An attempt to induce a histological ENL symptom in M.leprae-infected nude mice

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学位論文題目	AN ATTEMPT TO INDUCE A HISTOLOGICAL ENL SYMPTOM IN *M. LEPRAE*- INFECTED NUDE MICE （らい菌感染ヌードマウスに組織学的ならい性結節性紅斑の症状を誘導する試み）
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論文内容の要旨

Purpose of the study

Erythema nodosum leprosum (ENL) is a well known complication in lepromatous leprosy. It was described as early as 1912 by Murata. Inspite of extensive research concerning the lesions, the pathogenesis of ENL is a dispute among the researchers in leprosy.

It has been reported that PMN cells accumulation, acute vasculitis or necrotic granulation is common histological feature in the lesion of ENL. Besides, there is the suggestion that chemotherapy of leprosy especially DDS treatment may involved in the pathogenesis of ENL. According to Ridley et al., ENL is a reactional episode of lepromatous leprosy where large amount of *M. leprae* antigens and corresponding antibodies provide evidence for immune complex aetiology.

The congenitally athymic nude mouse is one of the ideal animal to provide experimental lepromatous leprosy even though its non-specific T cell mediated immunity is not comparable to the *M.leprae* specific immune deficiency seen in lepromatous leprosy patients.

In this study, *M. leprae*–infected nude mice which permit the growth of large number of *M. leprae* were used as a model of leproatous leprosy, and effect of anti-leprosy

drugs on histological symptoms of ENL in the lepromatous lesions was examined. DDS and rifampicin was chosen as the candidate which may induce ENL symptoms, and lamprene was expected to suppress ENL symptoms because it is well known this anti-leprosy drug can control ENL of the patients. Some serological works have also been conducted beside the histopathological and bacteriological observations.

Method of the study

Three kinds of experiments have been done. BALB/C-nu/nu mice were inoculated with *M. leprae* passaged through nude mice into both hind footpads. Inoculum size ranged from 1.5×10^6 to 1.0×10^8. The inoculated animals were maintained in vinyl isolators under SPF conditions.

1. Anti-leprosy drug treatment

a. Twenty five infected unde mice were devided into 4 groups.

Group 1 was untreated control group, Group 2 was given rifampicin orally 0.5 mg/mouse twice a week, Group 3 was given DDS containing diet (0.001%) daily, and Group 4 was given combined treatment of rifampicin and DDS as mentioned above. The anti-leprosy treatment was started after 47 weeks of *M. leprae* infection. Every week after the first day of treatment, one animal of each group was killed for histopathological observation.

b. Twenty two infected nude mice were divided into 2 groups.

Group one was untreated control and Group 2 was given DDS containing diet (0.001%) daily. The treatment was started after 45 weeks or 68 weeks of infection. The duration of treatment varied from 2 weeks up to 13 weeks. According to time course one mouse of each group was killed for histopathological and serological examination.

2. Anti-ENL treatment

Twelve infected mice were devided into 2 groups. One was untreated control and the other was given lamprene containing diet (0.003%) daily. The treatment started after 38 weeks of M. leprae infection. After 8 weeks of lamprene treatment the mice were killed for histopathological observation.

3. ENL prevention by lamprene treatment

Twelve infected nude mice were devided into 2 groups. One was untreated control

and the other was given lamprene containing diet (0.003%) daily for 26 or 30 weeks. The treatment started after 18 weeks of *M. leprae* infection. The animals were killed after the period of treatment was ended.

Histopathological studies.

Sections were stained by H.E. staining, and for observation of *M. leprae* Fite-Faraco's technique was adopted.

Bacterial enumeration.

Number of M.leprae in footpad was determined by modified method of Shepard.

Serological Test.

Direct immunofluorescence studies were carried out according to Kawamura's procedure. IgG, IgM and C_3 in infected organs was examined. Circulating immune complex was detected by C_{1q} solid phase assay by modified method of Smith. Anti-*M. leprae* antibody was detected by Nomaguchi's method.

Results of study.

Experiment 1-a.

Remarkable swelling of infected footpad of both treated and untreated group was noted macroscopically. In general there was no cosistant correlation between the swelling of footpad and the enlargement of spleen and inguinal lymphnode. The microscopic fundungs were nearly uniform in all cases of untreated and treated mice. Typical lepromatous lesion with large amount of acid fast bacilli was observed in all infeced footpads. In some sections a circumscript PMN cells accumlation was found. Defined vasculitis was rarely found, but in one of the control group defined vasculitis was found in the liver section. Almost all the lung specimens showed histopathologic changes which indicated bronchopneumonia with varying stage.

Experiment 1-b.

Histopathological findings.

The macroscopic and microscopic findings of the footpad were similar to the Experiment 1-a. Unfortunately several mice of both group died spontaneously during the experiment, making only 9 mice of the control group and 7 mice of the treated group were included in this experiment. PMN cells accumulation were found in 2 mice of treated group and 3 mice of the control group.

Serological findings.

It was found that 2 of the DDS treated mice had high concentration of immune complex (IC) in their sera, whereas only one untreated mouse had comparable IC but rather lower concentration in its serum. One of those two DDS treated mice had also

a high anti-*M. leprae* antibody in its serum. Immune complexes deposit in the footpad tissues of both groups could not be detected by immunofluorescence method.

Experiment 2.

All of the footpads of the untreated group showed accumulation of PMN cells and other inflammatory components, whereas only 3 out of 5 animals from lamprene treated group showed PMN cells accumulation. CI and anti-*M. leprae* antibody was not detected in the sera of mice from both groups.

Experiment 3.

About 10 times higher amount of acid fast bacilli were harvested from the untreated group compared to the lamprene treated group. Besides 3 out of 6 lamprene treated animals showed healing process without PMN cells infiltration in their footpads.

Summary of the study

1) It has been demonstrated that *M. leprae* infected nude mice can constantly provide experimental model of lepromatous leprosy, and PMN cells accumulation was comonly observed in those lepromatous lesions.

2) The presence of PMN cells accumulation in M. leprae infected tissue alone is not sufficient in confiding the onset of ENL.

3) PMN cells accumlation in footpads of *M. leprae* infected nude mice could not be prevented by early lamprene treatment.

4) PMN cells accumlation was not modulated by DDS and rifampicin treatment and only slightly modulated by long duration of lamprene treatment.

5) Early and long duration of lamprene treatment showed marked healing process in the tissue.

6) Since *M. leprae*-infected nude mice did not show immune complex deposit in infected footpads, and concentration of IC and anti-*M. leprae* antibody in the sera of those infected mice was usually very low, transfering of T cell subsets into nude mice was suggested for further study to explore the mechnism of ENL.

論文審査の結果の要旨

らい性結節性紅斑（Erythema nodosum leprosum; ENL）は，らいの合併症の中でも重要な位置を占めており，らいの治療の大きな障害となっている。ENL はらい腫型患者（lepromatous type）に発生し，その治療中にらいの症状の軽減に伴い，皮膚，末梢神経，紅彩，毛様体，強膜，関節，睾丸等に急性炎症の形で出現する。皮膚結節は圧痛を伴い，時に膿瘍を形成し，体温上昇の

ほか，後遺症として末梢神経の麻痺，失明，関節強直，男子不妊症を残す。

ENLの発生機序としては，現在免疫複合体病説が有力である。ENLは，病理組織学的には血管周囲の多型核白血球（PMN cells）の集合として特徴づけられ，その場にはらい菌由来の抗原のほか，免疫グロブリン及び補体も検出される。しかしENLの発生には未だ不明な点が多い。らいの化学療法剤であるDiaminodiphenylsulfone（DDS）やリファンピシンがENLの発生を促し，逆に副腎皮質ホルモンがENL症状を軽減させることは前述の免疫複合体病因論を支持するが，lamprene やサリドマイドがENLに抑制的に働く事実は，未だ説明されていない。

これらの問題を解決するためのENLの研究には動物実験モデルを作る事が不可欠であるが，申請者は，実際的な観点から，ヌードマウスの足蹠にらい菌を接種することによりらい腫型病変を発生させ，これにDDS，リファンピシン，或いはlampreneを投与し，PMN細胞集団巣及び血中免疫複合体に対する影響等を調べた。その結果は以下のように要約される。

1. DDSまたはリファンピシンを単独又は一緒に投与した場合，PMN cells集団巣の発生の，有意の増強は見られなかった。また，らい病変部のIgG，IgM，C_3の沈着も，DDSの投与による血中の免疫複合体及び抗体の有意上昇も認められなかった。

2. ENLを鎮静させるlamprene を，らい感染後38週後から8週間投与したところ，対照群では6匹中全部にPMN cells集団巣を認めたのに対し，lamprene投与群では5匹中3匹にのみ認められた。血中免疫複合体及び抗体は，全例に認め得なかった。

3. 比較的早期からlamprene投与を開始し長期間（44週以上）治療を続けた場合でもPMN cells集団巣の発生を完全に抑制することはできなかった。しかしlamprene投与により，らい菌の増殖は有意に抑制され，6匹中3匹には明らかな治癒機転を認めた。

以上の成績から，申請者が研究当初に期待したように，らい菌感染ヌードマウスにDDSまたはリファンピシンを投与して，組織学的なENLを誘導する試み，及びENLの発生機序として重視される免疫複合体を，病巣内に検出する試みは不成功に終ったと云わざるを得ない。然し申請者の一連の成績は，PMN cells集団巣の存在を以てENL発生の証拠として来たこれまでの考えに，疑問を投げかける結果となった。今後，ENLの発生機序を明らかにするため，ヌードマウスにT細胞のサブセットを移入する実験が必要とされる。

以上述べたように，本研究は，らい腫型らい患者の治療の際に大きな障害となる結節性紅斑の動物実験モデルを作ることを目的とした，非常に意欲的なものである。得られた成績には，必ずしも十分に期待通りのものとは云えない部分もあるが，本研究で提示された方法論及び研究成果が今後のENLの研究に与えるインパクトは非常に大きなものがあり，価値ある集積と認める。よって本研究者は医学博士の学位を得る資格があると認める。