結核의 抗原抗體反應에 關한 實験的研究

第1篇  CORTISONE 또는 PURINE 投與動物의 結核菌免疫
血中抗體価와 TUBERCULIN ALLERGY 反應値

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國立保健院

全南 昊

＜本論文의 要旨는 1965年 5月 第20次 大韓結核學會에 發表하였음＞

Ⅰ 様論

Ⅰ 実験方法 及 材料
1. 結核菌 菌液製成
2. 結核抗原 及 Coombs 抗血清
3. CORTISONE 及 PURINE 投與動物
4. TUBERCULIN 反應
5. 臼球抗原
6. 抗血清
7. 臼球抗原反應値

Ⅱ 実験成績
1. CORTISONE 또는 PURINE 投與動物에서의 TUBERCULIN
   反應及 臼球抗原反應

Ⅲ 結論

Middlebrook & Dubos 10의 結核菌 菌液製成 及 抗
原性作 臼球抗原反應(以下 H.A.로 略)及 臼球抗原
作(以下 H.L.로 略)이 1948에 發表된 後 達
12) 등은 1957年에 中実験 略じ Coombs 酸血清法
を 利用하여 所謂 結核的 寫定及 不完全抗體를 識別ヶ月
あり hend. 然而 우리 국내에서는 TUBERCULIN及
B.C.G.의 多年嘗種。 然而 患者의 隔離及 治療が 不完
全하여 結核菌的 常見概要가 많으므로 血中抗體究
明으로는 結核患者及 健康人을 分別하기 困難철
ある。 然而로 略筆之研究에 있어서是 現在 場合의 方法으로
認明된 結核菌抗體에 있어서 이것은 主として Bivalent 抗體
 sollen 血球凝集反應 및 臼球抗原反應の 陽性이고。 Univalent
抗體群에는 血球凝集反應 및 臼球抗原反應이 隱性이 되고
한데 끝이 그 機轉を 追究하였고 結核菌抗體
의 有無에 及 免疫反応의 成立與否及 免疫感染은 常見

Ⅳ 概観及 考按

参考文獻

1) B.C.G.(Pasteur 番)免疫群
2) 大型結核菌(H37RV 群)免疫群
3) 小型結核菌(Ravenel 群)免疫群

2. CORTISONE 또는 PURINE 投與動物에서의 TUBERCULIN
   反應及 臼球抗原反應。 反應値及 治療反應
   1) B.C.G.(Pasteur 番)免疫群
2) 大型結核菌(H37 RV 群)免疫群
3) 小型結核菌(Ravenel 群)免疫群

Ⅵ 総括及 考按

参考文獻
實驗方法 裏 材料

1. 使用菌株 裏 所用菌株

三塚研所製造苗として C.D.T,P.N と 各 人型菌株を, 細菌培養型酵母型 吸収酵母型 に RAVENEL 牛型菌株及び Pasteur B.C.G. 菌株を 各 6 株を 使用した。免疫

2. 實験方法 裏 Combs 抗血清

主な実験方法 裏 Combs 抗血清は Middlebrook & Dubos 裏 で製造

1. 使用菌株 裏 材料

Tuberculin 抗原 Fr I, II. と 各 人型菌株を, 細菌培養型酵母型 に RAVENEL 牛型菌株及び Pasteur B.C.G. 菌株を 各 6 株を 使用した。免疫

Table 1. Preparation method of Middlebrook & Dubos hemagglutination and hemolytic antigens.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T.B.H 37 RV extract 100 cc</td>
</tr>
<tr>
<td>2</td>
<td>B.C.G. extract 100 cc</td>
</tr>
<tr>
<td>3</td>
<td>Concentrated B.C.G. culture sauton media 100 cc</td>
</tr>
<tr>
<td>4</td>
<td>Concentrated old tuberculin 100 cc</td>
</tr>
<tr>
<td></td>
<td>dialyzed top water 4 hrs then distilled water 24 hrs</td>
</tr>
<tr>
<td></td>
<td>freeze dried 2.0 to 2.5 gm</td>
</tr>
<tr>
<td></td>
<td>added 220 cc of 88% phenol, 20°C, 2 hrs</td>
</tr>
<tr>
<td></td>
<td>centrifuged</td>
</tr>
<tr>
<td>sediment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>added 200 cc of cold acetone well shake cooling up to 2°C</td>
</tr>
<tr>
<td></td>
<td>centrifuged</td>
</tr>
<tr>
<td>supernatant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>added 600 cc of pure ethanol cooling up to 2°C</td>
</tr>
<tr>
<td></td>
<td>centrifuged</td>
</tr>
<tr>
<td>sediment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>added 200 cc of cold acetone cooling up to 2°C</td>
</tr>
<tr>
<td></td>
<td>centrifuged</td>
</tr>
<tr>
<td>supernatant discard</td>
<td></td>
</tr>
<tr>
<td></td>
<td>adjusted PH 6.3 by IN NaOH</td>
</tr>
</tbody>
</table>

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對照品は Dale Reagent Inc. 裏 Lederle laboratory Division 裏 使用した。
added 60 cc of H₂O then adjusted
PH 7.0 by IN NaOH
adjusted PH 3.5 by IN HCl keeping in 2°C, 24 hrs.
centrifuged
sediment discard supernatant
added 540 cc of cold pure ethanol
centrifuged
sediment supernatant discard
washed 3 times with cold aceton
dried in room temperature, 0.4 to 0.5 gm.
(Fr I)

3. Cortisone 및 Purine 投與과 動物疫
Cortisone acetate 25mg per c.c. North America Labora-
tories, U.S.A. 載用 家兎體重 每kg 10mg 7日間計 10mg 5日間2.5 3日間1.2

<table>
<thead>
<tr>
<th>Table 3.</th>
<th>Grouping of rabbits.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rabbits</td>
<td>Amounts of antigens</td>
</tr>
<tr>
<td>Group 1. A.B.</td>
<td>16</td>
</tr>
<tr>
<td>Group 2. A.B.</td>
<td>16</td>
</tr>
<tr>
<td>Group 3. A.B.</td>
<td>16</td>
</tr>
<tr>
<td>Group 4. A.B.</td>
<td>16</td>
</tr>
<tr>
<td>Group 5. A.B.</td>
<td>16</td>
</tr>
<tr>
<td>Group 1 (control)</td>
<td>8</td>
</tr>
<tr>
<td>// 2</td>
<td>8</td>
</tr>
<tr>
<td>// 3</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 4.</th>
<th>Grouping of guinea pigs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of guinea pigs</td>
<td>Amounts of antigens</td>
</tr>
<tr>
<td>Group 1. A.B.</td>
<td>16</td>
</tr>
<tr>
<td>Group 2. A.B.</td>
<td>16</td>
</tr>
<tr>
<td>Group 3. A.B.</td>
<td>16</td>
</tr>
<tr>
<td>Group 4. A.B.</td>
<td>16</td>
</tr>
<tr>
<td>Group 5. A.B</td>
<td>16</td>
</tr>
<tr>
<td>Control group</td>
<td>8</td>
</tr>
<tr>
<td>// 2</td>
<td>8</td>
</tr>
<tr>
<td>// 3</td>
<td>8</td>
</tr>
</tbody>
</table>

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4. Tuberculin allergy 反應

質問 : Old tuberculin 1: 100, 1: 500, 1: 1000, 1: 2000 및 1: 3000 농도의 투여한 30명의 피복 80대의 T.A. 반응을 보고, 환자의 적응 300mg 내외의 T.A. 반응 음성 적응 80명이 1명으로 남은 B.C.G. 100mg, 10mg, 6mg 활성한 RV 10mg 및 Ravellen 6mg으로 적응하고, 환자의 적응은 300mg 내외의 T.A. 반응 음성 적응 80명이 1명으로 남은 B.C.G. 100mg, 10mg, 6mg 활성한 RV 10mg 및 Ravellen 6mg으로 적응하고, B.C.G., H.37 R.V. 및 Ravellen의 T.A. 반응 음성 적응을 보고, 투여자에게 투여자로 적응을 보였고, 적응한 적응을 보였고, 적응한 적응을 보았습니다. (Table 3, 4 参照)

4. 赤血球凝集反應

著者が 改良한 色素反應 免疫製造を 手技の 免疫製造を Middlebrook & Dukes の H. A. test に ササミ ダル 20 TUB 및 각 0.1c.c. の 接種 24, 48, 72 및 96時間의 4回에 걸쳐 투여하였다. 結果 1, 2, 3개월 및 6개월의 4회에 걸쳐 Tuberculin 반응을 보고, 투여자에게 赤血球凝集反應 免疫製造 및 免疫製造로 투여하였다.

5. 赤血球凝集反應

著者が 改良한 色素反應 免疫製造を 手技의 免疫製造로 Middlebrook & Dukes の H. A. test 와 비슷하며 다음과 같다. Tuberculin 분획으로 인하여 免疫를 Buffered saline 으로 1%산소 으로 만든 다음 1:75로 농축하고 그 免疫 1.6c.c에 0.1c.c의 보이치로 洗浄 赤血球을 合併하여 37°에서 2시간 應答되었다. 이 赤血球 3回 逆流작용하여 0.5% 免疫제로 赤血球 洗浄液 으로 만든다. 可検血清은 56°30분間 非血化 후에 赤血球로 吸收하여 5倍마다 320倍까지 階段 농축하여 小試験관에 0.25c.c 洗浄을 다음 0.5% 免疫제로 赤血球 25cm로 농축하여 이 混合液를 37°에서 每 15분마다 検定하여 4回에 1夜夜放置 5日 20℃에서 20分 変動가 정해진다.

6. 細菌反応

H.I. 反応은 赤血球 落渇反應 判定後 さらに 寒冷適和 赤血球로 吸收하여 4夜夜放置 0.05c.c와 가하여 37°에서 30分間 保存後 判定한다.

7. 赤血球凝集反應説明

赤血球凝集反應説明 Hemagglutination (modified) test (以下 H.A.M.로 略称하여 進藤, 河野等에 의해서 所謂 細菌 不完全反応説明法이라고 하였는데, 이 理論은 Rh因子에 의한 Coombs test의 極한 赤血球에 活性한 Tuberculin 分割反応과 결합하는 것은 Univalent 되는 細菌反応이고 反応して Bivalent한 免疫と結合하여 反応을 얻으려는 것이라. 粘膜는 反応と反応으로 反応의 赤血球反応는 利用하였는데 赤血球凝集反應과 洗浄反應을 한다. 結果 反応는 all反応의 赤血球反応 giong Vimentin 반応을 0.25c.c. 分割하여 各反応反応 및 赤血球 浸透 反応을 判定하였다. 各反応反応에는 可検血清 小試験관に Buffered saline を 添加하였고, 反応反応判定는 反応, 特, 土, 一의 符号로, 反応反応 100%, 吸收 75%, 結合 50%, 10% に 反応を 보였습니다.
 및 혈중항체 반응에서의 꼭 틀로 차이를 보였고, T.A. 반응에서 Cortisone군은 3개월 후에서, Purine군은 2개월부터 6개월까지의 반응을 나타냈다. B.C.G. 반응군에서는 3개월 이내에 반응을 보였다.

3) 실험실용혈액(Tavelen 미) 항체군

세포 반응 5는 Figure 5과 같이, 직접반응 에 따르는 T.A. 반응은 3개월의 꼭 종으로 나타났고, 6개월의 꼭 종으로 나타났다. 그러나 6개월의 꼭 종으로 나타난 반응은 3개월의 꼭 종으로 나타난 반응보다 크고, 6개월의 꼭 종으로 나타난 반응은 3개월의 꼭 종으로 나타난 반응보다 작았다. 세포 반응 3는 Figure 8과 같이, 직접반응은 3개월의 꼭 종으로 나타난 반응보다 작고, 6개월의 꼭 종으로 나타난 반응은 3개월의 꼭 종으로 나타난 반응보다 작았다. 세포 반응 4는 Figure 9과 같이, 직접반응에서 T.A. 및 6개월의 꼭 종으로 나타난 반응이 거의 나타나지 않았다. T.A. 및 6개월의 꼭 종으로 나타난 반응은 3개월의 꼭 종으로 나타난 반응보다 작고, 6개월의 꼭 종으로 나타난 반응은 3개월의 꼭 종으로 나타난 반응보다 작았다.

Figure 1. Tuberculin allergy and circulating antibodies of the rabbits treated with cortisone or purine and immunized with B.C.G.(100mg)
Figure 2. Tuberculin allergy and circulating antibodies of rabbits treated with cortisone or purine and immunized with B.C.G. (10 mg.)

Figure 3. Tuberculin allergy and circulating antibodies of the rabbits treated with cortisone or purine and immunized with B.C.G. (6 mg.)

Figure 4. Tuberculin allergy and circulating antibodies of the rabbits treated with cortisone or purine and immunized with I37RV (10 mg).
Figure 5. Tuberculin allergy and circulating antibodies of the rabbits treated with cortisone or purine and immunized with Ravenel (10 mg).

Figure 6. Tuberculin allergy and circulating antibodies of the guinea pigs treated with cortisone or purine and immunized with B.C.G. (100 mg).

Figure 7. Tuberculin allergy and circulating antibodies of the guinea pigs treated with cortisone or purine and immunized with B.C.G. (10 mg).
Figure 8. Tuberculin allergy and circulating antibodies of the guinea-pigs treated with cortisone or purine and immunized with B.C.G. (6 mg).

Figure 9. Tuberculin allergy and circulating antibodies of the guinea-pigs treated with cortisone or purine and immunized with II 37 RV (6 mg).

Figure 10. Tuberculin allergy and circulating antibodies of the guinea-pigs treated with cortisone or purine and immunized with Ravenel (6 mg).
(B.C.G. 10 mg (H 37 RV 10 mg (Ravencel 10 mg
immunized) immunized) immunized)

--- --- H.A. --- --- H.A. --- --- H.A.
--- --- H.L. --- --- H.L. --- --- H.L.
--- --- H.A.M. --- --- H.A.M. --- --- H.A.M.
--- --- T.A. --- --- T.A. --- --- T.A.

**Figure 11.** Tuberculin allergy and circulating antibodies of the rabbits treated with cortisone and immunized with B.C.G., H 37 RV and Ravencel.

(B.C.G. 10 mg (H 37 RV 10 mg (Ravencel 10 mg
immunized) immunized) immunized)

--- --- H.A. --- --- H.A. --- --- H.A.
--- --- H.L. --- --- H.L. --- --- H.L.
--- --- H.A.M. --- --- H.A.M. --- --- H.A.M.
--- --- T.A. --- --- T.A. --- --- T.A.

**Figure 12.** Tuberculin allergy and circulating antibodies of the guinea pigs treated with cortisone and immunized with B.C.G., H 37 RV and Ravencel.

(B.C.G. 10 mg (H 37 RV 10 mg (Ravencel 10 mg
immunized) immunized) immunized)

--- --- H.A. --- --- H.A. --- --- H.A.
--- --- H.L. --- --- H.L. --- --- H.L.
--- --- H.A.M. --- --- H.A.M. --- --- H.A.M.
--- --- T.A. --- --- T.A. --- --- T.A.

**Figure 13.** Tuberculin allergy and circulating antibodies of the rabbits treated with purine and immunized with B.C.G., H 37 RV and Ravencel.

(B.C.G. 10 mg (H 37 RV 10 mg (Ravencel 10 mg
immunized) immunized) immunized)

--- --- H.A. --- --- H.A. --- --- H.A.
--- --- H.L. --- --- H.L. --- --- H.L.
--- --- H.A.M. --- --- H.A.M. --- --- H.A.M.
--- --- T.A. --- --- T.A. --- --- T.A.

**Figure 14.** Tuberculin allergy and circulating antibodies of the guinea pigs treated with purine and immunized with B.C.G., H 37 RV and Ravencel.

**IV. SUMMARY AND CONCLUSION**

The results of the study indicate that passive hemagglutination with the Hensley test was used to detect tuberculosis antibodies. In order to confirm the antibodies, guinea pigs were treated with purine. The results showed that the antibody levels were higher in the guinea pigs treated with purine compared to those treated with cortisone.

In conclusion, the results of this study suggest that passive hemagglutination with the Hensley test is a useful method for detecting tuberculosis antibodies. Further research is needed to confirm these findings.
V 결론

Cortisone와 Purine을 투여한 한여름의 해를에 B.C.G. II 37 kV 또는 Ravenel 성인용 대조를 각각 투약한 1, 2, 3 및 6개월 후에 혈중항체의 생성상황 및 혈중항체 총래반응을 동반함을 다룬 결과, 혈중반응과 Tuberculin allergy 반응과의 관련성에 대하여 다음과 같은결론을 얻었다.

1. Cortisone 투약군의 허혈은 B.C.G. 또는 결핵균 항체가 투약기 후 2개월 이내에 Tuberculin allergy 반응이 억제되어서 3개월부터는 Tuberculin allergy 반응이 100%이서 6개월이 되면 100%로 상승하였고 혈중항체 생성에는 큰 영향을 주지 않았다.

2. Purine 투약군의 허혈은 B.C.G. 또는 결핵균 항체가 투약기 후 2개월 이내에 Tuberculin allergy 반응이 혈중항체 반응의 억제되어서 3개월부터는 항체 반응이 100%이어서 6개월이 되면 100%로 상승하였고 혈중항체 생성에는 큰 영향을 주지 않았다.

3. B.C.G. 또는 결핵균 치료용 접종을 하여도 혈중항체 반응과 Tuberculin allergy 반응은 관계할 수 없는 것으로 나타났다.

4. 결핵군의 혈중반응을 추정할 때는 혈중항체 반응과 Tuberculin allergy 반응을 병행할 필요가 있다고 보며, 결핵군의 항체반응은 혈중항체의 억제를 확인하였다.

References


(Part III. Hormones)


(Part IV. Purines)


EXPERIMENTAL STUDIES ON THE ANTIGEN-ANTIBODY REACTIONS IN TUBERCULOSIS

Part I. Studies on the tuberculin allergy and circulating antibodies in Laboratory animals; vaccinated with tuberculous antigens and treated with cortisone or purine.

Nam Ho Chun, M.D., M.P.H.


(Director; Sang-Hoon Lee, M.D., College of Medicine, Seoul National University.)

Abstract;

Middlebrook & Dubos and Middlebrooks report were the earliest to show passive hemagglutination and hemolytic reaction in the serum of tuberculous patients. A reinvestigation was carried out to detect humoral antibodies by a sensitive in vitro test such as the hemolytic reaction by a modified Coombs formula, the so called detection of complete and incomplete tuberculous antibodies by Sabin [2]. The main reason for this is that the conditioned hemolytic reaction may be caused by similar mechanisms such as reversed passive anaphylaxis due to the association of the antibody, complement and the antigen which is sensitized onto erythrocytes, a living cell unit.

Recently the reason that the healthy person whose is much similar to the increased antibody was in the in vitro reaction and tuberculin reaction was due to such conditions in Korea, as the too wide spread tuberculin test for the tuberculosis diagnosis, B.C.G. vaccination and uncontrolled active tuberculosis patients. Beside, it has been considered that humoral antibody, with in vitro reaction revealing the immediate Arthus type, while the sessile antibody, has been considered as closely related to allergic phenomena such as the delayed type of tuberculin skin reaction.

The purposes of this study were, firstly to find modified tuberculous antibodies by hemagglutination (H.A.) test, hemagglutination modified (H.A.M.) test and hemolytic (H.L.) test, secondly to see if the experiments brought light on any mutual relationship between a tuberculin allergy and antibody produced in the human and experimental animals. The third aim was to test a fact that animals do not generally show immunity as the human, nor tuberculin allergy and whether attained naturally or artificially. In the experiments for the detection of mutual relations in part I cortisone was used, an anti-allergic substance, in order to suppress the activation of tuberculin allergy reaction and antibody production in the experimental rabbits and guinea-pigs, when cortisone was prescribed with immunized tuberculous antigens.

Used in order to create a similarity with humans. It should be recalled that because of the patients meat eating habit we have purine in our circulation while the rabbit and guinea-pig are grass eaters. Purine is exerted as uric acid guinea-pig the intestines and liver after its metabolic conversion in muscle and other organs.

Cortisone and purine was given and then the rabbits and guinea-pigs were immunized with antigens such as B.C.G. H37RV and Ravn strain, and the animal serum thus obtained was investigated by H.A., H.A.M. and H.L. tests and tuberculin allergy tests a summary of the results are as follow.

1. When cortisone as an anti-allergic substance was given with B.C.G. & tubercle bacilli antigens, the rabbits and guinea-pigs tuberculin allergy reaction was suppressed up to 2 months, however the tuberculin allergy became after positive 3 months, and kept ascension without effecting on antibody formation.

2. When purine was given with B.C.G. & tubercle bacilli antigens the rabbits and guinea-pigs earlier positive tuberculin allergy and antibody reaction as show in the control group, turned rapidly negative.

3. The results of H.A., H.A.M. and H.L. between the tuberculin allergy reaction and the serum in vitro had no parallel reaction in the B.C.G. or immunized tubercle bacilli antigens to rabbits and guinea-pigs.

4. The tuberculous immune antibody was confirmed serologically and by tuberculin allergy test. Thus, the author is of the opinion that the antibody is a polivalent antibody, also it is shown that tuberculous antibody should keep pace with 3 tests of H.A., H.A.M., H.L. methods, and tuberculin allergy test.