Breakdown of Friend Virus-induced Tolerance and Development of Runting Syndrome in Rats

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SUMMARY

The effects of the inoculation of lymphoid cells into syngeneic rats made Friend virus (FV) tolerant by neonatal injections of FV were studied. The inoculation of lymphoid cells from immune donors brought about the runting syndrome in five of seven recipients, while the inoculation of lymphoid cells from normal donors induced the runting syndrome in one of five recipients. The titer of FV in the blood decreased markedly by the inoculation of lymphoid cells, but immunological tolerance to Friend lymphoma cells was not broken in the survivors. The repeated inoculation of lymphoid cells from immune donors induced the runting syndrome in all cases. The inoculation of immune lymphoid cells into young tolerant rats induced a low incidence of the runting syndrome and a high incidence of resistance to Friend lymphoma transplants. The major pathological findings in the runted rats were atrophy of the thymus and enlargement of the spleen with a depletion of lymphocytes. The pathogenic mechanism of the runting syndrome in the FV-tolerant rats induced by inoculation of syngeneic lymphoid cells was discussed.

INTRODUCTION

Studies with murine leukemias induced by Gross (1), Moloney leukemia (8, 9), and Graffi (6) viruses have suggested that newborn mice exposed to the leukemia virus were more susceptible to a subsequent challenge with syngeneic tumor transplants or were unable to produce circulating antibodies to the leukemia antigens. Therefore, they were considered to be immunologically specifically tolerant to the tumor-specific cellular and/or virion antigens.

Where immunological tolerance to FV-2-induced transplantation antigen in rats is concerned, we have reported that neonatal injections of high doses of FV (10^6 to 10^8 MID50/rat) always resulted in the development of a state of complete tolerance in the rats, as judged by the persistence of a constant viremia and the lethal growth of Friend lymphomas (10–14). These FV-tolerant rats developed splenic lymphomas about 200 days after the neonatal injection of FV (10).

The current study represents an attempt to determine whether immunological tolerance in rats given neonatal injections of FV can be broken by the inoculation of immunocompetent cells from syngeneic donors.

MATERIALS AND METHODS

Rats. An inbred strain of male and female WKA/Mk rats was used. These rats are the offspring of parents maintained by consecutive brother-sister mating for more than 200 generations at the Laboratory for the Breeding of Experimental Animals, Hokkaido University, Sapporo, Japan.

FV. The FV used has been serially maintained in DHS/Mk mice by the injection of spleen homogenates. The FV was recovered from Friend-diseased spleens by the method of Chenaille et al. (5). Usually, 0.1 to 0.3 ml FV suspension at a titer of 10^6 to 10^8 MID50/ml was injected i.p. and s.c. into newborn rats. The titer unit, expressed as MID50/ml, is the dose at which 50% of the mice are positive for the development of Friend disease as measured by the spleen focus assay method of Axelrad and Sleeves (2) and is calculated according to the cumulative method of Reed and Muench.

Friend Lymphoma. Transplantable Friend lymphoma (WFT-13) was induced in WKA/Mk rats at 172 days after the neonatal injection of FV. This tumor grew well either in rats made FV tolerant by the neonatal injection of FV or in immunologically depressed rats but failed to grow lethally even in syngeneic normal adult rats. Detailed characteristics of Friend lymphomas have been reported elsewhere (10, 11, 13, 14).

Serum. Serum was obtained from rats immunized repeatedly with WFT-13 cells by cardiac puncture under ether anesthesia. After the blood clotted at room temperature, the serum was separated by centrifugation at 3000 rpm for 30 min.

Titration of FV in Blood. At different ages, 3 to 4 rats that were given neonatal injections of FV were bled by cardiac puncture under ether anesthesia. After the blood clotted at room temperature, the serum was separated by centrifugation at 3000 rpm for 30 min.

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fixed in Bouin's fluid, and the foci on the surface of each spleen were counted with the naked eye. Virus titers were expressed as MIDs/ml as mentioned above.

**Cell Preparations.** Donor rats for the injection of lymphoid cells were killed by ether. Spleens and lymph nodes were minced with fine scissors and gently homogenized with a loose-fitting glass TenBroeck homogenizer. The crude spleen and lymph node cell suspensions were filtered through surgical gauze and were washed 3 times at 1000 rpm for 5 min, after which 1 to 5 x 10^8 cells/ml were resuspended in Eagle's minimal essential medium. Cell viability was determined by lack of permeability to trypan blue. All suspensions had 95% or more viable cells.

**Autopsy and Microscopic Examination.** For histological examination, the spleen, thymus, lymph nodes, and other tissues were fixed in a 10% formalin solution and embedded in paraffin, and 4-µm-thick sections were prepared. Hematoxylin and eosin staining was used routinely for all sections.

**RESULTS**

**Changes of Body Weight.** Two months after neonatal injections of high doses of FV, 43 FV-tolerant rats were divided into 4 groups as follows. In Group 1, 7 rats were inoculated twice at 5-day intervals with spleen and lymph node cells (5 x 10^7) obtained from rats preimmunized repeatedly with WFT-13 cells. In Group 2, 5 rats were inoculated twice at 5-day intervals with spleen and lymph node cells (5 x 10^7) obtained from normal rats. In Group 3, 7 rats were inoculated 4 times at 4-day intervals with sera obtained from rats immunized with WFT-13 cells. In Group 4, 7 rats were not inoculated as a control group.

Clinical symptoms and body weights were recorded in individual rats of the 4 groups at different stages following the above inoculations. Six days after the 1st inoculation, all rats in the 1st group and 1 in the 2nd group showed a cessation of body weight gain, diarrhea, ruffled hair, anemia, hunched posture, etc. Five of the 7 rats in the 1st group and 1 of the 5 rats in the 2nd group died of the runting syndrome from 11 to 18 days after the 1st inoculations. The body weight of the runted rats in the late stages was less than one-half of that of the control rats (Chart 1). Typical runting syndrome was not observed in the rats of the 3rd group although 2 of them showed a loss of body weight from 12 to 18 days after the 1st inoculation with immune sera.

**Titer of FV in the Blood.** The induction of tolerance to FV-specific transplantation antigen in newborn rats must depend not only on the dose of the virus injected but also on the degree of virus multiplication. For determination of the titer of the virus in the rats of the 4 groups described above, all rats from each group were bled by cardiac puncture at different ages, and the blood was pooled according to groups for the titration of the virus. After initial inoculations, the virus titer curves in each group were recorded in Chart 2. In the 1st group, the titer of the FV decreased rapidly and could not be detected at 6 days. In the 2nd group, the titer of the virus decreased from 6 days, reaching its minimal level with a titer of 10^3.0 MIDs/ml at 12 days and thereafter gradually increased again. In the 3rd group, the titer of the virus decreased slowly and reached a plateau with a titer of 10^1.7 MIDs/ml at 6 days. In the control group, the titer of the virus remained nearly constant at a titer of 10^2.7 to 10^3.8 MIDs/ml.

**Transplantation of WFT-13 Cells.** For a determination of whether immunological tolerance to FV-specific transplan-
tation antigen was abolished, the surviving rats from each group received s.c. transplants of WFT-13 cells. The results are presented in Table 1. The 2 survivors in the 1st group rejected the WFT-13 transplants but then died of the running syndrome. Two of the 4 survivors in the 2nd group died from the growth of WFT-13 cells, while the other 2 rats resisted the WFT-13 challenge but thereafter succumbed to the running syndrome. All rats in the 3rd and 4th groups died from the growth of the WFT-13 transplants.

**Effects of Repeated Inoculations of Lymphoid Cells.** In the experiment described above, most of the FV-tolerant rats developed the running syndrome after the inoculation of lymphoid cells from rats preimmunized with WFT-13 cells. Some of them resisted the WFT-13 challenge but subsequently developed the running syndrome. Experiments were then carried out to investigate the still remaining possibility that the repeated inoculation of lymphoid cells might completely break a tolerant state, thereby inhibiting the development of the running syndrome. Nine FV-tolerant rats at 2 months of age were inoculated i.p. 5 times at 3-day intervals with $5 \times 10^7$ lymphoid cells per rat. As the result, all (9 of 9) tolerant rats developed severe running syndrome and died at a mean survival period of 20.0 days (Table 2).

**Inoculation of Lymphoid Cells into Young FV-tolerant Rats.** Spleen and lymph node cells from syngeneic rats immunized with or without WFT-13 cells were inoculated into young FV-tolerant rats at 21 days after neonatal injections of FV. The results are shown in Table 3. In the young tolerant rats inoculated with immune lymphoid cells ($5 \times 10^7$) twice at 5-day intervals, 3 of the 13 rats (23.1%) died of the running syndrome from 14 to 21 days after the 1st inoculation. Nine of the 10 remaining rats rejected the WFT-13 transplants and survived, while 1 rat succumbed to the running syndrome after rejecting the WFT-13 transplants. The 9 survivors did not develop primary lymphomas until more than 300 days. One of the 8 (12.5%) young tolerant rats inoculated with normal lymphoid cells developed the running syndrome. Of the remaining 7 rats, 5 died from the growth of WFT-13 cells and 2 developed the running syndrome after rejecting WFT-13 transplants. None of the 7 young tolerant rats not inoculated developed the running syndrome, and all of them died from the growth of WFT-13 transplants.

**Pathological Findings in the Runted Rats.** By macroscopic examination of the runted rats, the most common findings were atrophy of the thymus and enlargement of the spleen. However, the spleen appeared atrophic in severely runted rats or in rats that survived for a relatively long period after first exhibiting clinical symptoms. The degree of thymic atrophy varied individually, but many of the thymuses were one-half normal size or less. In some of the severely runted rats inoculated repeatedly with immune lymphoid cells, it was not possible to identify any surviving thymic tissue at necropsy. The size of lymph nodes varied from normal in most rats to atrophie in some extremely runted rats. The liver was anemic but normal in size. Under microscopic examination, the type of lesions observed in the thymus depended on the degree of the running syndrome and the length of survival after the initial inoculation. In general, the histological changes in the atrophic thymuses involved both their lymphoid component and epithelial framework. In the relatively long-surviving rats, which displayed mild symptoms of wasting, thymic lesions accompanied by a depletion of lymphocytes were located at cortical areas; but in the severely runted rats, individual lobules of the thymus were markedly small and corticomedullary differentiation disappeared (Figs. 2 and 3). In the cortex, thymocytes disappeared completely and the reticulum cells were altered by vacuolar degeneration (Fig. 4). In the medulla of the thymus, lymphocytes decreased and reticulum cells proliferated. The histological change of the spleen consisted of

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### Table 1

<table>
<thead>
<tr>
<th>Substances inoculated</th>
<th>Incidence of runting syndrome</th>
<th>Transplantation of WFT-13 cells in survivorsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune lymphoid cells</td>
<td>5/7 (71.0)%</td>
<td>0/2² (2/2)²</td>
</tr>
<tr>
<td>Normal lymphoid cells</td>
<td>1/5 (20.0)</td>
<td>2/4 (2/2)</td>
</tr>
<tr>
<td>Immune sera</td>
<td>0/7 (0)</td>
<td>7/7</td>
</tr>
<tr>
<td>None</td>
<td>0/7 (0)</td>
<td>7/7</td>
</tr>
</tbody>
</table>

a WFT-13 cells ($5 \times 10^7$) were transplanted s.c.

b Spleen and lymph node cells ($5 \times 10^7$) were injected i.p. twice at an interval of 5 days.

c Numbers in parentheses, percentage of incidence.

d Number of rats that died of tumor growth/number of rats challenged with WFT-13 cells.

e Number of rats that developed the runting syndrome/number of rats that rejected tumor.

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### Table 2

<table>
<thead>
<tr>
<th>Cells inoculated/ No. of cells</th>
<th>Route</th>
<th>Incidence of runting syndrome</th>
<th>Mean survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$5 \times 10^7$ (5 times)</td>
<td>i.p.</td>
<td>9/9</td>
<td>20.0</td>
</tr>
</tbody>
</table>

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### Table 3

<table>
<thead>
<tr>
<th>Cells inoculated</th>
<th>Incidence of runting syndrome</th>
<th>Transplantation of WFT-13a survivors in survivors</th>
<th>Incidence of primary tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune lymphoid cells</td>
<td>3/13 (23.1)%</td>
<td>0/10⁰ (1/10⁰)</td>
<td>0/9⁰</td>
</tr>
<tr>
<td>Normal lymphoid cells</td>
<td>1/8 (12.5)</td>
<td>5/7 (2/7)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0/7 (0)</td>
<td>7/7 (0/7)</td>
<td></td>
</tr>
</tbody>
</table>

a WFT-13 cells ($5 \times 10^7$) were transplanted s.c. interval of 5 days.

b Spleen and lymph node cells ($5 \times 10^7$) were injected i.p. twice at an interval of 5 days.

c Numbers in parentheses, percentage of incidence.

d Number of rats that died of tumor growth/number of rats challenged with WFT-13 cells.

e Number of rats that developed the running syndrome/number of rats that rejected tumor.

f Rats observed more than 300 days after infection.
atrophy of the white pulp with a marked depletion of lymphocytes and lymphoblasts in follicular areas. In most of the runted rats, the follicles were replaced by proliferating histiocytes and reticulum cells. Megakaryocytes increased relative to other cell components in the red pulp (Fig. 5). The lymph nodes displayed a loss of lymphocytes, and germinal centers varied in size from extremely small to prominent (Fig. 6). The bone marrow of the runted rats showed a decrease of erythroid cells and a relative increase of metamyelocytes. In other organs such as the kidney, lung, parotid gland, and testes, we could not discern histological changes except for cell-inflammatory infiltrations in the lungs of some severely runted rats. These histological changes of the lymphoid organs were very similar to the runting syndrome in the rats induced by neonatal injections of low doses of FV as reported previously (18).

DISCUSSION

Previously, we have reported that rats given neonatal injections of low doses of FV showed a high incidence of the runting syndrome, while rats given neonatal injections of high doses of FV grew normally as FV-tolerant rats (18). Histological and immunological studies of these runted rats have suggested that the pathogenesis of the runting syndrome induced by neonatal injections of low doses of FV might be ascribed to the specific immune reaction between immunocompetent cells of the host and their own tissues which had acquired FV-specific transplantation antigen (17). The pathogenic mechanism might be considered to be the same as that for rats rendered tolerant by the inoculation of syngeneic lymphoid cells. The inoculation of immune lymphoid cells into FV-tolerant rats brought about a high incidence of the runting syndrome, but the inoculation of immune sera did not induce the runting syndrome. This fact indicates that the development of this syndrome is not based on humoral immunity but on cell-mediated immunity in the host. The incidence of the runting syndrome in tolerant rats by the inoculation of specific immune lymphoid cells was higher than that in tolerant rats by the inoculation of normal lymphoid cells. Repeated inoculations of immune lymphoid cells brought about the runting syndrome in all tolerant rats. Furthermore, the clinical and histological findings in these runted rats were almost identical to those of classical runt disease. The evidence of this experiment suggests that an immune reaction similar to graft-versus-host reaction occurs between tolerant rats and syngeneic lymphoid cells. Graft-versus-host reaction is known to have developed in rodents given neonatal injections of allogeneic lymphoid cells or in F1 hybrid rodents receiving lymphoid cells of parental origin (4). In these models, the runt disease is believed to be produced by a specific reaction of immunocompetent donor cells recognizing the transplantation antigen of the host as foreign. The same is considered to hold true in tolerant rats receiving syngeneic lymphoid cells. In our experiments, inoculated lymphoid cells recognized the FV-specific transplantation antigen in the virus-infected cells as foreign, and the consequent specific immune reaction results in the development of the runting syndrome.

A few reports have described the breakdown of immunological tolerance induced by viral infection. Volkert and Hannover Larsen (19, 20) have shown that a viremic state in mice infected neonatally with lymphocytic choriomeningitis virus could be terminated by the adoptive inoculation of syngeneic immune lymphoid cells. Spärck and Volkert (16) have also demonstrated that the adoptive inoculation of syngeneic immune lymphoid cells could eliminate an oncogenic viremia and prevent the development of primary lymphomas in mice. In our studies, the inoculation of syngeneic immune lymphoid cells into tolerant rats could terminate a viremia but could not break immunological tolerance to WFT-13 cells. Most of the rats developed the runting syndrome and died. On the other hand, the inoculation of immune lymphoid cells into young tolerant rats could terminate a viremia and also abrogate immunological tolerance to WFT-13 cells. However, there were a few young tolerant rats that produced FV-specific cytotoxic antibody and were resistant to WFT-13 transplants (17). Almost all of these rats developed the runting syndrome and died within 2 months. This fact indicates that even rats given high doses of FV neonatally do not develop complete tolerance to WFT-13 cells in the early stages of infection. Therefore, the immunological tolerance in young tolerant rats might be broken easily by the inoculation of syngeneic lymphoid cells.

Finally, the cause of the runting syndrome in tolerant rats by the inoculation of lymphoid cells may be due to extensive damage to immature hematopoietic cells, which are considered to be the target cells of FV. If this is true, the additional inoculation of syngeneic bone marrow cells may prevent the development of the runting syndrome and abolish completely the immunologically tolerant state.

REFERENCES

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