

CHRONIC ALLOGENEIC DISEASE

II. DEVELOPMENT OF LYMPHOMAS*

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Immunocytes with antiself activity can be acquired experimentally if lymphoid cells from inbred animals are administered to their F₁ hybrids. Infant recipients of parental cells are usually killed by an acute graft-*versus*-host reaction, but this seldom happens when the cells are injected into mature F₁ hybrids. Instead, a chronic disorder resembling an autoimmune disease often develops. Three immunologic syndromes have been described thus far: immunohemolytic anemia (1) and glomerulonephritis (2) in mice, and polyarthritis with dermatitis and carditis in rats (3, 4). Moreover, a high incidence of lymphomas occurs in (C57Bl/6 × DBA/2)F₁ mice given C57Bl/6 spleen cells (5), a combination in which the immunologic lesion is immunohemolytic anemia (1). The present experiments were undertaken in (BALB/c × A/J)F₁ mice, which develop immune complex-mediated glomerulonephritis when inoculated with BALB/c spleen cells (2). Administration of parental cells to these hybrids triggered a sustained immunoproliferative reaction, and eventually a high incidence of reticulum cell sarcomas developed.

Materials and Methods

Animals.—BALB/c, A/J, and (BALB/c × A/J)F₁ (hereafter referred to as CAF₁) mice were obtained from Jackson Laboratories, Bar Harbor, Maine. Many of the animals were bred in our laboratory from Jackson parental stocks. The mice were housed in plastic cages and fed standard diets. Spleen cell donors were 6–8 wk old and the F₁ recipients were 6 wk old at the time of the first injection of parental cells.

Preparation of Spleen Cell Suspensions.—A previously described technique (2) was used.

Histopathology.—Specimens of all visible lymph nodes, spleen, liver, small bowel, kidney, lung, and thymus were obtained at autopsy. Tissues were fixed in 10% buffered formalin and stained with hematoxylin and eosin. Classification of neoplasms followed the nomenclature of Dunn (6).

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Electron Microscopy.—Fragments of tissue were fixed for 30 min in 6.5% glutaraldehyde (7), postfixed with 1% osmium tetroxide buffered with 0.1 M phosphate buffer (pH 7.2) and embedded in epon after dehydration in graded alcohols. Thick sections (0.5 μ) were prepared to select blocks, which were then cut with glass knives on a Porter-Blum microtome. Ultrathin sections were mounted on uncoated grids, stained with lead citrate 0.4% (8) and examined with an RCA EMU 3-C electron microscope (50 kv, objective aperture 50 μ).

Tissue Transplantation.—Cell suspensions containing $5\text{--}15 \times 10^6$ cells were prepared as described and injected intraperitoneally into litters of 1- to 2-day-old mice. The recipients were killed and autopsied at the time of appearance of an obvious tumor, or no later than 1 yr after they received the cell suspension.

Cytotoxicity Assay.—A method based on the release of radioisotope from ^{51}Cr -labeled mouse lymphocytes, described by Houghton (9) and Wigzell (10), was used. The tissue culture medium was Kaliss' buffer (11). Monospecific antisera against single H-2 specificities were

TABLE I
Incidence of Lymphomas in Experimental Mice

Age	No. of BALB/c spleen cells administered ($\times 10^6$)				
	10	50	100	150	200
<i>months</i>					
3-5	—	—	—	3/46	—
6	0/17	0/15	—	5/33	7/19
12	0/19	2/17	3/14	20/73	19/44
18	—	—	—	31/56	—
24	—	—	—	14/18	—

Numerator, number of neoplasms; denominator, total number of mice in the group.

obtained from Dr. George Snell, Bar Harbor, Maine. Tumor cells previously frozen at -90°C were tested for their ability to absorb antibody activity from the monospecific antisera. The antiserum was diluted so as to produce about 80% of the maximum ^{51}Cr released from labeled target cells. Suspensions containing increasing numbers of tumor cells (in 25 μl) or normal cells were added to the selected dilution of antiserum (i.e., that dilution producing 80% of the maximum release of ^{51}Cr from the target cells). The mixture was incubated for 15 min, after which labeled target cells and complement (selected normal rabbit serum) were added. In five instances fresh tumor cells labeled with ^{51}Cr were used as the target cells. Tumors selected for analysis by this method consisted of a solid mass of neoplastic tissue. Particular care was taken to exclude from study any tumors embedded in or surrounded by normal-appearing (by gross and microscopic examination) lymphoid tissue.

Assays for Chimerism. The spleen assay (12, 13) method of Simonsen and Jensen was used.

RESULTS

Control Mice. There were 324 control CAF_1 mice, of which 241 were untreated, 45 were injected with 150×10^6 syngeneic spleen cells, and 38 were inoculated with 150×10^6 frozen and thawed BALB/c spleen cells. The 241 untreated animals were killed when 6, 12, 18, or 24 months old. These subgroups contained 58, 50, 68, and 65 mice respectively. The 45 CAF_1 mice treated with 150×10^6 syngeneic spleen cells were killed when 6 (16 mice) or 12 (29 mice)

months old. The third group of control CAF₁ mice, which were injected with 150×10^6 BALB/c spleen cells that had been subjected to three cycles of freez-

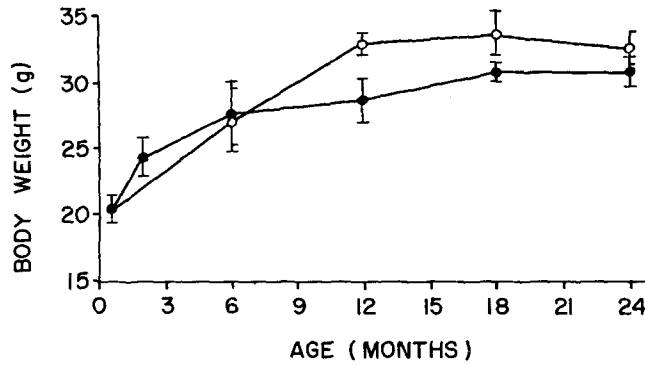


FIG. 1. Body weights of control (○—○) and experimental (●—●) animals. In this and succeeding figures, the horizontal bars indicate 1 SE.

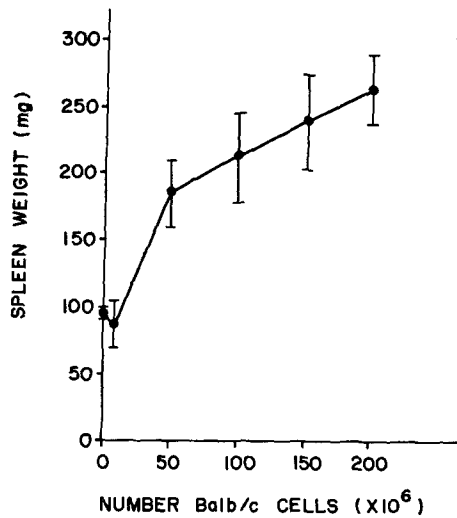


FIG. 2. Spleen weights of CAF₁ mice 1 yr after receiving the indicated amounts of BALB/c spleen cells.

ing and thawing, was killed at 6 (16 mice) or 12 (22 mice) months of age. As there were no differences among the various groups with regard to body and organ weights, histology, and tumor incidence, the results were pooled (Figs. 1-4, 7-9).

54 BALB/c mice were autopsied, 25 at the age of 1 yr and 29 at the age of

18 months. Three (12%) of the 1 year old mice and three (10.2%) of the 18 month old mice had reticulum cell sarcomas, type B. Four alveologenic carcinomas were found, three of them in 18 month old mice. A hemangioepithelioma was discovered in the liver of a 12 month old BALB/c animal.

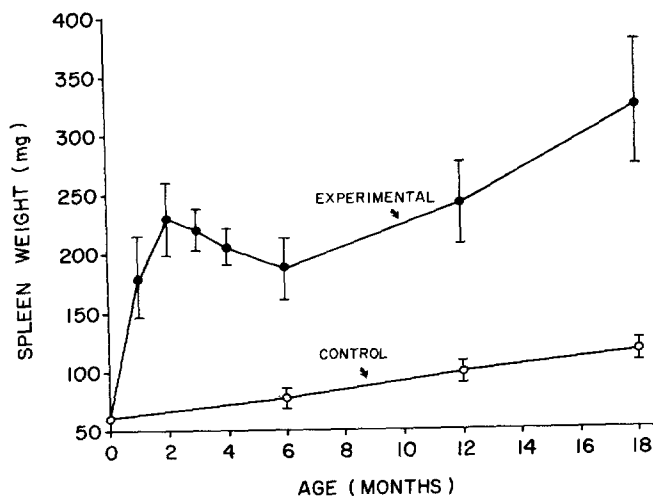


FIG. 3. Spleen weights of groups of CAF₁ mice given 150×10^6 BALB/c spleen cells.

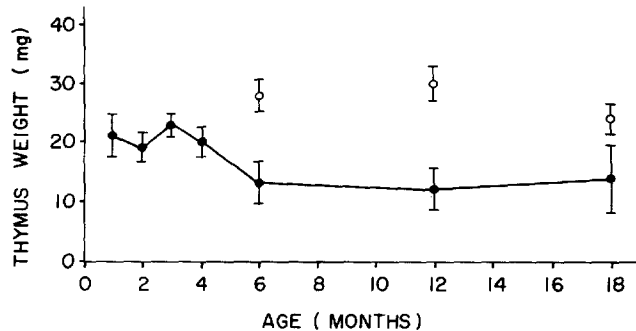
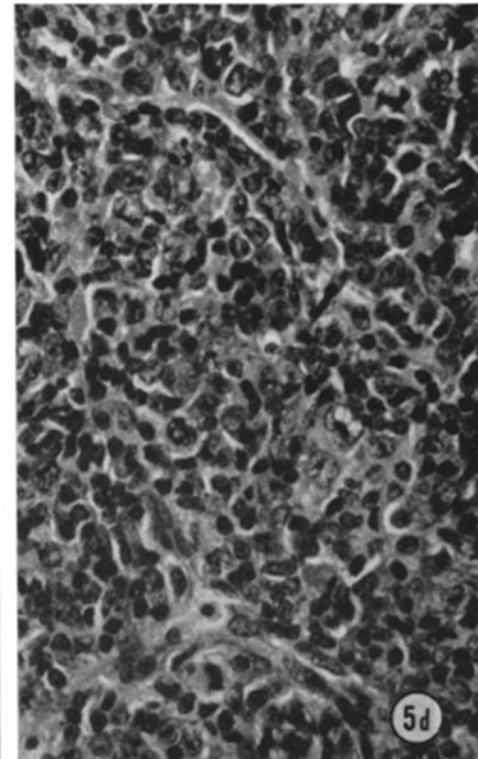
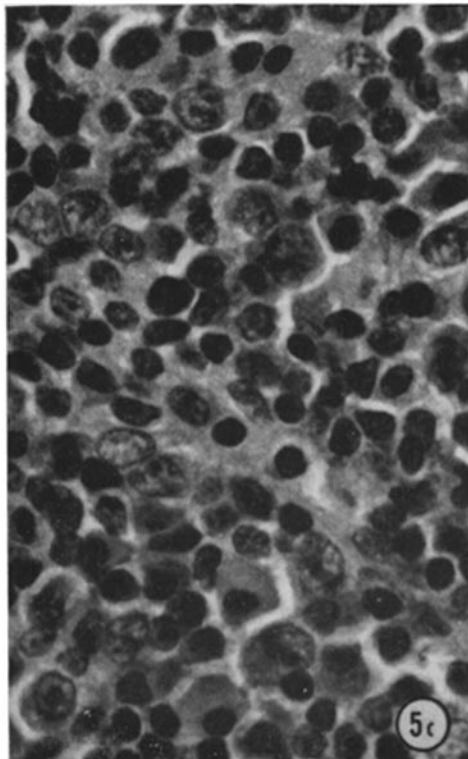
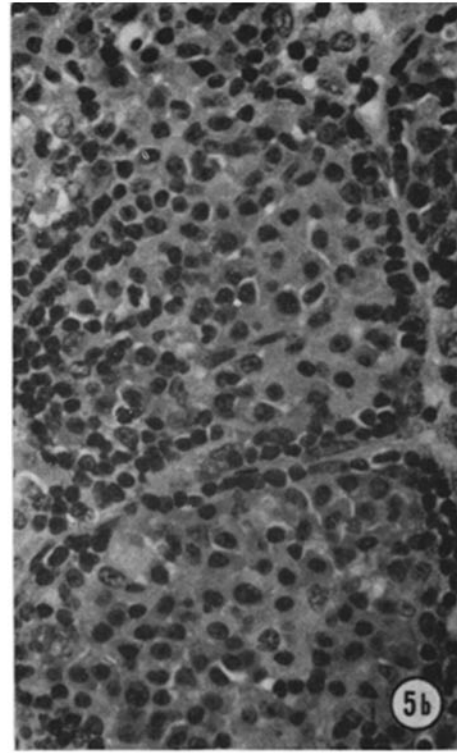
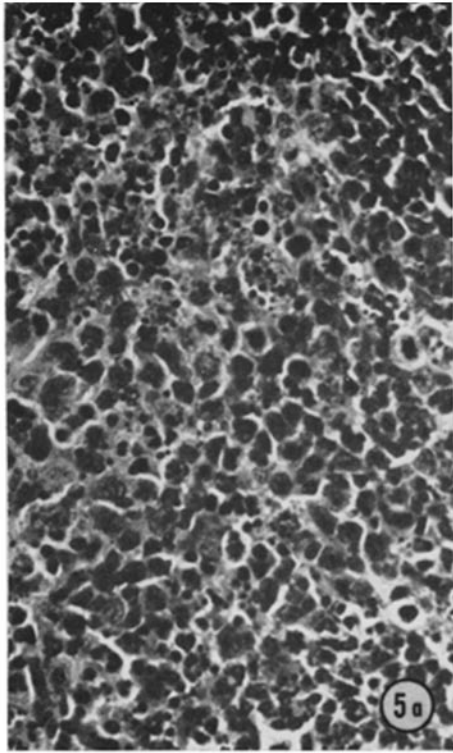


FIG. 4. Thymus weights of groups of CAF₁ mice given 150×10^6 spleen cells (●—●) compared with those of control CAF₁ mice (○—○).

Experimental Mice. 371 CAF₁ mice were divided into the groups shown in Table I. Another 49 mice, not included in that table, were killed less than 3

FIG. 5. Preneoplastic stage of chronic allogeneic disease. (a) Germinal center (spleen) containing many tingible bodies and nuclear dust. $\times 250$. (b) Nodular collections of plasma cells in a mesenteric lymph node. $\times 250$. (c) Pleomorphic change, with a mixture of plasma cells and reticulum cells. $\times 520$. (d) Prelymphoma, characterized by irregular sheets of pleomorphic tissue containing lymphocytes, plasma cells, and reticulum cells. $\times 250$.



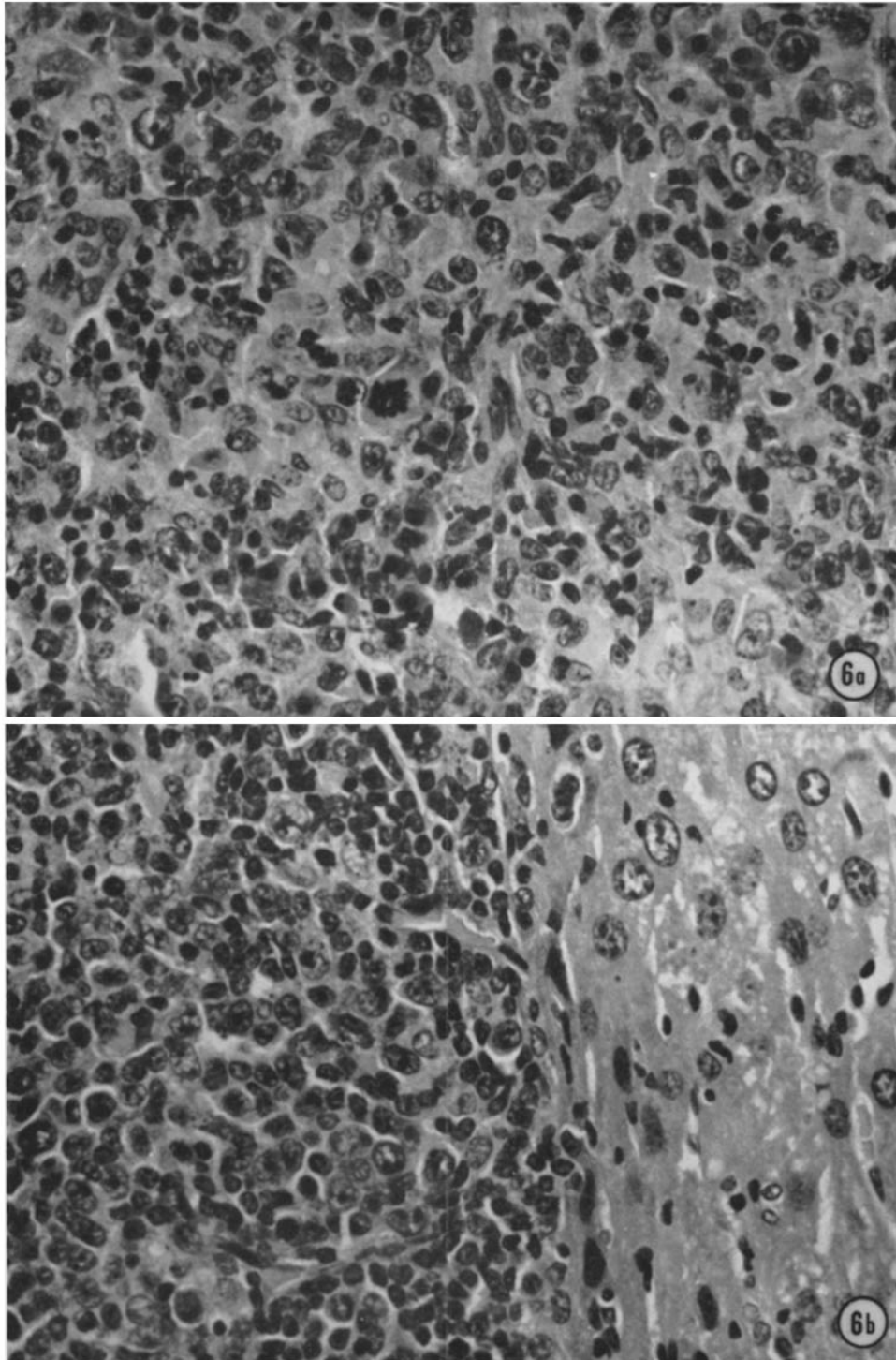


FIG. 6. Malignant stage of chronic allogeneic disease. (a) Reticulum cell sarcoma, type B, in spleen. $\times 250$. (b) Reticulum cell sarcoma, type B, invading liver. $\times 250$.

months after the administration of BALB/c spleen cells in order to obtain tissues for histologic studies.

None of the experimental mice developed signs of runt disease. Body weight (Fig. 1) was slightly reduced in the control mice at 1 yr, but at all other times there were no significant differences in body weight between control and experimental mice.

Splenomegaly was an outstanding feature of the disease. The degree of splenic enlargement was proportional to the number of BALB/c spleen cells adminis-

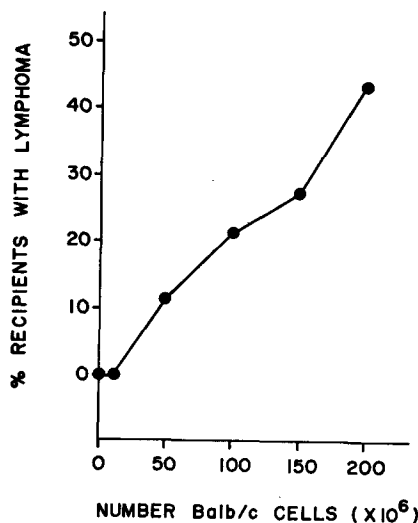


FIG. 7. Incidence of lymphomas in CAF₁ mice 1 yr after receiving the indicated number of BALB/c spleen cells.

tered (Fig. 2). The spleen enlarged early in the course of the disease and remained enlarged for the duration of the study (Fig. 3). Splenomegaly was usually accompanied by an enlargement of lymph nodes. Cervical, axillary, periaortic, and mesenteric lymph nodes were most frequently involved. The weight of the thymus was reduced, especially in the older mice (Fig. 4).

Four kinds of histologic changes occurred in the spleen and lymph nodes. Early in the disease, at 1–3 months, the lymphoid follicles were greatly enlarged. The bulk of the follicle consisted of a huge germinal center, which was filled with nuclear debris. Much of the debris lay free in the intercellular space, but a considerable amount was phagocytized. The result was a striking picture of myriad tingible bodies surrounded by nuclear dust (Fig. 5a). The red pulp of the spleen was often packed with nucleated red blood cells and megakaryocytes.

The next phase began 3–6 months after the injection of BALB/c spleen cells,

and was characterized by intense plasmacytosis. Sheets and nodules of plasma cells filled the medullary portions of the lymph nodes, and frequently extended into their cortical zones (Fig. 5b). In the spleen, plasma cells were abundant in the perifollicular zone of the red pulp. This, together with the enlarged follicles, produced a marked triple-zoning effect. In some specimens mast cells were abundant. Not every lymph node of a given mouse was abnormal; the mesen-

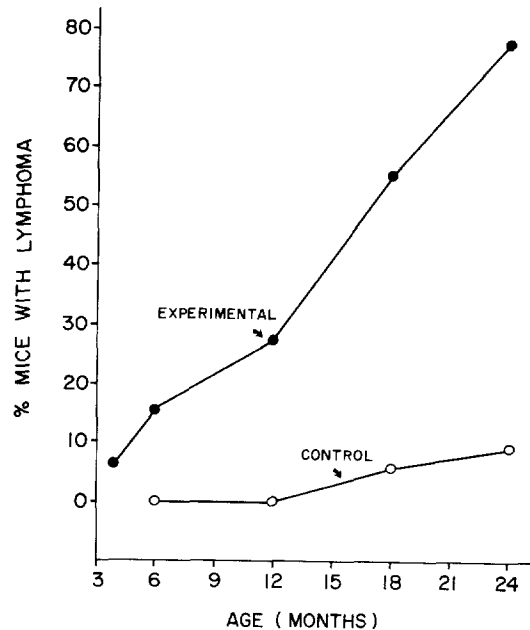


FIG. 8. Incidence of lymphomas in groups of CAF₁ mice given 150×10^6 BALB/c cells (●—●) compared with that in control CAF₁ mice (○—○).

teric lymph node was most frequently affected, and in some animals it was the only lymph node with important changes.

6 months or longer after the injection of parental spleen cells a new element was seen. This was the appearance of numerous reticulum cells, and in many instances the previously described nodular collections of plasmocytes were replaced by irregular sheets of tissue consisting of plasma cells and reticulum cells (Fig. 5c). Whereas the former changes were readily recognized as intense hyperplasia of lymphoid tissue, many examples of this third pattern were difficult to classify. Its pleomorphic aspect suggested neoplasm, but preservation of the architecture of the specimen did not corroborate this. This lesion, which was tentatively diagnosed as "prelymphoma," was present in 32 mice (Fig. 5d).

The fourth histologic pattern was that of a malignant lymphoma (Fig. 6).

The incidence of lymphomas was proportional to the number of BALB/c spleen cells administered (Fig. 7). The development of lymphomas in 226 CAF₁ mice given 150×10^6 BALB/c spleen cells is compared with that in 324 control CAF₁ mice in Figs. 8 and 9.

A total of 93 lymphoid tumors developed. Of these, 77 were reticulum cell sarcomas, type B; 1 was a type A reticulum cell sarcoma; 5 were lymphoblastic lymphomas; 1 was a mastocytoma; 9 were unclassified. One animal had a type

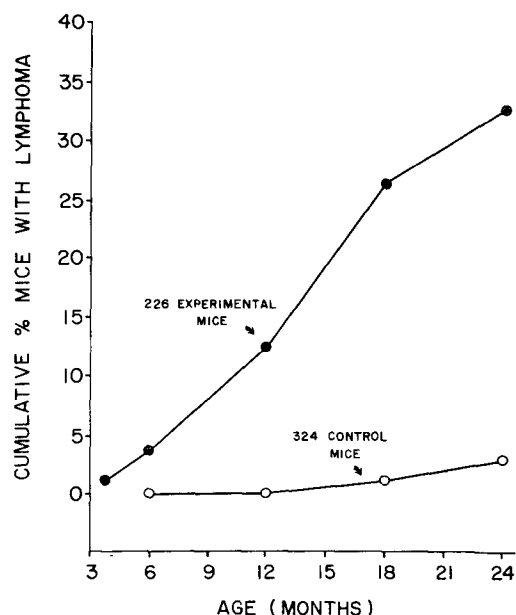


FIG. 9. Cumulative incidence of lymphomas in experimental and control mice.

B neoplasm in its spleen and a lymphoblastic lymphoma in its mesenteric lymph node. Another mouse had an unclassifiable neoplasm in its spleen and a lymphoblastic tumor in a cervical lymph node. Of the 77 type B reticulum cell sarcomas, 20 were confined to the spleen, 10 occurred only in lymph nodes, 20 involved both spleen and lymph nodes, and 27 had metastasized to nonlymphoid organs, usually the liver, kidney, and lung. The 9 unclassified lymphomas were characterized by whorls of reticulum cells alternating with solid sheets of megakaryocytes. In five of these cases, megakaryocytes were also present in lymph nodes, liver, kidney, and lung. In no instance was a thymoma observed.

Electron Microscopy.—Electron microscopy corroborated the observations made by light microscopy. The hyperplastic phase of the disease was characterized by three striking features: (a) numerous macrophages containing

extremely fine debris (Fig. 10*a*); (*b*) numerous mast cells (Fig. 10*b*); and (*c*) abundant plasma cells. The neoplasms were comprised of lymphoblasts (Fig. 11*a*), among which were numerous and conspicuous reticular cells (Fig. 11*b*). The over-all picture was typical of a reticulum cell sarcoma.

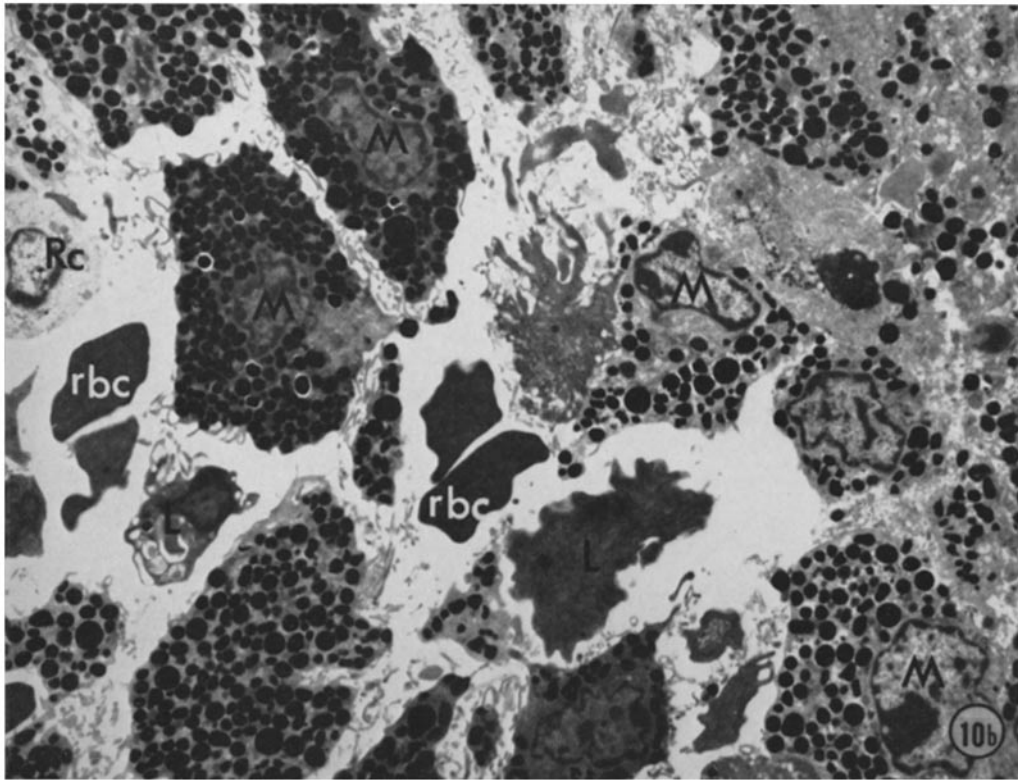
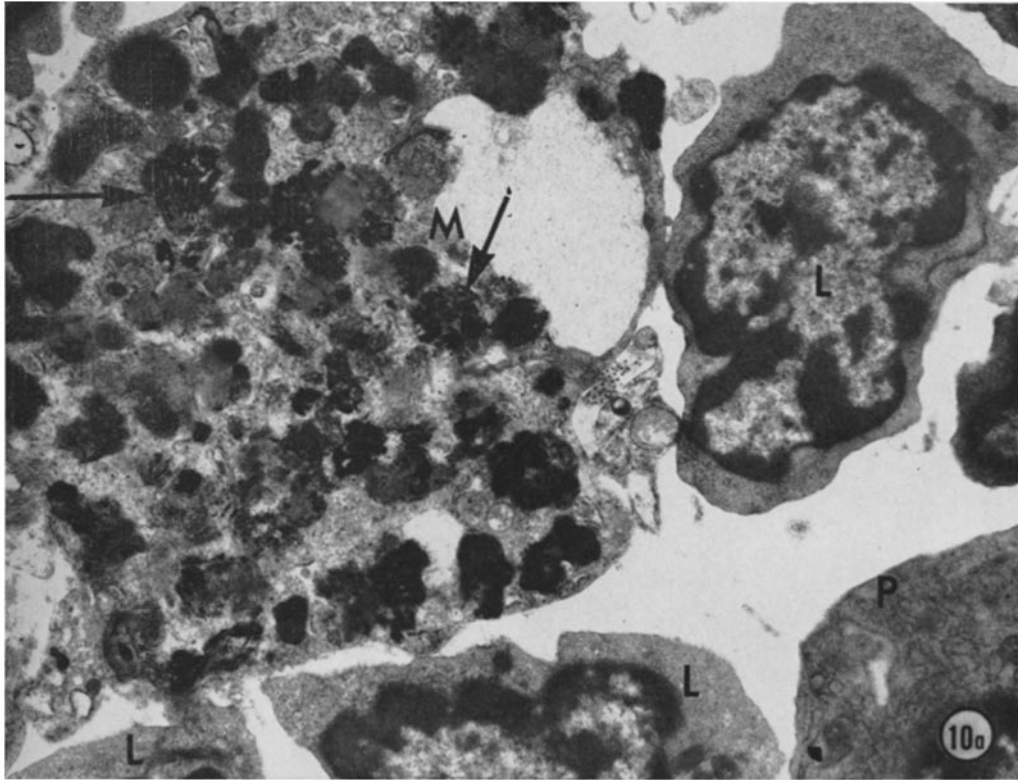
Despite a fastidious search through numerous specimens, no virus particles were seen.

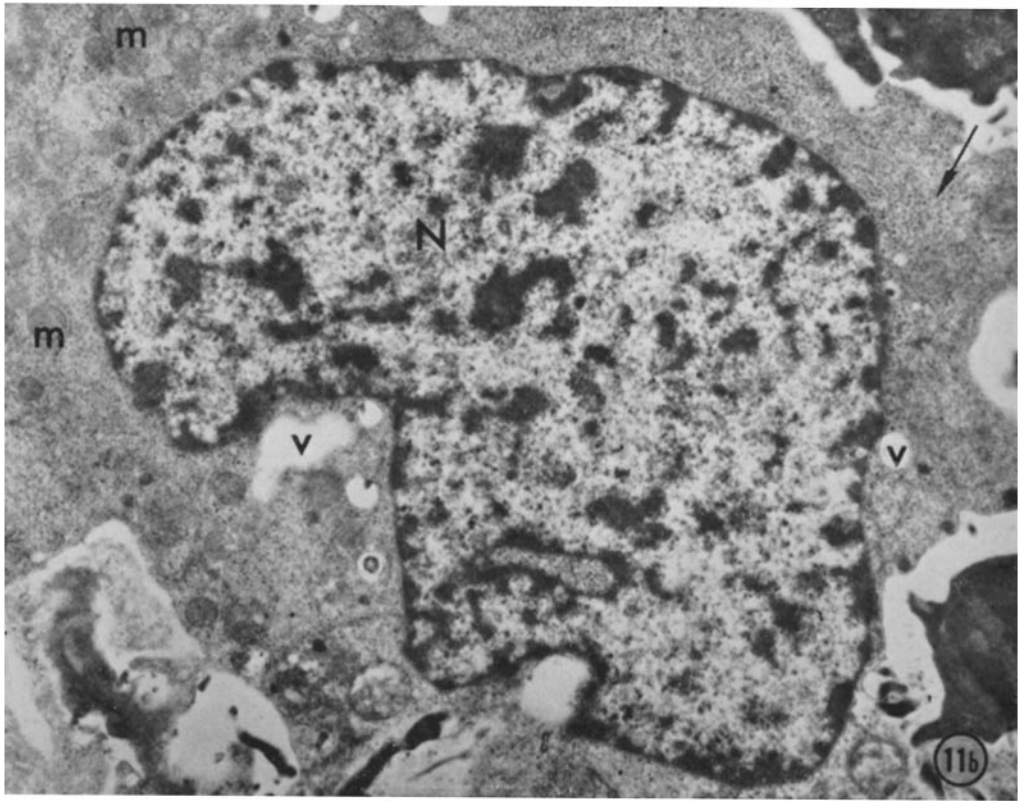
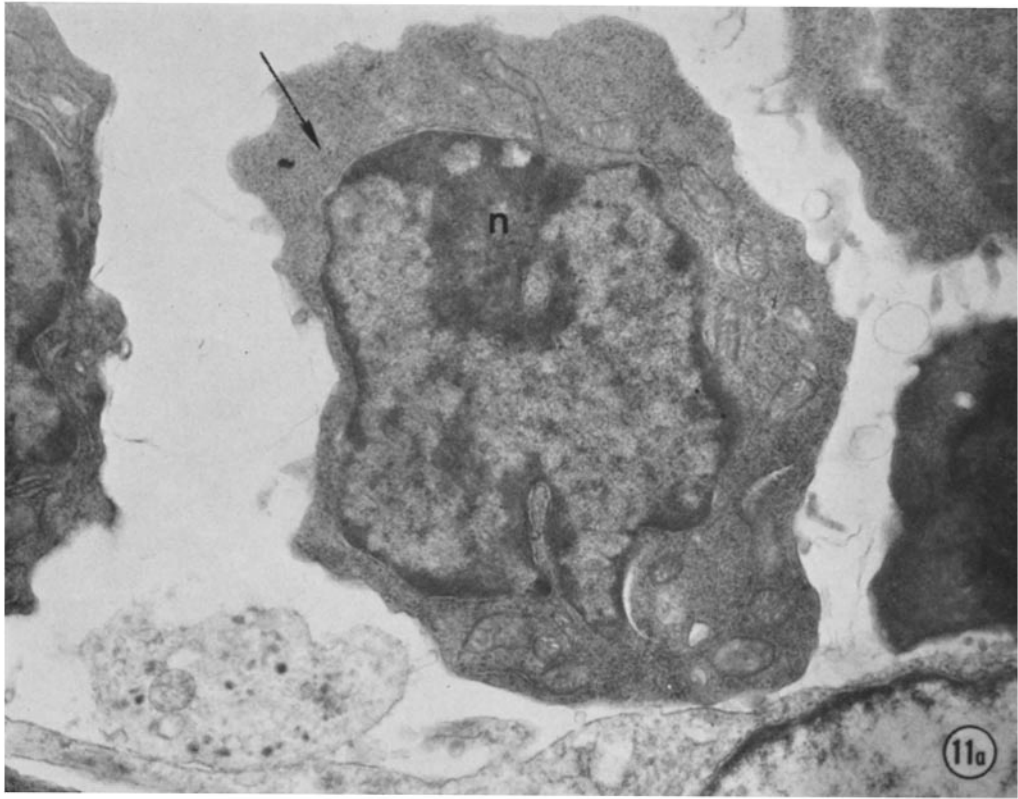
Transplantation of Tumors.—Cell suspensions of the spleens of 51 experimental mice were injected into litters of infant syngeneic mice. The donors were selected because, at the time of autopsy, their spleens were greatly enlarged. Subsequent histologic analysis of these transplanted tissues revealed the following diagnoses: hyperplasia in 17, prelymphoma in 5, malignant lymphoma in 29. Reticulum cell sarcomas developed in recipients of: 2 of the 17 hyperplastic spleens, 3 of the 5 spleens diagnosed as prelymphoma, and 19 of the 29 spleens containing malignant lymphoma. In the latter group, seven spleens diagnosed as unclassified lymphoma were transplanted and four of these took in the recipients; in each instance the neoplasm in the recipient had the morphology typical of a reticulum cell sarcoma, type B. Primary neoplasms were also injected simultaneously into syngeneic and either parental or allogeneic infants, or into both. In 11 instances the tumor grew in at least one kind of recipient. The results are shown in Table II. In the two cases where the primary tumor took in both CAF₁ and BALB/c animals, it also grew in allogeneic mice.

Cytotoxicity Tests.—The cells of eight tumors were tested for their ability to absorb antibody activity from alloantisera. Fig. 12 shows the results of the antiserum being directed against H-2.5, a specificity present in the A/J parent of CAF₁ and absent from its BALB/c parent. In each case, the tumor cells were capable of absorbing antibody activity from this serum. Specificity of the test is demonstrated in Fig. 13, which shows that the tumor cells failed to absorb antibody activity from an antiserum directed against H-2.2, a specificity found on C57B1/10Sn cells, but absent from CAF₁ cells. Live cells from five tumors were labeled with ⁵¹Cr and were reacted directly with alloantisera directed against specificities present on BALB/c cells, but not on A/J cells (H-2.31); or present on A/J cells, but not on BALB/c cells (H-2.1, H-2.5, H-2.11, H-2.23); or not present on either BALB/c or A/J cells (H-2.2). The results (Table III) show that the tumors contained the specificities contained in CAF₁ cells.

Assays for Chimerism.—The spleens of 52 experimental mice were analyzed for the presence of BALB/c cells by the spleen assay method of Simonsen. In

FIG. 10. Preneoplastic phase of chronic allogeneic disease. (*a*) A portion of the cytoplasm of a macrophage (*M*) is shown. Note the abundant debris and its fine, intricate pattern (arrows). A plasma cell (*P*) and several lymphocytes (*L*) are also seen. $\times 8000$. (*b*) A nest of mast cells (*M*), among which rare lymphocytes (*L*), reticular cells (*RC*), and red blood cells (*rbc*) are visible. $\times 3500$.





this technique, about 10×10^6 spleen cells of the suspected chimera are inoculated into 8-day-old syngeneic F_1 hybrids. 1 wk later the infant recipients are killed and their spleen and body weights compared with those of uninjected litter mates by means of a spleen index, which is the ratio of spleen to body weight. An index of 1.00 indicates no change in spleen weight; doubling of spleen weight yields an index of 2.00. If parental cells with anti- F_1 reactivity are present in the donor inoculum, splenomegaly occurs in the test mice. In the parent-hybrid combinations studied, the minimum spleen index of significance is 1.5. The 52 experimental CAF₁ mice had received BALB/c spleen cells at times

TABLE II
Transplantation of Tumors into Syngeneic, Parental, or Allogeneic Mice

Tumor No.	Growth in		
	CAF ₁	BALB/c	Allogeneic mice
C36-6	+*	0‡	ND§
C76-45	+	+	+
C18-40	+	+	+
C117-69	+	ND	+
C117-66	+	0	ND
C17-5	+	0	ND
CV17-69	+	0	0
C18-42	+	0	0
C18-13	+	ND	+
C125-42	+	0	ND
CV126-40	+	0	ND

* +, take.

‡ 0, no take.

§ ND, not done.

varying from 1 wk to 1 yr prior to the assay. In only one instance did the inoculation of spleen cells from the experimental mice produce a significant spleen index (Fig. 14).

The spleens of an additional 23 experimental mice were analyzed by means of the discriminant spleen assay. In this technique, spleen cells of the putative chimera are injected simultaneously into syngeneic and allogeneic F_1 hybrids; both test hybrids are semiallogeneic with respect to the parental donor of the original experimental animal. For example, the test hybrids used were (BALB/c

FIG. 11. Neoplastic phase of chronic allogeneic disease. (a) Lymphoblast: the large nucleolus (*n*) and the dense cytoplasm studded with ribosomes (arrow) are characteristic of this type of cell. $\times 7200$. (b) Reticular cell: the nucleus (*N*) is irregularly shaped and has a finely scattered chromatin, but the large nucleolus typical of this cell is not seen here. The cytoplasm is packed with ribosomes (arrows) and contains numerous mitochondria (*m*) and vacuoles (*v*). $\times 8000$.

\times A/J) F_1 and (BALB/c \times C57B1/6) F_1 , and the experimental animals, (BALB \times A/J) F_1 , had been injected with BALB/c spleen cells. 6 of 23 CAF $_1$ mice analyzed in this way had received the BALB/c spleen cells 24 hr previously. In each of the mice so studied a significant spleen index occurred in the (BALB/c \times C57B1/6) F_1 recipient, but not in the (BALB/c \times A/J) F_1 recipient (Fig. 15).

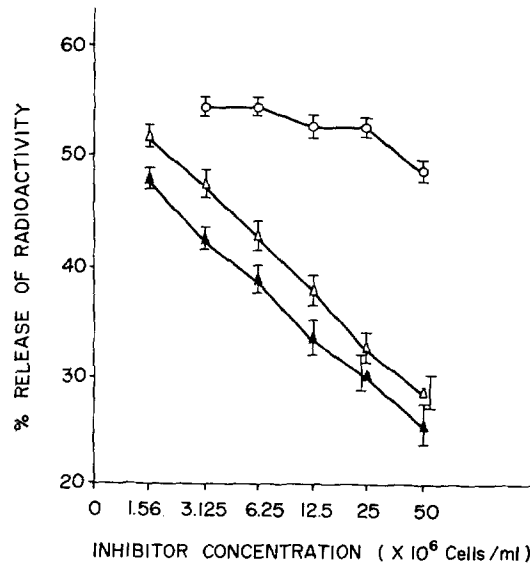


FIG. 12. Cytotoxicity assay based on release of ^{51}Cr from A/J target cells. The alloantiserum was directed against H-2.5, a specificity present on A/J cells, but not BALB/c cells. Absorption by normal CAF $_1$ lymphocytes is shown by the open triangles; absorption by the tumors is indicated by the closed triangles. Results obtained when BALB/c cells were used for absorption are shown by the open circles.

The results shown in Figs. 14 and 15 indicate that the donor (BALB/c) cells lost their ability to react against CAF $_1$ antigens within 24 hr of their inoculation. However, some BALB/c cells persisted in their CAF $_1$ hosts, and they were detectable in 23 out of 23 cases by their reactivity against (BALB/c \times C57B1/6) F_1 antigens (Fig. 15, right panel).

Since the lower limit of sensitivity of the spleen assay is between 5 and 10×10^6 cells, another method was tried in an attempt to detect BALB/c cells with antihost activity in the experimental mice. Adult CAF $_1$ mice were given 950 R total body x-irradiation and, within 6 hr, 70×10^6 spleen cells from CAF $_1$ mice that had been injected with 200×10^6 BALB/c spleen cells 1 month earlier. If BALB/c cells with anti-CAF $_1$ activity were present in the restoring

inoculum, a graft-*versus*-host reaction could occur in the irradiated test animal. The survival of these mice was compared with four other groups of lethally irradiated CAF₁ mice: (a) 12 mice given no other treatment; (b) 12 mice restored with 70×10^6 normal CAF₁ spleen cells; (c) 12 mice restored with a mixture of 60×10^6 CAF₁ spleen cells plus 10×10^6 BALB/c spleen cells; (d) 12 mice restored with a mixture of 60×10^6 CAF₁ spleen cells plus 10×10^6 spleen cells from BALB/c mice sensitized by two doses of 150×10^6 CAF₁

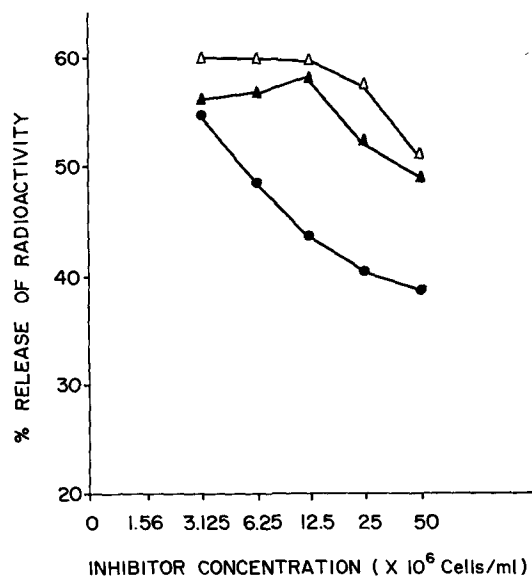


FIG. 13. Cytotoxicity assay employing an alloantiserum against H-2.2, a specificity present on C57B1/10Sn cells, but absent from CAF₁ cells. Absorption with C57B1/10Sn cells is shown by the closed circles. Cells from two CAF₁ tumors (open and closed triangles) failed to absorb antibody activity from this serum.

spleen cells. All mice of group (a) were dead within 10 days; all mice of group (b) survived longer than 3 months. Results of the remaining groups are shown in Fig. 16. The irradiated recipients of spleen cells from experimental CAF₁ mice developed typical secondary disease, and 75% of them were dead within a month. This result indicates that BALB/c cells with anti-CAF₁ activity were present in the experimental mice.

DISCUSSION

The incidence of lymphoid neoplasms in our group of 18-month-old BALB/c mice was 10.3%, which corresponds with Deringer's (14) results in 182 retired breeders of the subline BALB/cAnDe. Lymphomas occurred in CAF₁ mice

even less frequently. At the age of 24 months, 3 out of 65 (4.5%) of the animals had lymphomas, and the cumulative incidence in 324 mice studied over a 2 yr period was only 3%. By contrast, the incidence of lymphomas in CAF₁ mice injected with BALB/c spleen cells was much higher. For example, more than half the 18-month-old animals treated in this manner had lymphoid neoplasms.

These results confirm and extend our previous experiments in (C57B1/6 × DBA/2)F₁ mice injected with C57B1/6 spleen cells (5). Studies of others have also shown that the administration of allogeneic or semiallogeneic lymphoid cells results in a high incidence of neoplasms. Walford and Hildemann (15) injected newborn C3H mice with spleen cells from adult mice of a congenic

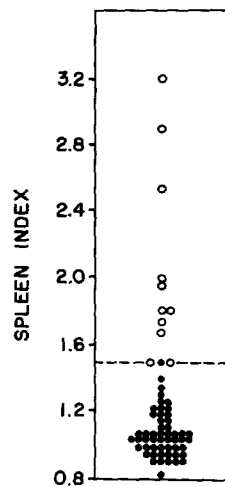


FIG. 14. Results of Simonsen assay, in which spleen cells from either BALB/c (open circles) or experimental CAF₁ (closed circles) were injected into newborn CAF₁ mice.

strain, C3H.K, differing from the recipient line only at the weak H-1 histocompatibility locus. Both the rate of appearance and the incidence of lymphomas were increased in the recipients. Walford (16) later extended these experiments by injecting either C3H (H-1^a) or C3H.K (H-1^b) spleen cells into either C3H or C3H.K newborn mice. A high incidence of lymphomas was found only in recipients of allogeneic spleen cells. Keast and Stanley (17), however, did not find an increased incidence of neoplasms in PH mice injected at birth with allogeneic spleen cells. Dawson, Cauchi, and Field (18) reported the development of neoplasms in F₁ hybrid rats given parental spleen cells; 17% of the animals developed tumors, most commonly fibrosarcomas. Unfortunately, they failed to mention the incidence of tumors in untreated animals of the same strain. A high proportion of semi-inbred Holtzman rats develop lymphosarcomas when joined in parabiosis (19). Of 242 pairs of rats examined

from 7-29 months after the parabiotic union, 44% had neoplasms. None of a large series of single, control rats had developed a tumor within that time. It is of considerable interest that when one of the parabionts was treated with an otherwise lethal dose of x-irradiation just prior to the anastomosis, neither the irradiated animals nor their partners developed lymphosarcomas (20). This suggests that an interaction among radiosensitive cells (lymphocytes?) of the allogeneic partners is required for development of the lymphomas.

As part of his extensive studies on the pathogenesis of leukemia in AKR mice, which uniformly develop a thymic lymphoma around the age of 1 yr, Metcalf (21) found that the incidence of tumors was increased in F₁ hybrid recip-

TABLE III
Cytotoxic Test Using CAF₁ Tumors as Target Cells

Source of Target Cells	C' plus normal mouse serum	C' plus alloantiserum vs. H-2 specificities					
		2	1	5	11	23	31
Normal BALB/c.....	24.4*	18.4	38.5	18.5	18.6	30.9	76.6
Normal CAF ₁	26.9	25.1	68.9	72.5	41.6	71.6	71.0
CAF ₁ Tumors							
CV109-4.....	33.4	28.0	69.4	64.2	49.2	67.3	71.7
CV109-5.....	29.7	26.9	68.1	63.2	45.7	68.3	73.6
CV117-90-1C†-1.....	30.7	28.2	69.6	70.5	48.0	70.2	70.1
CV117-69-2C†-1.....	25.5	21.2	67.7	72.1	46.8	71.0	72.2
CV117-69-2C†-2.....	26.5	25.1	68.9	70.8	44.4	72.6	70.4

* % release of total radioactivity.

† After serial passage in syngeneic recipients.

ipients of parental AKR spleen cells. Most of the neoplasms were derived from AKR cells, as judged by transplantation tests. When the results were analyzed in terms of the age of the injected cells, the incidence of leukemia in the recipients coincided with that in the donor line. Metcalf concluded that the AKR cells persisted in the F₁ and ultimately became neoplastic, just as they would have if left *in situ*. Our experimental model thus differs from his in two important ways: the donors we used have a low incidence of neoplasms, and the tumors we found originated in cells of the host.

Two lines of evidence demonstrate the origin of the neoplasms in CAF₁ mice injected with BALB/c spleen cells. In seven of the nine cases where it was possible to transplant tumor cells simultaneously into infant F₁ and BALB/c mice, only the F₁ mice accepted the tumor. In the two other instances the tumor grew in allogeneic as well as parental recipients, suggesting the loss of histocompatibility antigens. Perhaps more convincing are the results of the cytotoxicity assay. Every tumor examined by this method possessed H-2 antigens characteristic of the F₁ host.

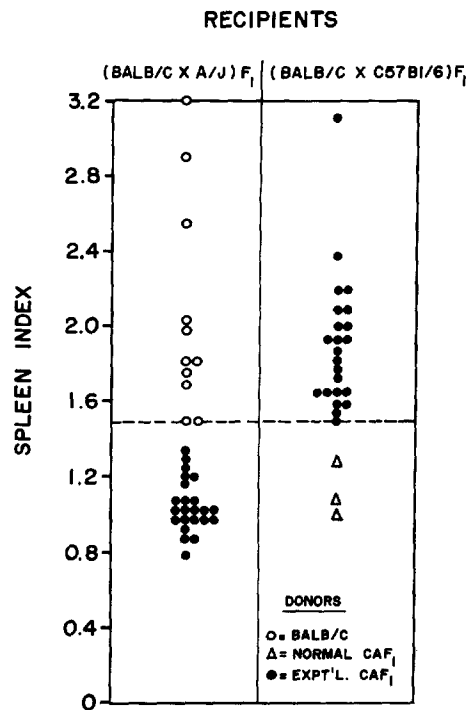


FIG. 15. Discriminant spleen assays using as donors normal BALB/c, normal CAF₁, or experimental CAF₁ mice. Spleen cells from the experimental mice failed to cause splenomegaly in syngeneic mice (lower left panel), but did so in (BALB/c × C57B1/6)F₁ recipients (upper right panel). Spleen cells from normal CAF₁ mice do not cause splenomegaly in (BALB/c × C57B1/6)F₁ animals, presumably because they are rejected.

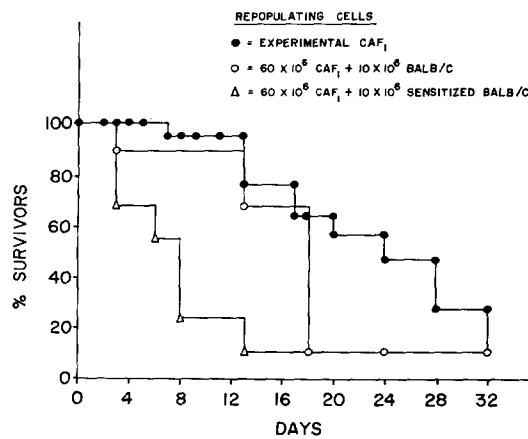


FIG. 16. Assay for chimerism using heavily irradiated, normal CAF₁ recipients. The dying mice developed characteristic signs of an acute graft-versus-host reaction.

How normal lymphoid cells provoke the development of lymphomas in their semiallogeneic hosts is unknown. Debilitating runt disease may be a contributing factor. It must be emphasized, however, that we have chosen experimental conditions that do not lead to the development of runt disease. For this reason, we believe that Keast's (22) experiments, in which he induced "runt-ing" with allogeneic spleen cells, cortisone, bacterial vaccines, or virus inoculations and failed to produce a high incidence of neoplasms, are irrelevant to our results. Atrophy of the thymus, which occurs in chronic allogeneic disease, could impair the recipient's resistance to neoplastic cells. However, destruction of the thymus was usually incomplete, the paracortical (thymus-dependent) (23) zone of the lymph nodes was not depleted of lymphocytes, and no correlation was found between thymus weight and the incidence of lymphoma.

The persistent splenomegaly in chronic allogeneic disease is most likely due to proliferation of host cells because the spleen assay results, which confirm an earlier report by Simonsen (24), indicate that the donor inoculum rapidly acquires tolerance to host antigens. However, when irradiated F_1 recipients were used in the bioassay (Fig. 13), parental cells with antihost activity could be detected. Their number was probably less than 10^7 , which is below the limits of detection of the spleen cell assay. Since 200×10^6 BALB/c cells were administered to the experimental animals, we estimate that about 95% of the parental inoculum loses anti-CAF₁ activity within 24 hr of its inoculation. The host's lymphoid reaction, which has also been observed in acute allogeneic disease (25), is an unexpected one. According to orthodox transplantation genetics, a hybrid-antiparent response cannot occur. However, certain phenomena, such as poor growth of parental line tumors in F_1 hosts (26) and the deficient growth of parental bone marrow in heavily irradiated F_1 animals (27), suggest the likelihood of such a response. Myburgh (28) has recently reported the development of anemia in both parabionts of a combination in which one member of the pair was an F_1 hybrid and the other a parental line mouse. He attributed this result to a hybrid-antiparent reaction. It is unlikely that the marked lymphoproliferative reaction we observed is due to this mechanism, which is too weak to cause rejection of parental skin grafts by F_1 hybrid recipients (29).

To what are these animals responding? From the morphology of their lymphoid tissue, the instigator should be a powerful and persistent antigen; this raises the question whether an antigenic stimulus can lead to neoplasia. Metcalf (30) injected C3H mice once a week for months with either *Salmonella adelaide* flagellar antigen, bovine serum albumin, or saline. Animals given saline injections had a 13% incidence of lymphomas; 19% of those given the *Salmonella* antigen had tumors; and 31% of the mice challenged repeatedly with bovine serum albumin had reticular neoplasms, many of which were plasma cell tumors. Balls and Ruben (31) have made a fascinating study in cold-blooded vertebrates of the induction of tumors by foreign tissues. They implanted

fragments of normal kidneys from European newts (*Triturus cristatus*) into the forelimbs of African clawed toads (*Xenopus laevis*). Half the recipients developed a lymphosarcoma at the site of the implant following its rejection. In other experiments they found that implants of allogeneic liver, spleen, and muscle, as well as xenogeneic kidney, had the same effect. Implants into the forelimb of an adenocarcinoma from *Rana pipiens* also induced lymphosarcoma in *Xenopus laevis*, but implantation of the adenocarcinoma into the anterior chamber of the eye failed to induce tumors. This indicates that the lymphosarcomas in the recipients were not due simply to transfer of a virus because there is no immunologic response to avascular intraocular transplants¹ (32). The development of neoplasms in recipients of human renal allografts is just now emerging (33). This has been attributed to immunosuppression, but the fact that the majority of these tumors arise in lymphoid tissue seems not entirely explainable in this way. Could these lymphomas be another demonstration of the consequences of a prolonged immune response?

The possibility that the tumors we observed are caused by a virus must be considered. Transmission of an infective virus from donor to host seems unlikely as the sole cause of the tumors, since frozen and thawed BALB/c cells were ineffective in producing disease. Furthermore, infective mouse leukemia virus can rarely be demonstrated in the tissues of BALB/c mice younger than 2 months of age.² Walford (16) also discounted this possibility because, in his work, augmented tumor development occurred only when lymphoid cells were transplanted across a histocompatibility barrier. It is conceivable that the parental graft injures host cells, thereby activating a latent virus. Continued proliferation of such a virus could provide the antigen responsible for the massive hyperplasia of lymphoid tissue. It may be unnecessary to postulate the presence of a conventional oncogenic virus; all that may be needed is a self-replicating antigen of sufficient immunogenicity to stimulate the host's lymphoid tissue. Thus far, no viruses have been seen by electron microscopy, and attempts to transmit the tumors by injecting cell-free filtrates of affected tissues into newborn mice have failed. But such negative results are inconclusive, and work along these lines is continuing.

The similarities between chronic allogeneic disease and the spontaneous disorder of New Zealand Black (NZB) mice and their hybrids is striking. In both cases, severe glomerulonephritis of the immune deposit variety develops (34), and in both instances there is a high incidence of malignant lymphomas (35). Chronic allogeneic disease is induced by immunocytes that are introduced into the animal with a needle and syringe. NZB disease occurs spontaneously, perhaps because of a unique genetic abnormality. In both chronic allogeneic

¹ In a forthcoming paper we will show that enclosure of immunocompetent parental cells in a cell-impermeable chamber does not provoke disease in F₁ recipients.

² Rowe, W. Personal communication.

disease and NZB disease, marked hyperplasia of lymphoid tissue precedes the development of lymphomas (36). Murine leukemia virus has been implicated in NZB disease (37), but the specificity of this finding is unclear since though almost all mice carry these agents, only NZB mice spontaneously develop autoimmune disease (38). A more anterior defect may exist in these animals, and we propose that, whatever its nature, it is shared by animals undergoing chronic allogeneic reactions.

Finally, immunocompetent cells with antiself activity must now be added to the list of factors that can trigger the development of a neoplasm. Whether or not analogous cells can arise spontaneously, by mutation or as the result of a viral or chemical action, remains to be demonstrated. Fortunately, new techniques are now available to test this hypothesis.

SUMMARY

The incidence of lymphomas in 371 CAF₁ mice injected with BALB/c spleen cells was compared with that in 324 control CAF₁ mice. A high incidence of lymphomas was found in the treated mice, but not in the control animals. The development of the neoplasms was a function of the number of parental cells administered. Analyses of the injected mice for the presence of donor cells indicated that the majority of the injected parental cells lost antihost activity, probably within 24 hr of their administration. The tumors were of host origin, as judged by transplantation tests and antigenic analysis of tumor cells.

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