Absence of Insulitis and Overt Diabetes in Athymic Nude Mice with NOD Genetic Background

Susumu MAKINO, Minoru HARADA*, Yoshio KISHIMOTO, and Yoshiyuki HAYASHI


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NOD mice spontaneously develop diabetic syndrome similar to that of insulin-dependent diabetes mellitus in man. Insulitis, i.e., lymphocytic infiltration into the pancreatic islets, is the etiologic pathological lesion in the development of diabetes mellitus in NOD mice. In the present study, we examined the role of the T cell in the development of insulitis and overt diabetes in NOD mice using NOD athymic and euthymic congenic mice. None of the NOD athymic mice developed insulitis at 9 weeks of age or overt diabetes up to 30 weeks of age. In contrast, NOD euthymic littermates showed almost the same incidences of insulitis and overt diabetes as those of NOD mice. These observations suggest that T cells are essential for the development of insulitis and overt diabetes in NOD mice.

The non-obese diabetic (NOD) mouse established in our laboratory is an inbred strain which spontaneously develops diabetic syndrome similar to that of insulin-dependent diabetes mellitus in man [1, 2, 11, 17, 19]. A salient feature of the diabetic syndrome is profound insulitis, i.e., lymphocytic infiltration into the pancreatic islets [1, 11]. It is also a common characteristic in insulin-dependent diabetes mellitus in man [3, 8], cattle [5], Chinese hamster [20], and rat [15]. Thus, we hypothesized that it would be the primary pathological lesion implicated in the pathogenesis of diabetes mellitus in NOD mice. Recently, based on breeding experiments between NOD and C57 BL/6J mice, we found that two recessive genes located on independent autosomal chromosomes are necessary for the development of insulitis in NOD mice [12]. We also reported that the treatments with anti-thymocyte serum or anti-Thy 1, 2 antibody markedly suppress the development of overt diabetes in NOD mice [6]. These findings demonstrate that under the genetic controls, thymus-dependent cell-mediated autoimmune mechanisms may be responsible for the pathogenesis of insulitis in NOD mice.

In order to elucidate the involvement of cell-mediated autoimmune mechanisms, particularly of T lymphocyte-mediated reactions, we attempted to produce NOD athymic nude congenic mice. Although the athymic nude mice used in the present study have not completely been replaced by the NOD genetic background, we present interesting evidence here that the
NOD athymic nude mice do not develop either insulitis or overt diabetes.

To breed NOD athymic nude congenic mice, we used NOD mice after the 20th generation of our nucleus stock which shows a constant incidence of insulitis and overt diabetes \[11\] and BALB/c-\(\text{nu/nu}\) mice which have been maintained in our laboratory after they were received from Dr. K. Esaki, the Central Institute for Experimental Animals, in 1974.

NOD athymic nude congenic mice were developed by the following scheme. The BALB/c-\(\text{nu/nu}\) mouse was mated with the NOD mouse to obtain the N1 generation. This generation was then intercrossed to obtain athymic nude (\(\text{nu/nu}\)) mice of the N1F1 generation. These athymic nude (\(\text{nu/nu}\)) mice were chosen to backcross again to the NOD mouse. These cross-intercross cycles were repeated. In the present experiments, we used NOD athymic nude (\(\text{nu/nu}\)) mice and their euthymic (\(\text{nu/+ or +/+}\)) littermates of the N4F1 and N5F1 generations.

All the mice were bred in an SPF animal facility at constant temperature (24±1°C) and humidity (55±10%), and allowed access to a commercial mouse diet CA-1 (Clea Japan, Inc., Tokyo) and tap water autoclaved at 121°C, for 7 min. and for 35 min., respectively.

Both the athymic and euthymic mice of the N4F1 and N5F1 generations were tested for glucosuria weekly up to 30 weeks of age and defined as having overt diabetes when the urinary glucose was 3+ or higher with Tes-Tape (Eli-Lilly, Indianapolis).

Also, different animals of the same generations were sacrificed at 9 weeks of age for histological observations. The pancreas, lacrimal glands and submandibular glands were removed under sodium pentobarbital (60 mg/kg, i. p.) anesthesia, fixed in 10% buffered formalin solution, and stained with hematoxylin and eosin. All the islets in five sections from each block of pancreas were examined for the presence of insulitis.

Table 1 shows the incidences of insulitis and overt diabetes in the original NOD mice and the NOD athymic (\(\text{nu/nu}\)) and euthymic (\(\text{nu/+ or +/+}\)) congenic mice. None of the NOD athymic mice developed insulitis at 9 weeks of age or overt diabetes up to 30 weeks of age. In contrast, NOD euthymic mice.

Table 1. Incidences of insulitis and overt diabetes in NOD athymic (\(\text{nu/nu}\)) and euthymic (\(\text{nu/+ or +/+}\)) mice

<table>
<thead>
<tr>
<th>Sex</th>
<th>NOD Insulitis at 9 weeks</th>
<th>NOD euthymic Overt diabetes up to 30 weeks</th>
<th>NOD athymic</th>
</tr>
</thead>
<tbody>
<tr>
<td>♀</td>
<td>95.7% (23)</td>
<td>90.6% (32)</td>
<td>0% (23)</td>
</tr>
<tr>
<td>♂</td>
<td>92.3% (26)</td>
<td>87.8% (41)</td>
<td>0% (16)</td>
</tr>
<tr>
<td>♀</td>
<td>79.1% (67)</td>
<td>86.1% (36)</td>
<td>0% (16)</td>
</tr>
<tr>
<td>♂</td>
<td>20.5% (44)</td>
<td>56.3% (16)</td>
<td>0% (15)</td>
</tr>
</tbody>
</table>

Numbers in the parentheses show the number of mice examined.

Table 2. Incidences of lymphocytic infiltration in the lacrimal and submandibular glands in NOD athymic (\(\text{nu/nu}\)) and euthymic (\(\text{nu/+ or +/+}\)) mice

<table>
<thead>
<tr>
<th>Organ</th>
<th>Sex</th>
<th>NOD</th>
<th>NOD euthymic</th>
<th>NOD athymic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacrimal glands</td>
<td>♀</td>
<td>4%  (23)</td>
<td>10% (10)</td>
<td>0% (10)</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>100% (26)</td>
<td>100% (10)</td>
<td>0% (10)</td>
</tr>
<tr>
<td>Submandibular glands</td>
<td>♀</td>
<td>74% (23)</td>
<td>70% (10)</td>
<td>0% (10)</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>12% (26)</td>
<td>10% (10)</td>
<td>0% (10)</td>
</tr>
</tbody>
</table>

Mice at 9 weeks of age were examined. Numbers in the parentheses show the number of mice examined.
developed insulitis with incidences of 90.6% in females and 87.8% in males. They developed also overt diabetes with incidences of 86.1% in females and 56.3% in males. These incidences of insulitis and overt diabetes in NOD euthymic females and males were nearly the same or even higher in some cases in comparison with those of NOD mice of the nucleus stock.

The infiltration of lymphocytes is also observed with high frequency in the lacrimal and submandibular glands of NOD mice [7]. Thus, we examined the histological changes in these organs of NOD athymic and euthymic mice. As shown in Table 2, none of the NOD athymic mice developed lymphocytic infiltration in the lacrimal and submandibular glands, while the NOD euthymic ones showed lymphocytic infiltration in both organs. Again, there was no difference in the incidences of lymphocytic infiltration in each organ between NOD euthymic and NOD mice of either sex. These findings mean that the two recessive genes controlling the development of insulitis [12] had been completely transferred to mice of the N4 F1 generation regardless of the presence or absence of the thymus. Therefore, the fact that NOD athymic mice displayed no insulitis provides definite evidence that T lymphocytes are essential for the development of insulitis in NOD mice. In addition, NOD athymic mice showed the absence of overt diabetes, which strongly supports our hypothesis that insulitis is the primary change leading to the development of diabetes in NOD mice [10, 12].

Similarly, the finding that lymphocytic infiltration into the lacrimal and submandibular glands were observed in the euthymic mice but not at all in the athymic littermates suggests that lymphocytic infiltration in various glandular tissues of NOD mice is also produced by T cell-mediated autoimmunity.

Recently, Ogawa et al. [16] demonstrated that neonatal thymectomy in NOD mice reduces the frequency of insulitis from nearly 100% to 50%. Like et al. [9] have also reported a similar effect of neonatal thymectomy on insulitis in BB/W rats that spontaneously develop insulin-dependent diabetes mellitus [13, 15]. Neither paper explains why complete abolishment of insulitis was unsuccessful in thymectomized mice and rats. A possible mechanism is that T lymphocytes are distributed to the peripheral lymphoid tissues before or at birth, as shown in NZB mice [14, 21]. In this context, the NOD athymic nude congenic mice that we developed should be a very valuable tool for clarifying the role of the T cell in the pathogenesis of diabetes in NOD mice, because nu/nu genes prevent development of the thymic anlage [4, 18].

Now that athymic nude congenic mice with NOD background are available, passive transfer of insulitis and overt diabetes with T cells of littermates is attractive as the next research step. Investigation along this line is in progress in our laboratory.

Acknowledgements

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References

NOD マウスと同一の遺伝的背景を有する無胸腺ヌードマウスにおける insulitis および顕性糖尿の欠如

牧野 進・原田 稔*・岸本 嘉夫・林 幸之

塩野義製薬油日ラボラトリーズ
*塩野義研究所

NOD マウスはヒトのインスリン依存性糖尿病病と類似の糖尿病態を自然発症する。Insulitis（腎島のリンパ球浸潤）は NOD マウスの病因を考える上で重要な意味を持つ病変である。今回、我々は NOD ヌードコンジニックマウスを用い、insulitis および顕性糖尿発現における T cell の役割を調べた。NOD無胸腺マウスは、insulitis (9 過間) および顕性糖尿 (30 過間) のいずれも発現しなかったが、NOD 有胸腺マウスは、NOD マウスと同等の insulitis と顕性糖尿の発現を示した。これらの観察は、NOD マウスの insulitis および顕性糖尿発現に、T cell の存在が必須であることを示唆している。