SUMMARY

While rats given neonatal injections of high doses of Friend virus grew up as apparently normal rats (Friend virus-tolerant rats), most rats given low neonatal doses of Friend virus died from the development of the runting syndrome (runted rats). At the transplantation of Friend virus-induced tumor (WFT-3), all tolerant rats died from the growth of the WFT-3 cells, while some of the rats that had escaped the runting syndrome rejected the WFT-3 transplants. The cytotoxic antibody to Friend virus-specific cell surface antigen was not detectable in the Friend virus-tolerant rats but was detected in all the runted rats. The titer of the antibody detected reached its highest level from 7 to 10 days before the death of the runted rats. The runting syndrome was induced by the transfer of syngeneic immune or normal lymphoid cells and also by the transfer of runted lymphoid cells into the Friend virus-tolerant rats. The appearance of Friend virus-specific transplantation antigen in rats infected with Friend virus was proved by the method of local graft-versus-host reaction.

These experiments demonstrate that the pathogenesis of the runting syndrome induced in rats by Friend virus consists of continuous reactions between the cells that have acquired Friend virus-specific transplantation antigen and the immunologically competent cells of the host.

INTRODUCTION

In the preceding report (23), we demonstrated that rats given neonatal injections of FV developed symptoms similar to runt disease (3, 4) or wasting syndrome (19), such as loss of body weight, diarrhea, ruffled hair, anemia, hunched posture, and early death. It was not detected in rats given high doses of FV neonatally, but was observed only in rats given low doses of FV neonatally. When rats were given neonatal doses of FV, 10⁴ to 10⁶ 50% minimal infective doses per rat, 70% died of the runting syndrome about 30 days after infection. The autopsy and histological examination of the runted rats revealed characteristic lesions in the lymphoid organs. These lesions consisted mostly of atrophy of the thymus and enlargement of the spleen with marked depletion of lymphocytes. Development of this syndrome was reduced by X-irradiation 7 days after neonatal infection. It has been suggested that the development of the runting syndrome by FV injection might be based on immunological reactions in the host.

This report attempts, by means of immunological studies, to clarify the pathogenesis of the runting syndrome in rats given low doses of FV neonatally.

MATERIALS AND METHODS

Rats

An inbred strain of male and female Wistar King Aptekman/Mk (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 transfer of spleen homogenates. The FV was recovered from the FV-diseased spleens by the method used by Chenaile et al. (5). Usually, a 0.1- to 0.3-ml FV suspension at a titer of 10⁵ to 10⁶ 50% minimal infective doses per ml was injected i.p. and s.c. into newborn rats. The titer unit, expressed as 50% minimal infective doses per rat, is the dose at which 50% of the mice are positive for the development of Friend disease as determined by a spleen focus assay; it is calculated according to the cumulative Reed-Muench method.

FV-induced Tumors

Transplantable WFT-3 was induced in WKA/Mk rats after a neonatal injection of FV. This tumor grew well both in rats that had been made FV-tolerant by a neonatal injection of FV and in immunologically depressed rats, but it failed to grow lethally even in syngeneic normal adult rats. Detailed characteristics of this tumor have been reported elsewhere (14-17).

Sera

The rats were bled by cardiac puncture under ether anesthesia. After the blood clotted at room temperature, sera were separated by centrifugation at 3000 rpm for 30 min. Before use, the sera were inactivated at 56°C for 30 min.
RESULTS

Induction of Tolerance and Development of the Runt Syndome. The incidence of the runting syndrome in rats that received neonatal injections of various doses of FV was studied. WFT-3 was transplanted into rats that had escaped from the runting to determine whether they were immunologically tolerant to FV-specific transplantation antigen. In the rats given neonatal injections of FV, $10^{4.9}$ 50% minimal infective doses per rat or more, the incidence of the runting syndrome was very low (10.4 to 12.6%), and all the surviving rats were killed by the growth of the WFT-3 transplants. Among rats given neonatal injections of FV, $10^{3.1}$ to $10^{3.0}$ 50% minimal infective doses per rat, the incidence of the runting syndrome was 55 to 60% and all surviving rats were also killed by the growth of WFT-3 transplants. Among rats given neonatal injections of FV, $10^{1.0}$ 50% minimal infective doses per rat, the incidence of the runting syndrome was very low (5.5 to 6.0%), and all surviving rats were resistant to WFT-3 transplants, although temporary growth of tumors was observed. In the rats given neonatal injections of FV, $10^{0}$ 50% minimal infective doses per rat or less, the incidence of the runting syndrome was very low, and most of the survivors were resistant to WFT-3 transplants (Table 1).

Thus, rats that received various neonatal doses of FV could be placed generally into 3 groups: (a) a tolerant group given neonatal injections of FV, $10^{4.9}$ 50% minimal infective doses per rat or more; (b) a runting group given neonatal injections of FV, $10^{1.0}$ to $10^{3.0}$ 50% minimal infective doses per rat; (c) a nontolerant group given neonatal injections of FV, $10^{0}$ 50% minimal infective doses per rat or less.

FV-specific Cytotoxic Antibody in Rats Given Neonatal Injections of FV. Thirty-nine newborn rats were divided into 2 groups and were given neonatal injections of FV, $10^{4.0}$ to $10^{5.0}$ or $10^{1.0}$ to $10^{3.0}$ 50% minimal infective doses per rat. Three to 4 rats from both groups were sacrificed at 2, 3, 4, 5, 6, or 7 weeks after infection and their sera were tested for the presence or absence of the cytotoxic antibody to WFT-3 cells (Table 2). In the sera from rats that received high neonatal doses of FV ($10^{4.0}$ to $10^{5.0}$ 50% minimal infective doses per rat), the cytotoxic antibody to WFT-3 cells could not be detected in any of those tested until the 3rd week and could be detected in only 2 of the 4 rats at the 4th week after infection. The titer of the antibody detected were 1:2 and 1:4, respectively. One of these 2 rats showed typical symptoms of the runting syndrome at this time. After the 5th week, the antibody could not be detected in any of the rats tested.

In the sera from the rats given low neonatal doses of FV ($10^{1.0}$ to $10^{2.0}$ 50% minimal infective doses per rat), the antibody could be detected in 1 of the 3 rats at the 2nd week and could be detected in all rats tested from the 3rd week. A higher titer of the antibody was detected in the rats at the 3rd week after infection. At the 3rd and 4th weeks, most of the rats manifested the clinical symptoms of the runting syndrome. However, the rats surviving more than 5 weeks did not show the symptoms, although the antibody was detected.
The Runting Syndrome in FV-tolerant Rats by the Transfer of Syngeneic Lymphoid Cells. Induction of the runting syndrome in Fv-tolerant rats by the transfer of syngeneic lymphoid cells was studied. Forty-three tolerant and 21 normal 2-month-old rats were divided into 5 groups as follows. (a) Seven tolerant and 5 normal rats were given 2 i.p. injections at an interval of 5 days, with lymphoid cells from rats preimmunized with WFT-3 cells. (b) Ten tolerant and 3 normal rats were given injections of lymphoid cells from normal rats in the same manner as the 1st group. (c) Seven tolerant and 5 normal rats were given injections of lymphoid cells from normal rats in the same manner as the 1st group. (d) Twelve tolerant and 5 normal rats were given injections of lymphoid cells from tolerant rats. (e) Seven tolerant and 3 normal rats received no injection (controls). All surviving rats were tested for their susceptibility to WFT-3 transplants. The results are presented in Table 3. Five of the 7 tolerant rats in the 1st group developed the runting syndrome, displaying a loss of body weight, diarrhea, ruffled hair, hunched posture, etc., from 11 to 18 days after the 1st transfer. Histological examination of these runted rats revealed degenerative and destructive changes of the lymphatic organs. These clinical and histological findings were almost the same as those of the runting syndrome in rats given low neonatal doses of FV. The 2 survivors in the 1st group died from the development of the runting syndrome after rejecting transplants of WFT-3 cells. None of the normal rats in the 1st group showed the runting syndrome, and they were resistant to WFT-3 transplants. Two of the 10 tolerant rats in the 2nd group developed the runting syndrome and 5 of the 8 survivors died from growth of WFT-3 transplants. The other 3 survivors rejected the WFT-3 transplants and then died of the runting syndrome. Three of the 7 tolerant rats in the 3rd group died of the runting syndrome and 2 of the 4 survivors died of the growth of WFT-3 transplants. The other 2 survivors rejected the WFT-3 transplants and then died of the runting syndrome. One of the 12 tolerant rats in the 4th group died of the...
runting syndrome. All the remaining rats died from the growth of WFT-3 transplants, except for 1 rat that developed the runting syndrome and died. The 7 tolerant rats in the 5th group did not develop the runting syndrome and died from the growth of WFT-3 transplants. None of the normal rats in the 2nd, 3rd, 4th, and 5th groups developed the runting syndrome, and all of them were resistant to WFT-3 transplants.

The Runting Syndrome in FV-tolerant Rats by Repeated Transfer of Syngeneic Lymphoid Cells from Rats Immunized with WFT-3 or Allogeneic Hepatoma (AH66) Cells. For determination of whether the development of the runting syndrome in FV-tolerant rats by the transfer of syngeneic lymphoid cells was due to specific or nonspecific immune reactions, syngeneic lymphoid cells obtained from rats immunized with WFT-3 or AH66 cells into tolerant rats were repeatedly transferred (Table 4). All (9 of 9) tolerant rats that received injections of lymphoid cells from rats immunized with WFT-3 cells died of the runting syndrome 16 to 24 days after the 1st transfer. Three of the 8 tolerant rats (37.5%) implanted with lymphoid cells from rats immunized with AH66 cells developed the runting syndrome. Upon transplantation of WFT-3 cells, 4 of the 5 surviving rats died of WFT-3 tumor growth, and 1 rat developed the runting syndrome.

### Table 3

**Development of the runting syndrome in FV-tolerant and normal rats by the transfer of syngeneic lymphoid cells**

<table>
<thead>
<tr>
<th>Transferred materials</th>
<th>Recipients</th>
<th>Incidence of runting syndrome</th>
<th>Transplantation of WFT-3 in survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of rats that died of runt</td>
<td>No. of rats that died of tumor growth/</td>
</tr>
<tr>
<td></td>
<td></td>
<td>syndrome/no. of rats given</td>
<td>no. of rats challenged with</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lymphoid cells</td>
<td>WFT-3 cells</td>
</tr>
<tr>
<td>Immune lymphoid cells</td>
<td>Tolerant</td>
<td>5/7</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Normal lymphoid cells</td>
<td>Tolerant</td>
<td>2/10</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Runting lymphoid cells</td>
<td>Tolerant</td>
<td>3/7</td>
<td>42.8</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Tolerant lymphoid cells</td>
<td>Tolerant</td>
<td>1/12</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>None</td>
<td>Tolerant</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

* Cells (5 × 10⁷) were transplanted s.c.

### Table 4

**Induction of the runting syndrome in FV-tolerant rats by the transfer of syngeneic lymphoid cells from rats immunized with WFT-3 cells or AH66 cells**

<table>
<thead>
<tr>
<th>Tolerant rats given injections of lymphoid cells¹ from</th>
<th>Incidence of runting syndrome</th>
<th>Transplantation of WFT-3 in survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of rats that died of runt</td>
<td>No. of rats that died of tumor growth/</td>
</tr>
<tr>
<td></td>
<td>syndrome/no. of rats given</td>
<td>no. of rats challenged with</td>
</tr>
<tr>
<td></td>
<td>lymphoid cells</td>
<td>WFT-3 cells</td>
</tr>
<tr>
<td>Rats immunized with WFT-3 cells</td>
<td>9/9</td>
<td>100.0</td>
</tr>
<tr>
<td>Rats immunized with AH66 cells</td>
<td>3/8</td>
<td>37.5</td>
</tr>
<tr>
<td>Normal rats</td>
<td>3/10</td>
<td>30.0</td>
</tr>
<tr>
<td>Tolerant rats</td>
<td>1/10</td>
<td>10.0</td>
</tr>
</tbody>
</table>

* Spleen and lymph node cells (5 × 10⁷) were injected i.p., 5 times, at 5-day intervals.

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3099
N. Takeichi, N. Kuzumaki, and H. Kobayashi

syndrome and died. Three of the 10 tolerant rats given injections of lymphoid cells from normal rats developed the runting syndrome. After transplantation of WFT-3 cells, 5 of the 7 surviving rats died of the tumor growth and the remaining 2 rats developed the runting syndrome and died. Only 1 of the 10 (10%) tolerant rats given lymphoid cells from tolerant rats developed the runting syndrome, and all the remaining rats died of growth of WFT-3 transplants. The results suggest that the development of the runting syndrome is based on specific immune reactions between the tolerant hosts and transferred syngeneic lymphoid cells.

Detection of FV-specific Transplantation Antigen by Local GVHR. As mentioned above, the runting syndrome could be induced in FV-tolerant rats by the transfer of syngeneic lymphoid cells. It was presumed that similar immune reaction to GVHR in homologous disease developed between a new cellular antigen in the virus-infected cells and the transferred syngeneic lymphoid cells. Sixteen tolerant and 16 normal 2-month-old rats were divided into 4 groups corresponding to the 1st 4 groups shown in Table 3. In all 4 groups, 5 x 10^7 lymphoid cells from various donors were injected into the subcapsular space of the left kidney of the individual rats. Appearances of the GVHR were measured in terms of the renal index (Ki/Kc) and were confirmed by histological examination (Table 5). All (4 of 4) tolerant rats transferred with immune lymphoid cells developed white nodules under the subcapsular region of the kidney (Fig. 1), and the mean renal index (Ki/Kc) was 1.23, compared with 0.98 in the normal rats. By microscopic examination, a marked infiltration of mononuclear cells, such as lymphocytes and histiocytes, was observed in all cases from the subcapsular region to the parenchyma on the injected kidney (Fig. 2). The 4 tolerant rats that received injections of normal lymphoid cells showed slight increases in kidney weight, and the mean renal index was 1.04 compared with 0.84 in the normal rats. However, histologically, 2 of the 4 tolerant rats displayed a marked infiltration of mononuclear cells from the subcapsular region to the parenchyma of the left kidney. The other 2 rats showed cell infiltration only in the subcapsular region. The 4 tolerant rats given injections of runted lymphoid cells also showed increases in kidney weight and a renal index of 1.11. Two rats were classified histologically as Grade II, while the other 2 rats were classified as Grade I. In contrast, no increase in weight and infiltration of the cells was seen in the injected kidney of a donor-recipient combination of tolerant lymphoid cells to tolerant rats, as was true in the case of tolerant lymphoid cells to normal rats; nor was a GVHR observed in the kidneys of tolerant rats as a result of the transfer of sera from rats immunized with WFT-3 cells.

DISCUSSION

A few reports have described the development of the runting syndrome in animals by oncogenic virus infection. Polyoma virus was found to induce the runting syndrome in mice; this syndrome consisted of atrophy of the Pyer's patches and depletion of lymphocytes in the lymph nodes and spleen (24). Recently, Anderson et al. (1) reported that cats infected neonatally with feline leukemia virus had a higher mortality associated with atrophy of the thymus and lymphoid
depletion. The pathogenesis of these runting syndromes is not yet clear, although it is supposed that the cause of these syndromes is closely related to the appearance of a new cellular antigen or antigens in the virus-infected cells, which necessitated a continuous reaction of the lymphoid tissues. Our present experiments demonstrated some evidence about the pathogenic mechanism of the runting syndrome in rats caused by leukemia virus infection.

Newborn rats are generally known to be susceptible to leukemia virus, although adult rats are highly resistant. The rats given high neonatal doses of FV developed lymphomas after about 200 days. In the latent period, they were more susceptible to subsequent challenge with syngeneic lymphoma (WFT-3) cells or were unable to produce humoral antibodies to FV antigen and WFT-3 cells (15, 16). Therefore, they were considered to be immunologically specifically tolerant to FV-specific cellular and/or virion antigens. Conversely, rats given low neonatal doses of FV developed the runting syndrome markedly in about 30 days. Most of them produced the cytotoxic antibody to WFT-3 cells, and some of the rats that escaped death from runting were resistant to WFT-3 transplants. The highest titer of the antibody was detected 7 to 10 days before death from runting. In these rats, immunological tolerance to FV-specific cellular and/or virion antigens was established for a certain period after infection but soon was broken by the immune reaction of the host. A virus dose of 10^1.0 50% minimal infective doses per rat was the critical level in inducing the runting syndrome in newborn rats. In the same situation, the runting syndrome was induced in FV-tolerant rats by the transfer of syngeneic lymphoid cells. The incidence of the runting syndrome in tolerant rats by the transfer of specific immune lymphoid cells was higher than that produced in tolerant rats by the transfer of normal or nonspecific immune lymphoid cells. Transfer of lymphoid cells from runted rats into tolerant rats also brought about the runting syndrome. The appearance of the virus-specific transplantation antigen in the tolerant rats was confirmed by the method of local GVHR. These facts indicate that an

<table>
<thead>
<tr>
<th>Transferred materials</th>
<th>Recipients</th>
<th>Mean Ki/Kc</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune lymphoid cells</td>
<td>Tolerant</td>
<td>1.23</td>
<td>I, II, II, III</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0.98</td>
<td>0, 0, 0, 0</td>
</tr>
<tr>
<td>Normal lymphoid cells</td>
<td>Tolerant</td>
<td>1.04</td>
<td>I, II, II</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0.84</td>
<td>0, 0, 0, 0</td>
</tr>
<tr>
<td>Runted lymphoid cells</td>
<td>Tolerant</td>
<td>1.11</td>
<td>I, II, II</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0.97</td>
<td>0, 0, 0, 0</td>
</tr>
<tr>
<td>Tolerant lymphoid cells</td>
<td>Tolerant</td>
<td>0.98</td>
<td>0, 0, 0, 0</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>0.97</td>
<td>0, 0, 0, 0</td>
</tr>
</tbody>
</table>

注：

- Weight of kidney injected with lymphoid cells/weight of collateral kidney.
- Grade I, noninvasive lymphoid graft; Grade II, lymphoid graft with invasive tongue; Grade III, invasive destructive reactions.
- Spleen and lymph node cells (5 x 10^7) were injected.
Immune reaction similar to the GVHR occurred between tolerant hosts and transferred syngeneic lymphoid cells. From the experimental results presented, the rats given low neonatal doses of FV developed a specific immune reaction to their own tissues, which had acquired FV-specific transplantation antigen, and growth of their immune functions was followed by development of the running syndrome.

Recent studies have demonstrated the development of specific immune reactions in leukemogenesis by RNA viruses (2, 9). McCoy et al. (18) reported that C57BL/6 mice given relatively high neonatal doses of RLV developed detectable transplantation and serological immunity against transplantable Rauscher lymphoma cells. The results showed that transplantation resistance in C57BL/6 mice given neonatal injections of a $10^{-1}$ dilution of RLV developed and then disappeared more rapidly than in the mice given neonatal injections of a $10^{-4}$ dilution of RLV. However, they did not show whether mice given neonatal injections of an undiluted ($10^6$) dose of RLV developed specific immune reactions. In our studies, a few produced the cytotoxic antibody to WFT-3 cells in the early days after they were given neonatal injections of high doses of FV. Almost all of these rats developed the running syndrome and died within 2 months. Any remaining rats were unable to produce the cytotoxic antibody and transplantation resistance to WFT-3 cells during their lives. However, the possibility cannot be ignored that even the rats given high neonatal doses of FV do not develop complete tolerance to FV-specific cellular and/or virion antigens in the early stage of infection.

On the other hand, it has been reported that treatment with cortisol acetate (20), sterile bacterial vaccines, or bacterial infection (6, 7) was the cause of the running syndrome. Such cases are difficult to fully explain by the immunological mechanism. Keast et al. (11, 12, 13) demonstrated that the lesions in the intestines of runted mice might allow the diffusion of an abnormal amount of bacterial endotoxin into the circulation and later even permit bacteremia. This bacterial infection or its endotoxin might play an important role as a promoting factor in the genesis of the running syndrome in rats given low neonatal doses of FV.

Finally, the running syndrome induced in rats by FV infection consisted of immunological disorders in leukemogenesis. This syndrome serves as an experimental model for analysis of the interrelationship between immunological disorders and neoplasia.

REFERENCES

Fig. 1. The macroscopic appearance of GVHR on the 7th day after an injection of $5 \times 10^7$ lymphoid cells from the rats immunized with WFT-3 is shown on the right. The contralateral kidney given no injection is shown on the left as a control. X 8.

Fig. 2. Microscopic examination of the kidney shown in Fig. 1. There is massive infiltration of mononuclear cells and destruction of tubules. H & E, X 150.
Immunological Studies of Runting Syndrome in Rats Inoculated with Friend Virus

Noritoshi Takeichi, Noboru Kuzumaki and Hiroshi Kobayashi


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