Skin Test Reactivity to Mycobacterial Antigens in Patients with Pulmonary Tuberculosis and in Normal Population in Korea

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=국문초록=

한국인 폐결핵환자와 건강성인 및 국민학교 1학년아동의 결핵 및 비결핵성
항산균항원에 대한 피부반응

대한결핵협회 결핵연구원

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균양성(95명) 및 균음성(131명) 폐결핵환자와 건강성인(39명) 및 BCG 접종반응이 없는 6~7세 국민학교 1학년아동(1,162명)을 대상으로 tuberculin 단독 또는 tuberculin 과 비결핵성항산균항원을 동시에 피내주사한 다음 그 반응을 관찰한 바 다음과 같은 결과를 얻었다.

경시적 피부 반응양상은 다같이 않아 최대반응이 나타나는 시간이 다 달랐다.

균양성환자에서는 4~6시간에 반응이 나타나서 24시간에 최대에 이르는 조기반응형이 많이 관찰되지만(53%), 균음성환자 또는 건강성인 가운데서는 거의 대부분(58~68%)이 48시간에 최대반응을 나타내었다. 균양성 및 음성환자의 약 10%는 72시간까지 반응크기가 서서히 증가하는 지연형 반응을 보여주었다. 또 균양성 및 음성 폐결핵환자와 건강성인은 93~100%가 양성반응(10 mm 이상)을 나타내는데 비해 국민학교 아동은 9.9%만이 양성반응을 보여주었다. 평균경결반응의 크기는 대개 48시간에 최대에 달하는데, 폐결핵환자 및 건강성인의 48시간에서의 평균경결반응크기는 19.0~20.9 mm 인데 비해서 국민학교 아동은 15.4 mm 였다.

대부분의 균양성 환자가 *M. avium* 또는 *M. scrofulaceum* 항원에 대해 10 mm 이상의 경결반응을 보여주었다. 국민학교 1학년 아동에 대해 결핵균과 비결핵성항산균 항원으로 이중피부반응검사를 실시한 결과를 보면 *M. tuberculosis*, *M. scrofulaceum*, *M. avium*, *M. intracellulare*, *M. fortuitum*에 6 mm 이상의 반응을 보인 아동이 각각 11.5%, 20.6%, 13.3%, 13.7% 및 19.0%였으며 이들 각 항원에 대해서만 특이하게 반응하는 아동은 2.3%, 10.7%, 5.8%, 3.4% 및 11.4%였다. 10 mm 이상의 반응을 나타낸 아동 가운데서는 각각 1.9%, 5.2%, 3.6%, 1.7% 및 8.2%가 특이반응을 보여주어 비결핵성 항산균에 의한 감염이 상당히 있음을 알 수 있었다.

* 본 연구는 결핵연구원 1984년도 연구비에 의해 이루어졌음.

INTRODUCTION

The skin testing with tuberculin has long been used to identify the tuberculous infection mainly for the epidemiological and diagnostic purposes¹⁻³. However it has been recognized that a skin reaction to tuberculin does not necessarily indicate infection solely with *Mycobacterium tuberculosis*, but it may indicate cross reactions, caused by other mycobacterial infection, particularly among low-grade tuberculin reactors⁴⁻⁶. The delayed skin hypersensitivity to intradermal injection of tuberculin appears approximately 6 to 8 weeks after infection with *M. tuberculosis* and shows maximum reaction between at 48 and 72 hours. The reaction is recorded by measuring the size of induration instead of erythema although the latter reaction has equal immunological significance². It has been observed by several investigators that the time course of induration to tuberculin test is not identical in all reactors⁷⁻⁹. Lenzini et al⁷ classified the tuberculin reaction in tuberculosis patients into basically different two types, i.e., the typical delayed hypersensitivity which appears maximum reaction at 48~72 hours after intradermal injection of tuberculin and an early reaction (Jones-Mote hypersensitivity) peaking at 24 hours but disappearing at 48 hours. They, however, observed mixed reaction in many individuals, thereby constructing the third type of biphasic reaction pattern. A similar scheme of classification has been made by Stanford et al⁹ of which an early reaction peaking at 48 hours was called as Listeria type and the typical delayed reaction as the Koch-type. They suggest that the different types of reaction may be mediated by different mechanism and reflect different immunological implication especially in protection of host from infection.

We have investigated skin reactivity to tuberculin and other mycobacterial antigens in patients with pulmonary tuberculosis and in normal population in Korea. In addition, we have made an investigation on nontuberculous mycobacterial in-

fection in school children without BCG scar by simultaneous skin testing of human tuberculin with other mycobacterial sensitins.

MATERIALS AND METHODS

1. Sensitins

The following five sensitins were purchased from the Seruminstitut of Copenhagen, Denmark for this study.

a) *M. tuberculosis* sensitin RT 23 in tween diluent, 1 TU per 0.1 ml.

b) *M. scrofulaceum* sensitin RS 95, 0.1 mcg per 0.1 ml.

c) *M. avium* sensitin RS 10, 0.1 mcg per 0.1 ml.

d) *M. intracellulare* sensitin RS 23, 0.1 mcg per 0.1 ml.

e) *M. fortuitum* sensitin RS 20, 0.1 mcg per 0.1 ml.

All sensitins were used prior to their expiration dates and were refrigerated until the day of use.

2. Study population

Two hundreds and twenty six patients with pulmonary tuberculosis in Kong-Ju National Tuberculosis Hospital were tested simultaneously with tuberculin RT23 and *M. avium* sensitin RS 10 or *M. scrofulaceum* sensitin RS 95. Tubercle bacilli were demonstrated in the sputum specimens of 65 male and 30 female patients but not in the sputum specimens of 90 male and 41 female patients. Ages of 122 patients were between 20 and 39 years and 78 patients, 40 to 59 years old. Ages of few patients were less than 20 years or over 59 years.

Tuberculin tests were performed for 39 healthy adults who were 22 females and 17 males. Ages of 32 persons were 20 to 39 years and seven were 40 to 59 years old. Among 39 eligibles, only 27 individuals were available for observation of up to 72 hour tuberculin reaction.

Simultaneous skin testing of tuberculin with no-

Table 1. Tuberculin reaction of the patients with pulmonary tuberculosis, normal adults, and school children

Subjects	Number of testee	Induration Size			
		More than 5mm		More than 9mm	
		No. of reactors	Mean size & SE	No. of reactors	Mean size & SE
Sputum positives	95	95(100.0)	22.0±0.3	95(100.0)	22.0±0.3
Sputum negatives	131	130(99.2)	20.8±0.3	129(98.5)	20.9±0.3
Normal adults	39	36(92.3)	19.9±0.6	36(92.3)	19.9±0.6
School children	1,162	134(11.5)	15.2±0.4	115(9.9)	16.5±0.4

* Numbers in parenthesis represent per cent.

** Mean induration size was based on maximum reaction during a period of 24 to 72 hours observation.

nontuberculous mycobacterial sensitins were performed for 1st graders of primary school children who did not have BCG scar. Most of their age were 6 years but there were few of 7 years old children. Five hundreds and seventy four were male and 588, female.

3. Skin testing

All subjects were tested with intradermal injection of 1 TU of tuberculin RT 23 in a 0.1 ml volume on the volar surface of left forearm and not all but most of them were tested simultaneously with 0.1 mcg per 0.1 ml of nontuberculous mycobacterial sensitins on the right forearm. The skin reaction of patients and normal adults and parts of school children were observed at 4, 24, 48 and 72 hours following intradermal injection of sensitins.

RESULTS

Skin reaction to tuberculin PPD RT 23 of the patients with pulmonary tuberculosis, normal adults, and school children have been summarized in table 1. All sputum positive cases reacted to tuberculin with 10 mm or more of induration and their mean size was 22.0 mm. Of 131 sputum negative cases, 6 mm or more reaction was observed in 130 patients and 10 mm or more in 129 patients and their respective mean induration sizes were 20.8 and 20.9 mm. Mean induration of 36 positive

reactors out of 39 normal adults was 19.9 mm which seemed to be smaller than that of the patients with active disease. The number of reactors and their mean size were much small in school children showing 15.2 mm of mean induration in 11.5% of more than 5 mm reactors and 16.5 mm in 9.9% of more than 9 mm reactors as seen in table 1.

The time course of tuberculin reaction varied from person to person and changes of mean induration and erythema were summarized in table 2 and shown in figures 1~3. Here again the stronger and earlier reaction was observed in patients with active disease such as in sputum positive cases markedly at 24 hours reaction.

Largest mean induration appeared at 48 hours in all groups. Erythema reaction was much larger than induration but varied and not paralleled to the induration size. More than 9 mm induration was observed in 31.6% of sputum positive cases and 20.6% of sputum negative cases even at 4 hours reaction. But only one out of 12 positive reactors in school children whose reactions were observed from 4 to 72 hours after sensitin injection showed more than 9 mm reaction at 4 hours.

We could have classified the reaction patterns of positive reactors into three types according to the time at which the maximum reaction attained, even though it was not distinct in many cases. The type I reaction reached to maximum reaction at 24 hours and thereafter remained unchanged or

Table 2. Change of tuberculin reaction size after intradermal injection of sensitins

Reaction	Subjects	Reading time			
		4 hrs	24 hrs	48 hrs	72 hrs
Induration	Sputum positives	6.2±0.5*	19.7±0.4	20.9±0.3	19.9±0.3
	Sputum negatives	5.2±0.4	17.6±0.4	19.0±0.4	18.9±0.3
	Normal adults	—	16.9±0.9	19.7±0.8	18.9±0.8
	School children	1.8±0.9	11.8±1.2	15.4±0.7	15.5±0.7
Erythema	Sputum positives	10.1±0.4	23.9±0.9	31.9±0.9	29.8±1.1
	Sputum negatives	9.3±0.4	21.4±0.6	29.8±1.0	29.0±1.2
	Normal adults	—	22.1±1.3	33.3±1.9	36.8±2.9
	School children	2.9±1.0	14.4±1.3	23.5±2.2	24.7±2.6

* Mean induration(mm) and SE

Fig. 1. Tuerbculin reaction of normal adults. *Mean induration size and standard error(SE) at 48 hours.

steadily decreased. In the type II reaction the maximum reaction attained at 48 hours and then remained unchanged or slightly decreased. The type III reaction was characterized by a continuous increase of induration size up to 72 hours. As shown in table 3, the type I reaction was observed in 53.3% and 33.9% of sputum positive and negative

patients and in 28.0% and 25.0% of normal adults and school children. However the type II reaction was encountered more frequently in sputum negative patients or in normal individuals than in sputum positive cases. Approximately 10% of positive reactors showed type III reaction in both sputum positive and negative patients. From this ob-

Fig. 2. Tuberculin reaction of sputum positive patients with pulmonary tuberculosis.
*Mean induration and standard error.

servation it is clear that an early and large reaction appears more frequently in the patients with active disease. And the positive tuberculin reactors whose reaction size decreased at 72 hours outnumbered those who showed a largest reaction at that time, namely 79 over 24 among 250 reactors investigated. Consequently the reading of reaction at 48 hours instead of 72 hours permit to record largest reaction size in most cases.

Fig. 3. Tuberculin reaction of sputum negative patients with pulmonary tuberculosis.
*Mean induration and standard error.

The positive skin reaction (more than 9 mm induration) to *M. avium* sensitin was observed in 97.9% of sputum positive cases and in 84.6% of sputum negative cases and their mean size was 18.6 and 17.5 mm respectively as seen in table 4 and figures 4 and 5. And 41(9.9%) out of 414 school children tested showed more than 9 mm reaction to sensitin RS10 with mean size of 14.0 mm (table 4 and figure 5). The number of positive reactors and the reaction size were much more and larger in the sputum positive cases than in sputum negative cases suggesting some relationship with disease activity. The difference in relation with disease

Table 2. Reaction patterns for tuberculin positives

Subjects	Reaction type	Number of individuals	Reading time			
			4	24	48	72 hrs
Sputum positive cases	I	49(53.3)	7.9±0.8**	21.8±0.4	21.1±0.4	20.0±0.3
	II	34(37.0)	4.3±0.7	17.4±0.6	21.0±0.6	19.7±0.4
	III	9(9.8)	2.4±1.3	17.3±2.2	19.0±1.3	21.1±1.2
Sputum negative cases	I	41(33.9)	7.9±0.9	21.0±0.6	20.4±0.5	19.8±0.5
	II	68(56.2)	4.6±0.6	16.7±0.4	20.4±0.4	18.9±0.3
	III	12(9.9)	4.2±1.5	15.0±2.3	18.5±1.4	20.5±1.4
Normal adults	I	7(28.0)	—	20.7±1.1	20.0±0.8	19.6±1.1
	II	17(68.0)	—	15.4±1.3	19.8±1.1	19.6±0.7
	III	1(4.0)	—	3.0	11.0	14.0
School children	I	3(25.0)	5.3±2.4	14.3±1.5	14.7±1.5	13.3±1.7
	II	7(58.3)	0.7±0.4	12.3±1.4	16.4±0.8	16.6±0.9
	III	2(16.7)	0	6.5	13.0	15.0

* Numbers in parenthesis represent per cent.

** Mean induration(mm) and SE

ase activity was much more prominent when compared with those of tuberculin reaction. Linear relationship of reaction between tuberculin(RT 23) and avian sensitin (RS 10) of sputum positive or negative patients has been shown in scatter diagram of figure 4. In bacillary cases correlation coefficient r was given as 0.33 ($Y = -3.43 + 0.53 X$, standard error of estimators $SE = 4.14$) while in abacillary cases $r = 0.53$ ($Y = -1.77 + 0.81 X$, $SE = 5.77$), indicating low degree of relationship between two reactions.

There was no patient who reacted only to avian sensitin, but 10 sputum negatives and one sputum positive case showed less than 10 mm reaction. The results suggest that the patients with active disease showed a stronger cross reaction with avian sensitin than sputum negative patients.

The skin reaction to avian sensitin in school children was different from that of the patients with pulmonary tuberculosis. The reactors of 10 mm or more of induration to either tuberculin or avian sensitin or both were 12.8% of whom 6.3% reacted to both sensitins but 3.6% reacted significantly to avian sensitin and 2.9 % to tuberculin as

shown in table 5 and figure 5. If 6 mm or more of induration regarded as positive reaction, total reactors to either one or both sensitins should be 17.2% of whom the percentages of the reactors equally to both sensitins and specifically to avian sensitin or RT23 are 8.0%, 5.8% and 3.4% respectively. There are no generally accepted skin test criterion for the discrimination of tuberculous infection from nontuberculous mycobacterial infection. Our arbitrary criterion for specific reaction was based on circumstantial evidence that as seen in figure 5 the specific reactors to avian sensitin seemed to have been clustered to its end with very small reaction to tuberculin unless the induration size was larger than 9 mm.

The proportion of positive skin reaction to *M. scrofulaceum* sensitin RS 95 of the patients with pulmonary tuberculosis was even higher than that of avian sensitin. More than 97% of sputum positive or negative patient showed 10 mm or more of induration to *scrofulaceum* sensitin and their mean induration size was 19.9 mm or 19.1 mm respectively as seen in table 6 and figure 6. Both proportion of positive reactors and their mean indurat-

Table 4. The skin reactions to *M. avium* sensitin RS 10 of sputum positive and negative patients with pulmonary tuberculosis and of school children

Subjects	Number of testee	Induration size(mm)			
		More than 5 mm		More than 9 mm	
		No. reactors	Mean size & SE	No. reactors	Mean size & SE
Sputum positives	47	46(97.9)	18.6±0.6	46(97.9)	18.6±0.6
Sputum negatives	65	57(87.7)	17.1±0.6	55(84.6)	17.5±0.5
School children	414	55(13.3)	12.4±0.6	41(9.9)	14.0±0.5

* Numbers in parenthesis represent per cent.

Fig. 4. Scatter diagram of skin reactions to tuberculin RT 23 and avian sensitin RS 10 in sputum positive (47) and negative (65) patients.

Table 5. The reactors to tuberculin(PPDRT 23) and avian sensitin(PPDRS 10) in school children without BCG scar(414)

Positive reaction to	Induration size	
	More than 5mm	More than 9mm
Either one or both sensitins	71(17.2)	53(12.8)
Tuberculin (RT 23)	49(11.8)	37(8.9)
Specific to PPD RT 23	14(3.4)	12(2.9)
Avian sensitin (RS 10)	55(13.3)	41(9.9)
Specific to RS 10	24(5.8)	15(3.6)
Both sensitins equally	33(8.0)	26(6.3)

* Numbers in parenthesis represent per cent.

Fig. 5. Scatter diagram of skin reactions to tuberculin RT 23 and avian sensitin RS 10 in school children without BCG scar.

ion were small in school children showing 20.6% of more than 5 mm reactors and 14.0% of more than

9 mm reactors and their respective mean sizes were 12.2 mm and 14.4 mm. Correlation of tube-

Table 6. The skin reactions to *M. scrofulaceum* sensitin RS 95 of sputum positive and negative patients and of school children

Subjects	Number of testee	Induration size			
		More than 5 mm		More than 9 mm	
		No. reactors	Mean size & SE	No. reactors	Mean size & SE
Sputum positives	48	46(97.9)*	19.9(0.4)**	47(97.9)*	19.9(0.4)**
Sputum negatives	66	65(98.5)	19.1(0.5)	65(98.5)	19.1(0.5)
School children	272	56(20.6)	12.2(0.5)	38(14.0)	14.4(0.5)

* Numbers in parenthesis are per cent,
** Numbers in parenthesis are standard error.

Fig. 6. Scatter diagram of skin reactions to tuberculin RT 23 and scrofulacin RS 95 in sputum positive (48) and negative(66) patients.

rculin reaction with reaction to *M. scrofulaceum* sensitin has been shown in figure 7 and summarized in table 7. Forty two out of 272 children showed more than 9 mm reaction to either tuber-

Table 7. The skin reaction of 272 school children to tuberculin RT 23 and *M. scrofulaceum* sensitin

Positive reaction to	Reaction size(mm)	
	More than 5 mm	More than 9 mm
Either one or both sensitins	59(21.7)	42(15.4)
Tuberculin PPD RT 23	30(11.0)	28(10.3)
Specific to PPD RT 23	4(1.5)	4(1.5)
Scrofulacin PPD RS 95	56(20.6)	38(14.0)
Specific to PPD RS 95	29(10.7)	14(5.2)
Equal to both sensitins	26(9.6)	24(8.8)

* Numbers in parenthesis are per cent.

Fig. 7. Scatter diagram of skin reactions to tuberculin RT 23 and scrofulacin RS 95 in school children without BCG scar.

culin or scrofulacin or both. Tuberculin reactors were 28(10.3%) of whom tuberculin specific reaction was observed in four children(1.5%). If mo-

re than 5 mm reaction regarded as positive, 29 (10.7%) out of 272 children showed scrofulacin specific reaction.

Table 8. The skin reaction of 292 school children to tuberculin RT23 and *M. intracellulare* sensitin RS 23

Positive reaction to	Reaction size	
	More than 5 mm	More than 9 mm
Either one or both sensitins	43(14.7)	38(13.0)
Tuberculin PPD RT 23	33(11.3)	32(11.0)
Specific to PPD RT 23	4(1.4)	4(1.4)
Intracellulin PPD RS 23	40(13.7)	33(11.3)
Specific to PPD RS 23	10(3.4)	5(1.7)
Equal to both sensitins	29(9.9)	28(9.6)

* Numbers in parenthesis are per cent.

Fig. 8. Scatter diagram of skin reactions to tuberculin RT 23 and intracellulin RS 23 in school children without BCG scar.

The skin tests to *M. intracellulare* and *M. fortuitum* sensitins were performed for school children only. Positive skin reaction to *M. intracellulare* sensitin RS 23 was found in 33(11.3%) out of 292

Table 9. The skin reaction of 184 school children to tuberculin RT 23 and *M. fortuitum* sensitin RS 20

Positive reaction to	Reaction size	
	More than 5 mm	More than 9 mm
Either one or both sensitins	43(23.4)	33(17.9)
Tuberculin PPD RT 23	22(12.0)	18(9.8)
Specific to PPD RT 23	9(4.9)	6(3.3)
Fortuitin PPD RS 20	35(19.0)	27(14.7)
Specific to PPD RS 20	21(11.4)	15(8.2)
Equal to both sensitins	13(7.1)	12(6.5)

* Number in parenthesis are percent.

Fig. 9. Scatter diagram of skin reactions to tuberculin RT 23 and fortuitin RS 20 in school children without BCG scar.

children whose reaction size exceeded 9 mm and only five children (1.7%) reacted specifically as seen in table 8 and figure 8. Specific reactors are

3.4% if including 6~9 mm reactors.

M. fortuitum (PPD RS 20) reaction was observed in 27(14.7%) out of 184 children of whom

15(8.2%) children showed specific reaction which was highest of those of nontuberculous mycobacterial sensitins tested (table 9 and figure 9).

Children selected for this study were from two urban and six rural schools in Kang-Won, three rural schools in Choong-Buk, two rural schools in Chun-Buk, one rural school in Keong-Ki, and one urban school each in Pusan and Che-Ju provinces. Number of children in each area was too small to analyze the data by areas.

DISCUSSION

The skin tests with old tuberculin suffered from nonspecific reaction¹⁰⁻¹²⁾ apparently due to the prevalence of nontuberculous mycobacterial infection.

Seibert improved tuberculin to some extent by excluding the substances which caused nonspecific reaction¹²⁾. The development of biochemical and immunological procedures permitted to isolate highly purified antigen from crude mixtures but as yet the general agreement on their immunological specificity and reactivity was lacking¹²⁾. Recent advances in the production of monoclonal antibody are promising as it permits to foresee to provide more precise information about the antigenic structure of different tubercle bacilli and the means of antigen purification. Despite lack of the specificity, the tuberculin tests have been long and widely used to screen tuberculous infection by measuring induration at 48-72 hours after intradermal injection of 5 TU of tuberculin or bioequivalent doses. In general the mean induration of infected individuals is 16 mm and 95% of them distribute in the range of 8 to 24 mm³⁾. However there is no generally accepted skin test criterion for the discrimination of tuberculous infection from nontuberculous mycobacterial infection. In many countries, 10 mm induration has been used as a cutoff point although in areas where nontuberculous mycobacterial infections are very rare, even 5 mm could discriminate tuberculous infection, but 10 mm cutoff point may be unavoidable to include a significant number of nontuberculous infection in areas where such

infections are common³⁾.

The mean indurations of sputum positive and negative patients with pulmonary tuberculosis were 20.9 and 19.0 mm at 48 hours after intradermal injection of 1 TU of tuberculin RT 23 stabilized with tween 80 and 19.9 and 18.9 mm at 72 hours in this study.

The time course of tuberculin reaction was not identical in all reactors as investigated previously⁷⁻⁹⁾. The maximum reaction appeared distinctly at 24 hours in some reactors and decreased thereafter but it appeared beyond 48 hours in others as though most of reactors showed mixed type of reactions. Therefore mean induration of sputum positives turned out 22.0 mm if analyzed in their maximum reaction observed during a period of 24 to 72 hours and 20.8 mm in sputum negative cases and 19.9 mm in normal adults.

The first graders of primary school who did not have BCG scar reacted to tuberculin with much smaller size (16.5 mm) of induration when compared with those of patients with pulmonary tuberculosis and of normal adults. The older ages in testees, the higher of positive rate and also the larger in reaction size. This may be due to repeated exposures to the tuberculous and/or nontuberculous mycobacteria in older age groups or may be due to the less matured immunity of school children. Early reactions such as more than 9 mm induration at 4 hours or the maximum reaction at 24 hours was much more common in sputum positive cases. The 24 hours maximum reaction appeared in an over half (53%) of sputum positive cases while it appeared in 34% of bacillary cases, in 28% of normal adults and in 25% of school children. Hence it seems hardly to relate early skin reaction to the protective immunity in human being even if close relationship between early reaction and protective immunity had been clearly demonstrated elsewhere^{7,9,13)}. The 48 hours maximum reaction was observed in 56-68% of sputum negative patients and normal individuals but in 37% of bacillary cases apparently being contrary to the early reaction. The proportion of late reactors

Table 10. Isolation of nontuberculous mycobacteria from sputum specimens with or without clinical significance and from environmental source such as soil ^{14,15)}

Species or complex	Clinically significant isolates*	Clinically insignificant isolates ¹⁵⁾	Environmental isolates ¹⁴⁾
<i>M. scrofulaceum</i>	4(20.0)	15(15.5)	25(13.3)
<i>M. szulgai</i>	1(5.0)	0	0
<i>M. gordonae</i>	0	11(11.3)	12(6.4)
<i>M. flavescens</i>	0	3(3.1)	3(1.6)
Other scotochromogens	0	14(14.4)	0
<i>M. avium-intracellulare</i> complex	5(25.0)	9(9.3)	9(4.8)
<i>M. terrae</i> complex	1(5.0)	31(32.0)	58(30.9)
Other nonchromogens	0	9(9.3)	24(12.8)
<i>M. fortuitum</i>	6(30.0)	1(1.0)	55(29.3)
<i>M. chelonae</i>	2(10.0)	0	0
<i>M. smegmatis</i>	1(5.0)	0	0
Other rapid growers	0	4(4.1)	2(1.1)
Total isolates	20	97	188

Numbers in parenthesis are per cent. * This data was derived from clinical isolates of nontuberculous mycobacteria in our laboratory during a period of 1980 to 1983. Etiological relationship of those isolates has been confirmed by repeated isolation from a serial sputum specimens of the patients for over 6 months of period.

was same between sputum positive and negative patients.

It has been well known that in some tropical or subtropical areas nontuberculous mycobacterial infections exceed tuberculous infection and this may influence to the epidemiology of tuberculosis. Palmer et al¹¹⁾ consider high proportion of 5~11 mm reactors indicating high prevalence of nontuberculous mycobacterial infection and the typical bimodal distribution of tuberculin reaction in general population indicating absence of nontuberculous infections. This is not true in Korea because as much as 26% of more than 4 mm reactors in 6~7 years old school children fall in 5~11 mm reaction. Simultaneous skin testing of tuberculin with nontuberculous mycobacterial sensitins in school children without BCG scar revealed that prevalence of nontuberculous mycobacterial infections was unexpectedly high. More than 5 mm reactors to the sensitins of *M. tuberculosis*, *M. scrofulaceum*, *M. avium*, *M. intracellulare*, and *M. fortuitum* were 11.5%, 20.0%, 13.3%, 13.7%, and 19.0%

and specific reaction to the corresponding sensitins were 2.3%, 10.7%, 5.8%, 3.4%, and 11.4% respectively. More than 9 mm reactors to the above mentioned sensitins were 9.9%, 14.0%, 9.9%, 11.3%, and 14.7% respectively and their respective specific reactors were 1.9%, 5.2%, 3.6%, 1.7%, and 8.2%, clearly indicating that *M. scrofulaceum* and *M. fortuitum* infections seemed to exceed tuberculous infection in this age group (figure 10). Although we could not clarify whether 10 mm or more react to both sensitins or 6~9 mm reaction within 4 mm difference indicate infection with both *M. tuberculosis* and *M. avium* or *M. tuberculosis* infection crossreacting with avian sensitin, we assume they might have been infected with both organisms because the number of specific reactors to avian sensitin exceeded the tuberculin specific reactors. This finding was somewhat compatible with the frequency of isolation of nontuberculous mycobacteria from clinical sources with or without clinical significance and from environmental sources (soil) as seen in table 10^{14~15)}.

Fig. 10. Summary of the skin reactivity of school children to mycobacterial sensitins. Mean induration and standard error of positive reactors whose reactions were 10 mm or more to the corresponding sensitins. Shaded bar indicates positive rates and plain bar indicates proportion of specific reactors.

Several surveys on skin reactivity to nontuberculous mycobacterial sensitins in Korea had been made in 1965 and 1967^{16,17)}. In 1965 survey¹⁶⁾ the positive reactors to nontuberculous sensitins in an age group of 6~12 years turned out 0.9~6.2% whose reaction (erythema) were more than 25% larger than that of tuberculin reaction. Quality of sensitins used and criteria employed are different from that of the present study so that direct comparison is not possible. However low positive rate might have been resulted from high prevalence of tuberculous infection in that age group at that time. In other words, children in mid-1960s were at high risk of infection with *M. tuberculosis* which has much higher virulence than nontuberculous mycobacteria and thus nontuberculous mycobacterial infection is hardly to establish in the presence of tuberculous infection.

Prevalence of tuberculous infection in children of 6~7 years old was 31.5% in 1965 but it decreased down to 9.9% turned out in this study^{18,19)}. In 1967 survey¹⁷⁾ 20.4% of children of 0~13 years reacted 5 mm or larger induration to scotochromogenic sensitin than that to tuberculin and 15.9% to nonchromogenic sensitin and 10.5% to rapid grower sensitin. This and Haitian survey²⁰⁾ sho-

wed high proportion of *M. scrofulaceum* infection as it was in our study, but *M. fortuitum* infection was least common in both surveys contrasting with our findings in which *M. fortuitum* reactions were second common to scrofulacin reactions. Batty reactors in Indian children of an age group of 5~9 years were 40% in high prevalent areas and 10% in low prevalent areas²¹⁾.

SUMMARY

The skin reactions to tuberculin and nontuberculous mycobacterial sensitins in patients with pulmonary tuberculosis, in normal adults, and in school children without ECG scar have been investigated.

The time course of tuberculin reaction was not identical in all reactors but differed in the time of appearance of maximum reaction. Early reactions appearing within 4~6 hours or peaking at 24 hours were observed most commonly in bacillary cases(53%). The 48 hour-maximum reaction was found in 58~68% of sputum negative patients(aba-cillary cases) and normal individuals. Later reactors whose reaction increased continuously up to 72 hours were around 10% in both sputum positive and negative patients. More than 9 mm reactors

were 100% and 98% in bacillary and abacillary cases and 93% in normal adults but 9.9% in school children.

Mean induration size was largest at 48 hours in all subjects showing 19.0~20.9mm in patients and normal adults but 15.4 mm in school children. The Earlier and larger reaction was apparently common in patients with active disease.

Almost all of bacillary cases reacted to avian and scrofulacin sensitins with more than 9 mm induration. Simultaneous skin testing of tuberculin with nontuberculous mycobacterial sensitins in school children revealed that more than 5 mm reactors to sensitins of *M. tuberculosis*, *M. scrofulaceum*, *M. avium*, *M. intracellulare*, and *M. fortuitum* were 11.5%, 20.6%, 13.3%, 13.7%, and 19.0% and specific reactors to the corresponding sensitins were 2.3%, 10.7%, 5.8%, 3.4%, and 11.4% respectively. More than 9 mm reactors specific to the above mentioned sensitins were 1.9%, 5.2%, 3.6%, 1.7%, and 8.2% respectively.

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