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MALIGNANT LYMPHOMA AND AMYLOIDOSIS FOLLOWING GRAFT-VERSUS-HOST REACTION

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ABSTRACT

Persistent stimulation of immunocompetent cells, such as histocompatibility reactions, may be one of the causes of neoplastic proliferation of lymphoreticular tissues. In the present studies, the graft-versus-host reaction was induced in 18-week-old male (C 57 Bl/6 $J \times$ DBA/2 J)F_1 mice by two or four weekly intraperitoneal injections of approximately 80×10^6 male C 57 Bl/6 J or C 57 Bl/Na mouse lymphoid cells. Some of the recipient mice were treated with amethopterin.

The majority of mice dying soon after injection of parental lymphoid cells showed signs of runt diesase and most animals recovering from the disease survived and were alive at the age of 24 months, at the termination of the experiments. Histological studies of 94 mice of experimental groups revealed lymphomatous changes in 10 mice and a moderate to extensive amyloidosis in 22 mice, with no abnormal changes in 15 untreated control mice. Nine out of 10 lymphomas were Type B, only one being reticulum cell sarcoma, Type A. Five out of 9 Type B lymphomas were associated with amyloidosis. Comparative analysis of mortality rates due to runt disease, and incidences of lymphoma and amyloidosis in each experimental group showed that the resulting lymphoma incidences were apparently indifferent to the severity of runt disease.

The results obtained in the present studies, together with those in others, suggest that the overt graft-versus-host reaction may not be a necessary precondition for increased lymphomagenesis in parental-F_1 hybrid chimeras and that factors other than the graft-versus-host reaction itself may also be involved.

INTRODUCTION

Immunological theories of carcinogenesis have been postulated by several investigators[1)2)3)]. Persistent stimulation of immunocompetent cells, such as histoincompatibility reactions, may be one of the causes of neoplastic proliferation of lymphoreticular tissues. The graft-versus-host reaction, induced in F_1 hybrid mice by the administration of parental spleen cells, has been considered the ideal system for examination of the possible role of immunologic responses in the induction of malignant lymphomas[1)2)]. With this system, Schwartz and Beldotti[4)] and Walford[5)] succeeded in the induction of lymphomas.

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The histoincompatibility was strong (H-2 difference) in the case of Schwartz and Beldotti and weak (H-1 difference) in Walford's experiments. In neither of these studies was virus etiology completely ruled out.

That the lymphomas induced in the experiments of Schwartz and Beldotti were of host rather than donor origin[4] raised some doubt whether an immunologic reaction itself caused the induction of these lymphomas. Thus, an attempt was made to reexamine the question in the present studies using the donor-recipient combination identical with that used by Schwartz and Beldotti with some modifications.

MATERIALS AND METHODS

In the present studies, the graft-versus-host reaction was induced in 18-week-old male (C 57 Bl/6 J × DBA/2 J)F_1 mice (hereafter referred to as BDF_1) by two or four weekly intraperitoneal injections of approximately 80×10^6 male C 57 Bl/6 J or C 57 Bl/Na mouse lymphoid cells. The parental lymphoid cells were prepared as follows. Whole spleens and lymph nodes (cervical and mesenteric) were teased with fine forceps on a sterile stainless steel mesh immersed in chilled Hanks' solution. The cells passing through the mesh were collected and washed in the solution by gentle centrifugations and the resulting cell suspension was then diluted with the solution to contain the desired numbers of cells. Three groups of BDF_1 recipients were used: i) mice receiving two weekly injections of 80×10^6 cells, ii) mice receiving four weekly injections of the same dosage of cells and iii) mice receiving the same dose of cells as in ii) followed by five intraperitoneal injections of amethopterin (3 mg/kg of body weight). The drug was given every other day beginning the day after the last injection of parental lymphoid cells. Since C 57 Bl/Na, a subline of C 57 Bl/6 J and therefore used in the present studies as one of the donor strains, has been maintained by random breeding for years in the Laboratory Animals Center of our University and may not be considered inbred and identical with C 57 Bl/6 J, the groups of BDF_1 mice that received lymphoid cells from these C 57 Bl/Na mice were separately followed.

Experiments were terminated by sacrificing the surviving mice at the age of 24 months. Gross and microscopic examinations were performed on each mouse of all groups. Lymph nodes, liver, spleen and kidney were fixed with formalin and stained with H & E, Congo red and methyl violet. The latter two stainings were applied in the cases where hyaline deposits in the spleen and/or liver were present. In addition, examination of the sections stained with Congo red was carried out with the polarizing microscope in order to ascertain whether there was any birefringence[6].

RESULTS

The majority of mice dying soon after injection of parental lymphoid cells showed signs of runt disease and the cumulative mortality rate in each group is shown in Table 1. Most animals recovering from runt disease survived and were alive at the age of 24 months, at the termination of the experiments.

TABLE 1. Experimental Design and Incidence of Malignant Lymphoma and Amyloidosis

Parental lymphoid cells administered		Amethopterin	No. of BDF_1 mice	Mortality[a] (%)	No. of survivors	Malignant lymphoma No. (%)	Amyloidosis No. (%)
Donor strain	cell dose						
C 57 Bl/6 J	$80\times10^6\times2$	—	18	16.6	15	3(20)	6(40)
C 57 Bl/6 J	$80\times10^6\times4$	—	35	42.8	20	1 (5)	2(10)
C 57 Bl/Na	$80\times10^6\times4$	—	20	0	20	4(20)	6(30)
C 57 Bl/6 J	$80\times10^6\times4$	+	40	52.5	19	2(10.5)	5(26.3)
C 57 Bl/Na	$80\times10^6\times4$	+	20	0	20	0	3(15)
Untreated control mice			15	0	15	0	0

[a] Cumulative percent mortality during the first 150 experimental days, iudicative of fatal runt disease.

On gross examination, 22 out of the total 94 autopsied mice of experimetal groups showed abnormal changes including a moderate lymphoadenopathy and a moderate to extensive splenomegaly and/or hepatomegaly, whereas none of 15 untreated control mice showed any abnormal changes. There was no sign of infection and the thymus was invariably atrophied.

Before microscopic examination, these 22 mice were thought to be affected by malignant lymphomas. Unexpectedly, however, histological studies of them and the other apparently normal mice revealed lymphomatous changes only in 10 mice and a moderate to extensive amyloidosis in 22 mice. Nine out of 10 lymphomas were Type B, only one being reticulum cell sarcoma, Type A. Five out of 9 Type B lymphomas were associated with amyloidosis. Occurrence of lymphomas and amyloidosis in each experimental group is shown in Table 1. The highest incidence of lymphoma and amyloidosis was 20 and 40% respectively. None of the mice inoculated with C 57 Bl/Na lymphoid cells succumbed to runt disease and none of the mice inoculated with C 57 Bl/Na lymphoid cells and then treated with amethopterin developed lymphomas, while amyloidosis was observed throughout experimental groups irrespective of donor strain or amethopterin injection.

In the spleen of mice with amyloidosis, amyloid substance was deposited in the perifollicullar zones or mantles of the splenic follicles in mild to moderate cases while in extensive cases, was deposited diffusely, almost replacing

normal elements. In the liver, amyloid deposit was seen in sinusoids and within the liver cells. In these mice with amyloidosis, extramedullary granulocytopoietic hyperplasia, particularly in lymph nodes, was invariably observed, though in varying degree.

DISCUSSION

In the present studies, the highest incidence of malignant lymphomas was 20% and far lower than that reported by Schwartz and Beldotti[4]. Mean age at which lymphomas developed was around 24 months in our experiments and 8 or 12 months in the experiments of Schwartz and Beldotti. The severity of the graft-versus-host reaction decreases with age of host. In the present studies, 18-week-old BDF_1 mice were used and mortality rate due to runt disease in mice untreated with amethopterin was up to about 40%. This was much lower than that observed in the experiments of Schwartz and Beldotti in which 6-week-old BDF_1 mice were used. It is quite conceivable that the use of older host may be one of the causes of late occurrence and low incidence of lymphomas in the present studies. It is noteworthy, however, that the resulting lymphoma incidences are apparently indifferent to the severity of runt disease (Table 1).

Occurrence of amyloidosis in mouse radiation chimeras suffering from "secondary disease", a type of graft-versus-host reaction, has been reported by Bradbury and Micklem[7]. In the present studies, 22 out of 94 BDF_1 mice inoculated with parental lymdhoid cells, surviving the graft-versus-host reaction, developed a moderate to extensive amyloidosis. In Hodgkin's disease, secondary amyloidosis often develops[8]. In the present studies, 5 of 9 cases of type B lymphoma were associated with amyloidosis. In neither the study of Schwartz and Beldotti nor of Walford was there any description of whether amyloid deposit was present or absent.

While the experiments were in progress, two papers concering the graft-versus-host reaction and lymphomagenesis appeared: one by Rossi and Friend[9] and another by Cole and Nowell[10]. The former workers, using a combination of BALB/cJ and DBA/2J with several weak histocompatibility differences, failed to induce a significant number of malignant lymphomas, while the latter with a combination of C57L(H-2^a) and A/He(H-2^b), succeeded in induction of malignant lyphomas in a high incidence. The experiments of Rossi and Friend were complicated with unexpected development of mammary carcinoma and ours with amyloidosis. The results obtained in the present studies, together with those of others, suggest that the overt graft-versus-host reaction may not be a necessary precondition for increased lymphomagenesis in parental-F_1 hybrid chimeras and that factors other than the graft-versus-host reaction itself may also be involved.

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