An Attempt to Induce a Histological ENL Symptom in *M. leprae* Infected Nude Mice

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Erythema Nodosum Leprosum (ENL) is a well known complication in lepromatous leprosy. It was described as early as 1912 by Murata(1). Several authors have described the clinical and histopathological picture in the skin since then(2-6). Later on, Ridley et al.(7) described the immunopathology of ENL. Inspite of extensive research concerning the lesions, the pathogenesis of ENL is a dispute among the researchers in leprosy.

An ideal model of lepromatous leprosy in animal is one that permits the growth of large numbers of *M. leprae* and is severely impaired in its ability to mount an effective immune response against the organisms. The athymic nude mouse is one that suites to those requirements. Those nude mice have been used in leprosy research since 1976(8,9). Congenitally athymic nude mice are highly susceptible to infection with *M. leprae*. Although the nonspecific deficiency of T cell mediated immunity seen in nude mice is not comparable to the *M. leprae* specific deficiency seen in lepromatous leprosy patients, it has been suggested by some researchers that nude mice might be a suitable model for studying some aspects of the disease(8-11).

Anderson(12) proposed that granulocytes activation by interaction of immune complexes and complement is responsible for the development of ENL and its complication in individual with high bacillary load.

There is the suggestion also that dapsone therapy may involved in the pathogenesis of ENL since dapsone (DDS) caused increased migration of neutrophil(13).

Ridley et al.(6) reported that ENL with PMN cells infiltration, necrotic granulation or vasculitis is not always a permanent features of acute stage. They also found heamorrhagic or vesicular type of ENL.

In the present study, for the first time, Diaminodiphenyl sulfone (DDS) or Rifampicin and Lamprene respectively were tried for promoting or modulating ENL reaction in *M. leprae* infected nude mice (nu/nu), and if possible making effort to elucidate the pathogenesis of this reaction.

Based on the various findings in patients with ENL the criteria of the onset of ENL in this experiment were decided if more than one of the above mentioned histologic features were present. This particular experimental animal would be considered suffering from reactional lepromatous leprosy as in human.
Materials and Methods

Three kinds of experiments have been done. The animals involved in these experiments were nude mice (BALB/Cnu/nu) which were inoculated in both hind footpads with *M. leprae* either of Izumi, Kurume Naha or Thai strain ranging from $1.5 \times 10^6$ to $1 \times 10^8$ bacilli in each one. The nude mice were 6 weeks old when they were inoculated. The mice were maintained in vinyl isolators under Specific Pathogen Free (SPF) conditions.

1. **Anti-leprosy drug experiment**
   a. Twenty five infected nude mice were randomly placed into 4 groups: One group (7 mice) was left untreated and was referred as control group. The second group was given Rifampicin orally 0.5mg/mouse twice a week. The third group was given 0.001% DDS containing mice diet daily and the fourth group was given a combined treatment with DDS and Rifampicin. The anti-leprosy drug treatment was started after 47 weeks of *M. leprae* infection. Every week after the first day of treatment, one animal of each group was sacrificed for histopathological observation. The duration of treatment when the animals were killed varied from 1 week up to 6 weeks.
   b. Twenty two infected nude mice were randomly placed into 2 groups: One group was left untreated and was referred as control group. The treated group was given 0.001% DDS containing mice diet daily. Unfortunately several mice of both groups died spontaneously during experiment, making only 9 mice of the control group and 7 mice of the treated group were included in this experiment. The treatment was started when the mice were either 54 or 68 weeks after inoculation. Started from 2 weeks after the first day of treatment and then followed every 4 weeks, one mouse of each group was killed for histopathological or serological examination. The duration of treatment varied from 2 weeks up till 13 weeks.

2. **Anti-ENL drug experiment**
   Twelve infected nude mice were devided in 2 groups. One group consisted of 5 mice was given with 0.003% Lamprene containing mice diet daily and the other group was left untreated and was referred as control group. The treatment was started after 38 weeks of *M. leprae* inoculation. After 8 weeks of Lamprene treatment the mice were sacrificed for histopathological observation.

3. **ENL prevention by Lamprene treatment**
   Twelve infected nude mice were devided into 2 group. One group consisted of 6 mice was given with 0.003% Lamprene containing mice diet daily for 26 or 30 weeks long. The treatment was started after 18 weeks of *M. leprae* inoculation. The other half of mice left untreated and was referred as control group. The animals were sacrificed after the period of treatment was ended.

**Histopathological studies**

The killed animals were autopsied and various organs (inguinal lymphnode, liver, spleen, kidney, lung, footpad and in some cases also lip, snout, tail or skin lesion) were prepared for histopathological studies. Six micron section were cut and stained with H.E. and Fite-Faraco stain for *M. leprae*. 
Immunofluorescence studies (Experiment 1-b)

Five micron sections of hexane fixed frozen footpad tissues were cut with cryostat and prepared for IF studies. The procedures for direct IF studies according to Kawamura(14). Sections were treated with FTC-labelled IgG fraction goat anti-mouse IgG, FTC-labelled IgG fraction of goat anti-mouse IgM or FTC labelled IgG fraction of goat anti-mouse C3 antiserum (Cappel Laboratories) in a dilutions previously determined as optimal.

Fluorescence Microscopy

The sections were mounted on non-fluorescent microscopic slide glasses. Buffered glycerol (9 parts of glycerol to 1 part of 0.2M Na₂HPO₄ pH 9.0) was used as the mounting medium. After covering with a proper coverglass the section was immediately observed under a Nikon Optihot Fluorescence Microscope.

Bacterial enumeration

Tissue homogenate was prepared from the left side hindfoot of each killed mouse. The number of M. leprae in homoeinate was determined microscopically by using the modified Shepard’s technique(15).

Circulating Immune Complex assay (Experiment 1-b and 2)

Serum sample was collected and kept in deep freeze until use. Circulating Immune Complex (CIC) were detected by C₁q solid phase assay according to Smith procedures with slight modification(16). Human C₁q (4.66 mg/ml in 0.5 M Na carbonate buffer pH 9.0) was kindly provided by Dr Kunio Yonemasu from Nara Medical University

Anti-M. leprae antibody assay (Experiment 1-b and 2).

Serum sample was assayed by ELISA method according to Nomaguchi technique(17).

Results

Experiment 1-a

Remarkable swelling of infected footpad of both treated and control group was noted macroscopically. In general there was no consistent correlation between the swelling of the inoculated footpad and the enlargement of the spleen as well as the inguinal lymph node.

The microscopic findings were nearly uniform in all cases of untreated and treated mice. The epidermis of the footpad revealed flattening of the rete ridges. In some cases the epidermis presented areas of atrophy overlying the dermal lesion. A poorly defined thin band of connective tissue situated under the epidermis was observed. In the majority of cases a moderate number of spindle cells were observed in a macrophages granuloma situated beneath the epidermis and incorporating several striated muscle. Macrophages showed as large cells with abundant and foamy cytoplasm. These cellular elements represent true lepromatous cells. Some muscle fibers showed loss of striation vacuolation of their cytoplasm. Lymphocytes diffusely distributed among the macrophages. Mast cells and moderate numbers of plasma cells were also present. Acid fast stain showed large clumps of bacilli inside macrophages. The scattered accumulation of foamy macrophages showed varying numbers of neutrophils in close association with them. In some sections a circumspect PMN cells accumulation was
Plate 1  Circumscript PMN cells accumulation in the footpad of a nude mouse after 48 weeks inoculation with M. leprae and treated with Rifampicin for 4 weeks. H. E. staining, 40×.

Plate 2  Extented vasculitis in the liver of a nude mouse after 54 weeks inoculation with M. leprae (control group). H. E. staining, 10×.

found (Plate 1). Defined vasculitis were rarely found, but in one of the control group defined vasculitis was found in the liver section (Plate 2).

The lymph nodes and spleens showed hyperactive appearance which characterized by germinal centers development and scattered large number of typical multinucleated syncytial histiocytes surrounded by cell population consisted of mononuclear cells. Mast cells were seen among them. Acid fast bacilli (AFB) dissemination was not found in other examined organs. Almost all the lung specimens showed histopathologic changes which indicated bronchopneumonia with varying stages (Plate 3). Other organs seems to be histologically unchanged.
Plate 3  Acid Fast bacilli found in the infiltrated ung of a nude mouse after 54 weeks inoculation with M. leprae. Fite Faraco staining, 40×.

Experiment 1-b

Some of the mice had swollen spleens or lymph nodes. In general there was no consistent correlation between the swelling of the inoculated footpads and the enlargement of the spleens as well as the inguinal lymph nodes. Remarkable swelling of infected footpad of almost of the animals was noted macroscopically.

The microscopic findings of the footpad sections were similar to the Experiment 1-a. Infiltration of macrophages containing AFB were found in some of the spleens and lymph nodes as summarized in Table 1. There were also accumulation of mononuclear cells and macrophages containing AFB found around the portal and central vein of the liver of some of the animals. PMN cells accumulation were found in 2 mice from the treated group and 3 mice from the untreated animals. These findings are shown in Table 1. Several of the lungs showed a marked pathologic features of bronchopneumonia or bronchitis. A small collection of PMN cells were found in 3 untreated mice as summarized in Table 1 too.

Circulating and deposited Immune Complexes and anti-M. leprae antibody

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 (Fig. 1) One of those 2 DDS treated mice had also a high anti-M. leprae antibody in its serum (Fig. 2). Moreover these circulating IC had higher concentration than the concentration of IC found in the normal sera of uninfected nude mice referred as control.

Immune Complexes deposits in the footpad tissues of both groups could not be detected by immunofluorescence method.

Experiment 2

One of the mice in the control group showed wasting syndrome. It showed degenerative changes in its organs and no inflammatory reactions in the footpad was observed. Few bacilli
found in the footpad indicated poor growth of them. Only $1.3 \times 10^9$ bacilli could be harvested from the footpad of that mouse. For that reason, that mouse was excluded for the analysis of the experiment. Two other mice from the control group showed also degenerative appearance in their livers and kidneys, but their footpads still showed usual inflammatory reactions against the bacilli. AFB dissemination was not detected in the organs of both examined groups. All of the footpads of the untreated group showed accumulation of PMN cells among the lepromatous cells and other inflammatory components, whereas only 3 out of 5 animals from the Lamprene treated group showed PMN cells accumulation. The results are summarized in Table 2. The microscopic findings of the organs of both groups were similar to the findings in Experiment 1 except of the above mentioned cases.

Circulating ICs and anti-\textit{M. leprae} antibodies were undetected in the sera of mice from both groups.

\textbf{Experiment 3}

The population growth of the bacilli could be confirmed by the number of harvest from both groups. Only a few amount of AFB could be harvested in the footpads of mice from the treated group, whereas about 10 times higher amount of bacilli were harvested from the untreated group.

AFB dissemination in the organs of mice from both groups could not be detected by histological examination. Whereas 3 animals of the Lamprene treated group showed healing

<table>
<thead>
<tr>
<th>Group</th>
<th>DDS treatment Duration of \textit{M. leprae} in infection</th>
<th>Duration in weeks</th>
<th>AFB inoculum $10^9$×</th>
<th>AFB harvest $10^6$×</th>
<th>PMN acc</th>
<th>Lympnode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>56</td>
<td>2</td>
<td>6.5</td>
<td>20.0</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>6</td>
<td>6.5</td>
<td>4.2</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>10</td>
<td>6.5</td>
<td>14.0</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td>15</td>
<td>6.5</td>
<td>10.0</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>4</td>
<td>10.0</td>
<td>40.0</td>
<td>+</td>
<td>−</td>
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<tr>
<td></td>
<td>76</td>
<td>8</td>
<td>10.0</td>
<td>14.0</td>
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<td>−</td>
</tr>
<tr>
<td></td>
<td>81</td>
<td>13</td>
<td>10.0</td>
<td>25.0</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Non-treated</td>
<td>54</td>
<td>X</td>
<td>6.5</td>
<td>10.0</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>X</td>
<td>6.5</td>
<td>1.9</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>X</td>
<td>6.5</td>
<td>12.0</td>
<td>NA</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>X</td>
<td>10.0</td>
<td>5.1</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td>X</td>
<td>6.5</td>
<td>1.4</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>X</td>
<td>10.0</td>
<td>3.2</td>
<td>+</td>
<td>−</td>
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<tr>
<td></td>
<td>76</td>
<td>X</td>
<td>10.0</td>
<td>7.4</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>81</td>
<td>X</td>
<td>10.0</td>
<td>4.0</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>89</td>
<td>X</td>
<td>10.0</td>
<td>26.0</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

\textbf{Table 1} Effects of short DDS treatment on \textit{M. leprae}

\textbf{Notes:}

NA = Not available; Ab = anti-\textit{M. leprae} antibody concentration; IC = Immune complex concentration.
infected nude mice (nu/nu) Experiment 1-b.

<table>
<thead>
<tr>
<th>AFB dissemination and PMN accumulation</th>
<th>Serum OD</th>
</tr>
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<tbody>
<tr>
<td>Spleen</td>
<td>Lung</td>
</tr>
<tr>
<td>AFB</td>
<td>PMN</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
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<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Discussion

It has been reported by Anderson et al. that DDS per se causes stimulation of PMN cells in normal adults and individuals with LL in vitro. Subsequently they suggested that DDS was proinflammatory and might contribute to ENL and reverse immunity reactions by stimulating PMN cells motility and lymphocytes responsiveness to antigens respectively. However, the results of the first experiment with long infected nude mice (more than 47 weeks) and treated with DDS alone or combined with Rifampicin could not confirm the suggestion of Anderson. All of the treated mice involved in the first experiments showed the presence of PMN cells accumulation as many as the untreated mice did. In another work, Anderson et al. observed there was no inhibitory effects of Rifampicin intake on PMN cells migration over a 1-month period in individuals with LL. PMN cells infiltration could be found in the footpads of untreated as well as in the footpads of Lamprene treated mice after 44 weeks or 48 weeks of M. leprae inoculation as depicted on Table 3. This evidence leads us to the conclusion that PMN cells accumulation in the M. leprae infected nude mice is
Experimental animals

Fig. 1 Serum levels of Immune Complex in Normal Nude Mice (NM), Infected Nude Mice (IM) and DDS treated Infected Nude Mice (TIM) assayed by C1q binding assay according to Smith(16).

Conditions of ELISA test:
Coating substance: 5μg/ml C1q in 100μl
Antimouse-PO: 1000 × dil. 200μl
Substrate: 130μl
H2SO42N: 100μl
Sample sera: 20μl
The absorption was read at 490 nm by Titertek Multiskan MC

Fig. 2 Serum levels of anti-M. leprae Antibody in Normal Nude Mice (NM), Infected Nude Mice (IM) and DDS treated Infected Nude Mice (TIM) by ELISA test. (Nomaguchi(17)).

Conditions of ELISA test:
Antigen for coating: sonicated M. leprae Izumi strain, 10μg/ml, 75μl/well.
Sample sera: 100 × dilution
Substrate: Ortho-phenyldiamine (OPD) 0.1mg/ml
Conjugate: anti-mouse globulin peroxidase dil. 1000 ×

Table 2 Effect of 8 weeks Lamprene treatment on 38 weeks M. leprae (Kuruma Naha 6.5×10⁹) infected nude mice (nu/nu) to the present of PMN accumulation in the footpad. (Experiment 2.)

<table>
<thead>
<tr>
<th>Code</th>
<th>AFB harvest 10×⁹</th>
<th>PMN accumulation</th>
<th>Code</th>
<th>AFB harvest 10×⁹</th>
<th>PMN accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3363</td>
<td>1.3</td>
<td>*)</td>
<td>X</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>3453</td>
<td>2.8</td>
<td>+</td>
<td>4532</td>
<td>3.0</td>
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</tr>
<tr>
<td>3551</td>
<td>17.0</td>
<td>+</td>
<td>4652</td>
<td>2.1</td>
<td>+</td>
</tr>
<tr>
<td>3613</td>
<td>8.2</td>
<td>++</td>
<td>4737</td>
<td>1.9</td>
<td>+</td>
</tr>
<tr>
<td>3736</td>
<td>6.0</td>
<td>++</td>
<td>4790</td>
<td>1.5</td>
<td>++</td>
</tr>
<tr>
<td>4001</td>
<td>2.1</td>
<td>+</td>
<td>4875</td>
<td>1.7</td>
<td>−</td>
</tr>
<tr>
<td>4160</td>
<td>6.0</td>
<td>+</td>
<td>X</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*) Wasting syndrome. No dissemination of AFB to other organs.
Table 3 Effect of early Lamprene treatment on 18 weeks *M. leprae* (Thailand 53) infected nude mice (nu/nu) for 26-30 weeks to the present of PMN accumulation in the footpad. (Experiment 3.)

<table>
<thead>
<tr>
<th>Code</th>
<th>AFB harvest 10×8</th>
<th>PMN accumulation</th>
<th>Code</th>
<th>AFB harvest 10×8</th>
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<tr>
<td>A**</td>
<td>29.0</td>
<td>+</td>
<td>U***</td>
<td>0.55</td>
<td>+</td>
</tr>
<tr>
<td>B**</td>
<td>77.0</td>
<td>-</td>
<td>V***</td>
<td>0.21</td>
<td>+</td>
</tr>
<tr>
<td>C**</td>
<td>3.2</td>
<td>+</td>
<td>W***</td>
<td>0.37</td>
<td>+</td>
</tr>
<tr>
<td>D***</td>
<td>28.0</td>
<td>-</td>
<td>X**</td>
<td>0.22</td>
<td>-**</td>
</tr>
<tr>
<td>E***</td>
<td>4.9</td>
<td>+</td>
<td>Y**</td>
<td>0.70</td>
<td>-**</td>
</tr>
<tr>
<td>F***</td>
<td>7.2</td>
<td>+</td>
<td>Z**</td>
<td>0.25</td>
<td>-**</td>
</tr>
</tbody>
</table>

* With healing process.
*** were killed 44 weeks after infection.
**** were killed 48 weeks after infection.

not influenced and promoted by the anti-leprosy drugs treatment even if it is given at a very early stage of *M. leprae* infection. However, PMN cells infiltration and bacilli dissemination in internal organs was not observed in early infected animals. This indicates that PMN cells accumulation could only arise in apparently infected organs, although the PMN cells infiltration was not always concomitant with the presence of bacilli (Table 1).

In their report, Lancaster et al.(19) could not clarify the significance of PMN cells foci in the footpad and liver of one nude mouse sacrificed among the other experimental animals. In describing histoid lesions, Ridley et al. (20) noted that highly active lepromatous lesions might show similar reactional changes consisting of infiltration of neutrophil polymorphs and cellular disintegration without any systemic disturbance. Most obviously, the difference between exacerbation reaction (ER) and ENL is that ER occurs in hyperactive lepromas, ENL in regressing granuloma(20). Moreover, Ridley et al.(20) observed that in reacting lesions, immunoglobulin IgG was at low level, IgM was moderate and IgE was markedly raised. Since Immunoglobulin was not investigated in the tissues, ER in our experimental animals cannot be excluded. The significance of PMN cells accumulation in *M. leprae* infected nude mice needs further elucidation.

Clofazimine, known alternatively as Lamprene, is also a widely used antileprosy drug. However, clofazimine has no documented immunostimulatory properties as DDS has, and on the contrary has been reported to be useful in controlling both ENL21 and reversal immunity reaction21,22. Gatner et al.23 claimed that clofazimine inhibited the motility of PMN cells from normal adults and individuals with LL in vitro. This statement can only slightly be supported by the results of our second and third experiments (Table 2 and Table 3) in nude mice.

According to Ridley et al.(7) and Ridley et al.(24), ENL is a reactional episode of lepromatous leprosy where large amounts of mycobacterial antigens and corresponding antibodies provide evidence for IC aetiology. With the negative finding of IC in the footpad tissues and other
organ tissues and only 2 out of 6 mice showed circulating IC in their sera, it is hard to conclude from the results of our experiments that the *M. leprae* infected nude mice with PMN cells accumulation comparable to reactional episode of lepromatous leprosy in human. Moreover, Ridley in 1969(25) had noted that any highly active lepromatous lesion might show similar reactional changes consisting of infiltrate of neutrophil polymorphs and cellular disintegration. Diagnosis of the onset of ENL reaction is supported by the clinical features such as the appearance in the skin of painful red nodules, iridocyclitis, orchitis, fever and headache(26). Unfortunately this clinical manifestation is not easy to be observed in the experimental animals. In this respect the results of our experiments could not confirm the incidence of ENL in *M. leprae* infected nude mice and it needs further investigation. It is possible that T lymphocytes deficiency in athymic nude inhibits the appearance of reactional episode. This opinion is supported by the results of the study in human leprosy by Modlin et al.(27) and emphasized by Wallach et al.(28). Their studies proved the importance of T cell immunity in ENL. The significance of T cells in the reactional immunity in experimental animals was shown by other investigators. The finding of Nakamura and Yogi(29) which showed thymus cells grafting in nude mice enhanced the growth of *M. leprae* and developed a severe lepromatous lesion seems contradictory to the finding of Kohsaka et al.(30). The last investigators found that thymus transplantation was effective suppresively on the growth of *M. leprae* in nude mice. This discrepancy is apparently due to the relative influence of the thymus tissue. Moreover, Chen et al.(31) suggested that the absence of a thymus itself apparently leads to qualitative as well as quantitative deficiency in the T cell population.

The experimental findings of Nakamura et al.(29) at one hand and Kohsaka et al.(30) at the other hand indicate at least the influence of reaction against *M. leprae*. Moreover, Wallach et al.(32) have shown a significant correlation between the bacterial load and the helper/suppressor ratio.

Further investigation with nude mice would be interesting to explore the mechanism of ENL by transferring proportional T subsets in the animals.

**SUMMARY**

An attempt to induce ENL reaction in *M. leprae* infected athymic nude mice by using antileprosy drugs such as DDS and Rifampicin was unsuccessful. IC which is accepted as the etiology of ENL could not be detected in the tissues of the infected nude mice. The presence of PMN cells foci in the tissues alone is not sufficient in confiding the onset of ENL. Although we could not succeed in the induction of ENL in nude mice we may draw some conclusion from the experiments:

1) PMN cells accumulation in footpads could not be prevented by early Lampremne treatment.
2) PMN cells accumulation was not modulated DDS and Rifampicin treatment and only slightly modulated by long duration of Lampremne treatment.
3) Early and long duration of Lampremne treatment showed marked healing process in the tissues.
Further study for exploring the mechanism of ENL reaction by transferring of T subsets in nude mice is suggested.

Acknowledgment

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らい菌感染ヌードマウスに組織学的
ENL 症状を誘導する試み

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1983年に Lancaster らは、重症らい菌感染ヌードマウスの足跡に多型核白血球の集団巣が存在し，また肝にも ENL に類似した病巣が見られることを報告している。著者は上記の所見がヒトの ENL に相当するものかどうかを解明するために，らい菌感染ヌードマウスにらい菌を投与したとき，上記の多型白血球集団巣がどのように変化するか，また同病巣部に免疫複合体が存在するかどうかを検査した。

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 症状を誘導する試みは不成功に終わった。また，ENL の発生機序として重視されている免疫複合体をらい菌感染ヌードマウスの病巣内に検出することはできなかった。多型核白血球集団巣の存在だけでは ENL 発生の症状であると結論するには不充分である。ヌードマウスにおいて ENL を発生させるところは不成功に終わったが，本研究から以下の結論を下すことができた。

1）飼料に 0.003%の割合にランプレンを添加し，らい菌感染後18週の比較的早期からヌードマウスに投与しても，多型核白血球集団巣の発生は完全には抑制されなかった。

2）多型核白血球集団巣の発生は DDS またはリファンピシンの投与では増強されず，ランプレンの長期投与でやや軽減した。

3）早期から開始し，長期間持続したランプレンの投与は，ヌードマウスのらい菌性病巣部に明らかな治癒機転をもたらした。

ENL 反応の発生機序を更に解明するためには，今後ヌードマウスに T cell サブセットを移入して行う実験が有意義であろうということが示唆された。