The Experimental Inoculation with *Mycobacterium leprae* in Autoimmune Mice : Results of MRL/*lpr* Mice Inoculated into the Right Hind Foot (continued)

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Susceptibility to *Mycobacterium leprae* of nude mice has been studied on various strains, and the importance of genetic backgrounds in relation to the development of lepromatous lesions has been pointed out and reported especially NFS/N and N:NIH(s) nude mice were high susceptibility to *M. leprae*. Additionally, NOD hybrid nude mice developed by the transfer the Crj:CD-1(ICR) nude gene were high susceptibility to *M. leprae* while that of their original NOD(Non-obese diabetic) mice which were developed experimental animal for the study of insulin-dependent diabetes mellitus (IDDM) were confined to multiplication of *M. leprae*.^(1,2)

The autoimmune MRL/*lpr* mouse bearing *lpr* gene was established by Murphy and Roths, Jackson laboratory, U.S.A. has been a very useful model for study of spontaneous systemic lupus erythematosus(SLE). Many immunological abnormalities of T cells have been found as compared with NZB, (NZB×NZW)F1 and BXSB mice. MRL/*lpr* mouse develop massive generalized lymph node enlargement, more than 90% of the cells in the enlarged lymph nodes is positive for Thy-1⁺ antigen. Older MRL/*lpr* mouse has a necrotizing exudative polyarteritis. Also, MRL/*lpr* mouse was confirmed rheumatoid factors of both the IgM and IgG, this is an excellent experimental model rheumatoid arthritis (RA) as well as for human SLE.⁽³⁾

In the present study, we have undertaken a comparison of susceptibility to *M. leprae* of autoimmune MRL/*lpr* mice (model for SLE and RA) with autoimmune NOD mice (model for IDDM), descriptions were made as high susceptibility to *M. leprae* of MRL/*lpr* mice as well as previously reported by using F₂(*lpr/lpr*•+/+) mice of MRL/*lpr*×Crj:CD-1(ICR)·*nu*(M₁).⁽⁴⁾

Materials and Methods

MRL/*lpr* and NOD mice were obtained from CLEA, Tokyo. Crj:CD-1(ICR) mice were obtained from Charles River, Atsugi, Japan. Thirty MRL/*lpr* mice and 20 NOD mice of 12-16 weeks old, and 20 Crj:CD-1(ICR) mice of 6 weeks old were used. The inoculum size of *M. leprae* derived from the foot passage of nude mice was 5.8×10^6 bacilli. The inoculation was made at the right dorsal site of the hind foot. The MRL/*lpr* mice were maintained in a vinyl isolator under specific pathogen free conditions, and were provided with sterilized autoclavable commercial diet(CE-2, CLEA, JAPAN) and tap water *ad libitum*. NOD and Crj:CD-1(ICR) mice were housed in a conventional animal room.

For identification, the harvested acid fast bacilli (AFB) from the MRL/*lpr* mice 240 days after inoculation, were reinoculated into the right hind foot of Crj:CD-1 (ICR) mice in order to confirm the growth pattern (persistent infection) in the mice. The AFB obtained were cultured on a 1% Ogawa's medium and a modified Nemoto's egg yolk medium at 33°C and 37°C for 3 months. Acid-fast staining of the smears of AFB was carried out by pyridine extraction method as modified by us.⁽⁵⁾ Besides, the tissues obtained from infected MRL/*lpr* mice were fixed in buffered formaldehyde solution, and stained with Fite-Faraco's and Haematoxylin-Eosin.

Results

In this experiment, we used MRL/*lpr* mice of 20 male and 10 female with already developed massive generalized lymph node enlargement as shown in Fig. 1. Used NOD and Crj:CD-1 (ICR) mice were male of 10 and female of 10. The multiplication at 105 days and 240 days of *M. leprae* inoculated into the right hind foot of the MRL/*lpr*, NOD and Crj:CD-1 (ICR) mice were shown in Table 1. In the column of 240 days after inoculation, the bacillary some male MRL/*lpr* mice were increased to 10^8 - 10^9 bacilli per foot, whereas NOD mice was approximately 10^4 bacilli per foot showing decrease in number of *M. leprae*. Histopathological section of the infected foot of MRL/*lpr* mice the 240 days after inoculation indicated the presence of a number of bacilli and globi around blood vessels in subcutaneous tissue or muscular layer on Fite-Faraco's stain as shown in Fig. 2 or Fig. 3. Also, their mice were observed to have systemic degenerative vascular disease. As shown in Fig. 4, the coronary granulomatous arteritis was observed in the inoculated foot.

The AFB isolated from the MRL/*lpr* mice were distinguished *M. leprae* from other mycobacteria. Results of reinoculation in normal mice were no swelling nor any other macroscopic lesions and the multiplication was confined to the site of inoculation. The cultivation on 1% Ogawa's medium and modified Nemoto's egg yolk medium at 33°C and 37°C for 3 months was all negative.

Acid-fast stainability of this bacilli was lost by pyridine extraction test. Therefore, the AFB grown in experimental mice were identified as *M. leprae* from these findings.

Table 1 : Number of *M. leprae* in right hind footpads of MRL/*lpr*, NOD and Crj:CD-1 (ICR) mice

Background	Days after inoculation			
	105	240		
MRL/ <i>lpr</i>	7.3×10^5	1.4×10^8	8.8×10^8	2.3×10^9
	2.9×10^7	2.8×10^8	5.0×10^8	8.1×10^8
	5.6×10^6	8.0×10^7	7.2×10^7	9.0×10^8
	9.2×10^6	3.1×10^6	3.5×10^7	7.8×10^6
	5.0×10^5		4.5×10^7	
NOD	5.4×10^5	1.7×10^5	3.1×10^4	2.5×10^5
	3.8×10^4	9.1×10^4	5.3×10^4	1.8×10^4
	7.5×10^4		8.9×10^4	
Crj:CD-1 (ICR)	6.2×10^5	2.7×10^6	4.5×10^5	3.7×10^6
	7.8×10^5	4.5×10^5	1.7×10^5	6.5×10^5
	2.3×10^5	8.9×10^4	6.8×10^4	

Inoculum size - - 5.8×10^6 bacilli / hind footpad

Fig. 1 A MRL/*lpr* mouse showing the enlargement of axillary lymph node. On 110 days after birth.

Fig. 2 A large number of bacilli are observed around blood vessels in subcutaneous lesion in a MRL/*lpr* mouse. On 240 days after inoculation with *M. leprae*. Fite-Faraco's stain.

Fig. 3 ditto. A large number of bacilli are seen in muscular layer and subcutaneous tissue. Fite-Faraco's stain.

Fig. 4 ditto. Subcutaneous lesion of foot accompanied with arteritis in MRL/*lpr* mouse. H&E stain.

Discussion

The MRL/*lpr*, autoimmune mice bearing *lpr* gene has been a very useful model to study the spontaneous systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA)⁽³⁾. The NOD mouse is a model of human insulin-dependent diabetes mellitus (IDDM), the diabetic processes appear to reflect an autoimmune response to the β cells within the islets of the pancreas. T lymphocytes play an important role in the initiation of insulinitis. In the pancreas of NOD mice, a predominant infiltration by activated T lymphocytes, including helper/inducer and cytotoxic/suppressor T cell, was observed in insulinitis. Natural killer cells were also detected in the lesions. As for splenic T cell subsets, cytotoxic suppressor T cells were increased in NOD mice^(6,7). On the other hand, autoimmune MRL/*lpr* mice are also T cell dependent. However these T cells have the following characteristics. Morse et al.⁽⁸⁾ or Wofsy et al.⁽⁹⁾ reported that the proliferating cells in enlarged abnormal lymph nodes express Thy-1⁺, L3T4⁻ and Lyt-2⁻ (double negative of Th and Ts) and B220⁺, and prud'homme et al.⁽¹⁰⁾ suggested an association of L-BCDF (B cell differentiation factor) which were produced spontaneously from the proliferating unusual cells with autoimmunity. However, recently, Santoro et al.⁽¹¹⁾ or Jabs and Prendergast⁽¹²⁾ demonstrated that the presence of functional abnormalities in splenic or enlarged nodes L3T4⁺ cells from MRL/*lpr* mice. Moreover, Santoro et al.⁽¹³⁾ or Asano et al.⁽¹⁴⁾ have suggested an important role for the T cell subset which were failed to produce interleukin 2 (IL-2), expressing B220 and L3T4 in the pathogenesis of disease in *lpr*-bearing mice. Furthermore, interleukin 3 (IL-3), interleukin 6 (IL-6) production in MRL/*lpr* mice were markedly increased^(15,16). Functional abnormalities of macrophages in MRL/*lpr* mice have shown an increased frequency of Ia⁺ resident⁽¹⁷⁾, also Nose et al. demonstrated the presence of MRL/*lpr*-MAF (macrophage activating factor derived from MRL/*lpr*) which were produced spontaneously⁽¹⁸⁾. Okuyama et al.⁽¹⁹⁾ reported that the ability to induce DTH against exogenous antigenic stimulation of MRL/*lpr* mice decreased with age. In view of the findings stated above, it is interesting to consider that the MRL/*lpr* mice would support the multiplication of *M. leprae*, while that of NOD mice would not support the growth of *M. leprae*. Also, it was confirmed the association with a genetic factor, for example, a factor the cause vascular lesions may be necessary for the development of marked lesions in addition to accelerate *lpr* gene with autoimmune-prone background gene of MRL/*lpr* mice.

Summary

Using MRL/*lpr*, NOD and Crj:CD-1 (ICR) mice, inoculations of *M. leprae* were made into the right hind foot at a dose of 5.8×10^6 bacilli per foot in order to study the influence of the immunobiological characteristics of their mice on the growth of *M. leprae*. To summarize of the results, MRL/*lpr* mice showed the high susceptibility to *M. leprae*, while that of NOD mice were poor. In conclusion, the immunobiological characteristics of MRL/*lpr*, autoimmune mice bearing *lpr* gene had effect on the multiplication of *M. leprae*, this mouse is a suitable multibacillary model for the study of leprosy.

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自己免疫疾患モデルマウスへのらい菌接種：MRL/*lpr* マウスの 右後肢足内における成績について（続報）

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キーワード：らい菌，自己免疫マウス，*lpr* 遺伝子，MRL/*lpr*，NOD

MRL/*lpr* マウスは，ヒトの全身性エリテマトーデス，慢性関節リウマチのモデルマウスとして広く利用されている。このマウスは，1個の常染色体性劣性の *lpr* 遺伝子とその遺伝的背景の修飾をうけて，T細胞の異常増殖による全身性リンパ節腫脹を呈し，免疫複合体性腎炎，血管炎，慢性関節炎等を自然発症する。同じくT細胞依存性に臓器特異的自己免疫

疾患（やせ型インシュリン依存性糖尿病）を自然発症するNODマウスとともに，らい菌接種を行った結果，NODマウスがらい菌の増殖に抑制的だったのに対して，MRL/*lpr* マウスは高いらい菌感受性を示した。