelonei* and *terrae* T-13 possessed certain amount of antigens shared with *M. intracellulare* and *vice versa*. A close antigenic relationship of the strains of *M. intracellulare* was found to be with *M. avium* and *scrofulaceum* K30003. And it was not clearly understood why *M. scrofulaceum* K30001 was antigenically estranged from *M. intracellulare, M. kansasii* and *tuberculosis* reacted weakly with *M. intracellulare* antisera except N-260R antiserum reaction to *kansasii* and did not reduce the homologous reaction of *intracellulare* antisera, suggesting a paucity of antigenic sharing. Though there were considerable strain variation in heterologous reaction between strains of *M. intracellulare*, a large antigenic sharing was evidently intraspecific and most close antigenic relationship was found between K41014, N-260D, N-260R, and N-260T, of which they showed an equal closeness to each other except between N-260T and K41014. Intraspecific heterologous reaction was removed easily by the homologous absorption, whereas reduction of the homologous reaction by heterologous absorption was different depending on the extent of antigenic sharing. Reduction of N-260D antiserum homolgous reaction by intraspecific heterologous absorption was found in order with N-260T>N-260R=K41014>Fo3193T>N-245>N-279T, reduction of N-260R antiserum reaction with K41014=N-260T=260D>N-238>N-257R>N-281D=N-275T=Fo3193>N-279T>N-245>N-275R, reduction of N-260T antiserum reaction with N-260R>N-260D>K41014>Fo3193T>N-245>N-279T, and reduction of K41014 antiserum with N-260R>N-260D>N-238>N-257>N-260T=N-281D=N-275T>N-279T=N-245>N-275R.

M. avium antiserum did not contain much of interspecific cross-reacting antibody, for example it did not react at all with *M. chelonei, nonchromogenicum* NC-3 & NC-11, *terrae* T-7, and *triviale* V-4, and very weakly reacted with *fortuitum* F-19, *intracellulare* N-242D & N-257T, *scrofulaceum* K30001, *smegmatis* and *triviale* V-12. Relatively strong reaction was found with *M. intracellulare* N-260D and K41014. Most of cross-reacting antibody were removed by the homologous absorption suggesting abundance of shared antigens on *M. avium* cell surface. Abundance of shared antigens on *M. avium* cell surfaces were confirmed again by observing a great reduction of homologous reaction by the heterologous absorption with menbers of MAIS complex. Though *M. intracellulare* N-242D, N257T and N-281D reacted very weakly with *M. avium* antiserum, they reduced more than 63% of the homologous reaction, suggesting that a large amount of shared antigens were distributed on *M. avium* cell surface while poor on those three strains. Whether the same explanation could be applied for a considerable reduction of

homologous reaction by *M. chelonei, nonchromogenicum* NC-3, *terrae* T-13, and *triviale* V-12 is not known.

SUMMARY

Cell surface antigenic relationships between pathogenic mycobacteria have been investigated by the enzyme-linked immunosorbent assay using phenol-killed cells and their rabbits antisera.

Homologous and heterologous reactions of *Mycobacterium avium-intracellulare* antisera before and after homologous and heterologous absorption revealed a close antigenic relationship between strains of the same species and between species if they were members of *M. avium*(MA)-*intracellulare*(MI)-*scrofulaceum*(MG) complex. MAI sera showed a considerable reaction with *M. kansasii*(MK) and *tuberculosis*(MTB), but not with the other species. MA(K40004) antiserum reacted with other mycobacteria except few strains of MI and 50~89% of homologous reaction was reduced by heterologous absorption with cells of MI or MS. Intraspecific reaction of MI antisera was naturally stronger than interspecific reaction and different in extent due to a magnitude of antigenic sharing. Antigenic relationships between N-260D, N-260R, N-260T, and K41014 was somewhat closer than that with N-242D, N-257T, N-281D, and N-275T.

M. nonchromogenicum(MNC) antisera showed a strong interspecific reaction with exception of *M. chelonei*(MC) and *triviale*(MTV) to which they reacted weakly or none. Antigenic sharing with *M. terrae*(MTR) and MG(K30003) was next to intraspecific sharing. NC-3 shared antigens considerably with MA, MC, and *M. fortuitum*(MF) while NC-11 did not.

MTR antisera showed a strong cross-reaction with MI but their homologous reaction was not reduced by MI absorption indicating a paucity of shared antigen of MTR surface. Intraspecific antigenic sharing of course was large with on exception between T-8 and T-13. A considerable amount of antigenic sharing was also found with MNC, MC and MF. Unlike T-8 serum, T-13 antiserum strongly cross-reacted with MA, MG, MK, and MTB.

In general, antigenic relationships of mycobacteria, that have been elucidated in this study, well conformed to taxons delineated by the various biological and biochemical means.

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