B-Cell and T-Cell Lymphomas and Other Associated Diseases Induced by an Infectious DNA Viroid-like Agent in Hamsters (Mesocricetus auratus)

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Five epidemics of diffuse, poorly differentiated lymphocytic, immunoblastic, and plasmacytoid lymphoma induced by an infectious, horizontally transmitting viroidlike agent have occurred in two hamster facilities. Incidence summaries and pathologic characteristics of the lymphomas induced in LSH and LVG hamsters are presented. An elevated leukocyte count with a marked increase in neutrophils and a significant decrease in small mononuclear lymphocytes was detected in 5-week-old but not in 10- or 25-week-old LVG hamsters born in the facility contaminated with the lymphoma-inducing agent. Three-week-old LVG hamsters exposed to the contaminated facility showed no similar hematologic change at 5 weeks of age or 5 weeks of exposure. Several associated syndromes, including an intussusception disease, pyelonephritis, inflammatory bowel disease, and body warts associated with the presence of the causative viroidlike agent in the contaminated colonies are described. Details of the epidemiology of the disease, karyology, viral studies, and correlation with several epidemics in other laboratories are presented. (Am J Pathol 1983, 110:254–266)

HORIZONTALLY TRANSMITTING RETROVIRUSES are responsible for lymphomas in cats and cattle.[1,2] Several species of nonhuman primates develop lymphatic tumors following infection with *Herpesvirus saimiri*.[3] There is strong circumstantial evidence to support the claim that Burkitt's lymphoma may result from infection with Epstein–Barr herpesvirus.[4] With the exception of Burkitt's lymphoma, the search for oncogenic viruses in human lymphomas has failed to yield typical retroviruses or herpesviruses[5] so readily found in the lymphomas of animals. If oncogenic tumor viruses exist in man, they may be altered either structurally or in their reproductive cycle. Intuitively, many scientists still believe that some types of human lymphoma may eventually be linked to an infectious agent, because it is unlikely that the widspread production of lymphomas in subhuman primates and other vertebrates by oncogenic viruses would uniquely exclude *Homo sapiens*. Additionally, a number of human (Kuru, Jakob–Creutzfeldt, progressive multifocal leukoencephalopathy) and animal diseases (scrapie) appear to have viroid or prionlike infectious causes, and the pathogenesis of all of these is poorly understood.

An interesting animal model for spontaneous lymphoma induction by an atypical viroidlike agent that can be transmitted horizontally now exists. In 1975 we reported an unusual lymphoma with an apparent infectious cause in Syrian golden hamsters held in one of our animal facilities.[5] More than half of 1-day-old to 3-week-old hamsters placed in the facility developed lymphoma within 4–30 weeks of expo-

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sure. Animal-to-animal transmission of an agent seemed likely. It was extremely difficult to obtain the agent cell-free when test preparations were injected into newborn or adult hamsters.[6-9] The pathogenesis of the disease and the biologic source of infection were not understood. Several outbreaks of the disease have occurred recently in established hamster colonies.

Our studies over the past 5 years[6-9] have established that an infectious agent is involved, that immunologically mediated resistance to lymphoma (not the causative agent) developed among most of the older exposed, lymphoma-free hamsters that survived, that the agent did not form detectable, mature virions in lymphoma or normal tissues and was difficult to sediment, vulnerable to DNAse inactivation, resistant to protease and RNAse inactivation, UV-resistant, environmentally stable, and not reproducibly susceptible to antibody neutralization. The details of the transmission of the lymphoma by injection of protamine sulfate stabilized agent were outlined previously.[19] Based on these findings, a presumed viroidlike agent was suspected.[9] Viroids were initially defined by Diener as very small ($<10^5$ daltons) infectious, naturally transmitting RNA structures, free of capsid and envelope, which produce plant infections as intracellular parasites.[10] Recent projections extend this definition to infectious DNA capable of disease production in metazoans.[10,11]

The pathogenesis and histologic characterization of this unusual lymphoma disease of hamsters is of interest because of the peculiar infectious, viroid nature of the agent involved, the recognition that it has now occurred in other facilities, and the morphologic similarity of the tumors to human lymphoma. The lymphomas described here provide for an excellent animal model system for the study of lymphomas with a potential viroid cause and may provide the basis for a search for similar agents in human diseases.

Materials and Methods

Diseased and normal tissues have been harvested from the hamster colony for gross, histopathologic, and detailed electron-microscopic evaluation over a 5-year period, involving 5 distinct epizootics of lymphoma. In excess of 4800 different tumors have been examined from more than 13,000 hamsters under study. Research involving SV40 and adenoviruses 7 and 31 was ongoing in our facilities during the first four epizootics.

The chronology of the lymphoma epizootics is outlined in Table 1. The LVG/LAK (random bred) and LSH/LAK (inbred) strains of Syrian golden hamster (Charles River, Newfield, NJ) were used throughout. Hamsters of these strains at varying ages, from newborns delivered in the contaminated areas to 3-4-week-old weanlings born elsewhere, were placed into rooms in special containment trailers or in one facility where lymphoma-inducing agent transmission was active. Exposed hamsters were carefully examined on a regular basis for evidence of lymphoma or other diseases. Newborns were weaned at 21 days. Necropsies were routinely performed on all hamster remains discovered in daily cage checks. Hamsters are cannabilistic, and therefore necropsy was not always possible. At necropsy, tumors as well as major organs

Table 1—Chronology of a Hamster Epizootic Horizontally Transmissible Lymphoma

November 1972	First noted that a number of hamsters in a breeding colony had unusual tumors (Lakeview Hamstery stock, Newfield, NJ). LSH inbreds and LVG randombred hamsters involved. Tumor originally diagnosed as lymphosarcoma. No similar tumor ever seen in our animals, even among aged stock.
December 1972	Observed that lymphoma would transmit to normal hamsters brought into the area but not housed with diseased animals. Controls housed elsewhere were disease-free.
January 1973	Tumor of the intestine and liver reclassified as B-cell lymphoma—Dr. R. J. Lukes.
March 1973	New colony initiated after exhaustive sanitization (UV, disinfectants, and destruction of all hamsters).
April 1973	New hamster stock purchased from Lakeview Hamstery.
August 1973	New lymphomas appeared among fresh stock—control hamsters at another site are disease free.
February 1974	Sacrificed all animals—exhaustive cleaning (UV, virucidal gases), all hamster stock killed. New animal personnel employed, no hamster hosts for >7 weeks.
March 1974	New animal stock from Lakeview hamstery; disease reoccurred with high frequency within 4 months. Split stock. Control animals held at another facility were lymphoma-free for >2 years.
June 1976	Evidence of spread of lymphoma to new containment facility, which was established at another site in August 1976.
May 1977	Disease highly active. Thirty-one days from room exposure as newborn to first tumor. 100% incidence possible in LSH; 60-70% among LVG hamsters.
December 1977	Colony moved to another site in containment trailers and relocated in high containment facility. Disease is active there with 50-90% lymphoma incidence among facility-exposed newborn hamsters.

showing any overt diseased condition were prepared for histologic examination. All samples were coded and fixed in acetic acid–formalin–alcohol buffer for sectioning and staining. We evaluated each suspected tumor microscopically without knowledge of the specific source of the tissue to avoid the possibility of bias. Diagnoses were decoded and were compared with the gross necropsy report. Records were kept of any signs noted prior to death. The most common physical signs in lymphomatous hamsters were emaciation, weakness, lethargy, diarrhea, rectal and abdominal bleeding, and subcutaneous masses.

Selected lymphomas from lymph nodes, liver, and kidney were harvested, trypsinized, standardized to 5 $\times$ 10^6 viable, trypan-blue-excluded lymphoma cells per multiliter, and injected subcutaneously into syngeneic hamsters for testing for oncogenicity throughout the course of study.

The tissue for transmission electron microscopic examination was fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer, postfixed in osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon. Thin sections were cut with an LKB Ultrotome, stained with uranyl-acetate and lead citrate, and viewed in a Philips 301 transmission microscope.

Results

Neoplasm Incidence and Gross Appearance of the Lymphomas

The incidence of lymphomas during four epizootics in normal, nonexperimental hamsters and hamsters on test in various experimental series is shown in Table 2. A preponderance of intraperitoneal and liver lymphomas were detected. Thymic lymphomas constituted between 3% and 7% of all lymphomas observed over a 4-year period but were not noted in the first two epizootics. The average incidence of spontaneous lymphoma among newborn LSH or LVG hamsters was 53% in 11 independent determinations, each involving 50–75 test hamsters following natural exposure to the agent in room areas where lymphoma disease was active. The variation in incidence of lymphoma among groups of newborn hamsters of either strain placed in a containment trailer facility from 1977 to 1981 ranged from 28% and 96% total tumors in these trials. An early age of exposure (< 24 hours old) and the presence of at least 20–50% lymphoma incidence in previously exposed hamster groups in an animal room correlated directly with the highest prevalence of lymphoma detected in new exposure groups. Modest aerosol and/or ingestion transmission could be demonstrated with dried cage litter containing urine and feces. Transmission by direct handling with contaminated gloves of the animal caretaker may also be possible.

Table 2—Lymphomas in Hamsters in the Infected Colonies Between 1975 and 1979 in Two Strains of Hamster

Site of lymphoma	Number of total lymphomas detected	
	LVG/LAK	LSH/LAK
Intestinal or intraperitoneal	1208 (46%)*	647 (54%)
Liver	848 (33%)	282 (24%)
Kidney	154 (6%)	129 (11%)
Thymus	81 (3%)	92 (7%)
Cervical	102 (4%)	13 (1%)
Perirenal	123 (5%)	8 (<1%)
Stomach	66 (2%)	4 (<1%)
Eye	13 (<1%)	1 (<1%)
Inguinal	3 (<1%)	2 (1%)
Total lymphoma	2571	1178

* Percentage of total lymphomas observed at all sites.

With the exception of hepatic lymphomas, lymphoma cell injection rapidly produced subcutaneous tumors with histologic characteristics identical to those of tumors of young syngeneic LVG or LSH hamsters given injections of the cells. Liver lymphoma transplants produced subcutaneous nodules, but these usually regressed. Otherwise, failure of tumor establishment by transplantation seemed related directly to necrosis and poor viability of the tumor source material. To date, in spite of a continuing major effort to culture the various lymphoma tissues *in vitro* by the use of a great variety of techniques for culturing lymphocytic tumors, we have been totally unable to maintain the lymphoma cells in culture.

Hamsters with advanced lymphomas were distinguished readily from healthy animals by physical signs (enlarged abdomens, an emaciated head and shoulder area, and severe dehydration). The lymphomas arose at multiple sites in either localized form or in disseminated form. Periodically, more than 25% of new hamsters in a shipment from the supplier became ill with enteritis and died within the first 5 weeks after being housed with hamsters carrying the lymphoma-inducing agent. This was a key sign in several primary outbreaks in adjacent holding rooms or in facilities frequented by animal caretakers from contaminated facilities.

Hamsters with thymomas were thinner than their cage mates, and a characteristic dyspnea and rounded chest developed that made the diagnosis obvious before the animal underwent necropsy. These

animals generally died within 48 hours of detection of clinical signs.

On gross examination, lymphomas were soft, round, yellow-white masses, often with central necrosis. Cervical, axillary, and inguinal lymphomas were generally localized tumors. Visible metastases from nodal tumors were rare in the abdomen, although the spleen was frequently enlarged. Liver lymphomas were seen in the dorsocaudal lobes and frequently were associated with metastasis to the kidneys and the formation of hemorrhagic ascites. The majority of ascites cells were presumed nonviable because they stained readily with trypan blue dye. Attempts to disperse nodal tumors and establish ascites tumor lines *in vivo* following syngeneic passage uniformly failed. Intestinal lymphomas ranged from hyperproliferation of a single Peyer's patch to multiple nodules along the small intestine, to a single large mass involving intestine, liver, kidney, and connective tissue. Lymphomas involving several abdominal organs were referred to collectively as "intraperitoneal" lymphoma. Those tumors which were localized in a single organ were categorized under that organ: eg, hepatic, thymic, intestinal. Those tumors that appeared grossly to involve only two organs unequally (usually liver and kidney) were classified under the category of the organ that had the largest tumor mass.

Small Intestine

Two patterns were noted in the gut wall. Infiltration from the external surface representing the primary origin in a mesenteric lymph node was common. An intramural origin within the gut wall was also noted arising within the Peyer's patches of the small intestine. These tumors were frequently multiple, growing to a large size by the earliest time of detection and producing firm, rounded masses on the serosal surface of the intestine.

Lymph Node

Anaplasia was observed in a large number of lymph nodes during each epizootic. The most commonly detected nodes involved were the mesenteric, cervical, inguinal, and axillary nodes. When neoplasia was present, total obliteration of the normal nodal architecture occurred, with replacement by neoplastic lymphoid cells (see Figure 1). Tumor cells penetrated the capsule, with infiltration into surrounding loose connective tissue and eventual extension into adjacent organs. Even when lymphoma was not present, hyperplasia of the node was a frequent finding.

Liver

The liver was either the initial site of lymphoma development or, in disseminated disease, was only secondarily involved. When early liver lymphoma was detected, in the absence of disease elsewhere, multifocal infiltrates of lymphoma cells were confined to the portal triads, spreading then to the parenchymal areas. In later stages, massive infiltration of the entire liver was noted, with tumor expansion through the hepatic capsules.

Microscopic Features

The microscopic appearance of the hamster lymphomas presented somewhat variable morphologic characteristics (Figure 1). Many tumors were characterized by a monotonous proliferation of immature lymphoid cells. The nuclei were generally round to oval in shape, and occasionally cells with cleaved nuclei were identified. Occasional tumors demonstrated a predominance of the cleaved nuclear forms. Nucleoli were generally inconspicuous, and mitotic figures were variable in number (rare to frequent). The cytoplasm was scanty in amount. The lymphomas generally contained conspicuous amounts of karyorrhectic debris, and some tingible bodies could be localized within histiocytic cells. The histiocytes and associated karyorrhectic debris occasionally produced a "starry sky"morphologic appearance. No lymphomas demonstrated nodular morphologic features or preservation of the follicular architecture.

Occasional lymphomas demonstrated moderate pleomorphism within the neoplastic lymphoid cells. The nuclei were fascicular, and some had multiple nucleoli. The nuclear membrane was irregular in contour and the cytoplasm of the lymphoid cells was relatively increased. Occasional plasma cells and mature lymphoid cells could be identified within these lymphomas. These lesions were thought to represent immunoblastic sarcomas. Some lymphomas were composed almost exclusively of plasma cells and plasmacytoid cells of varying degrees of differentiation. These lesions were best classified as *plasmacytomas*. Most lymphomas, regardless of the histologic characteristics, arose in the large bowel, small bowel, and adjacent lymph nodes. The liver also demonstrated a high incidence of involvement with lymphoma. Other organs and anatomic sites were less frequently involved (see Table 2).

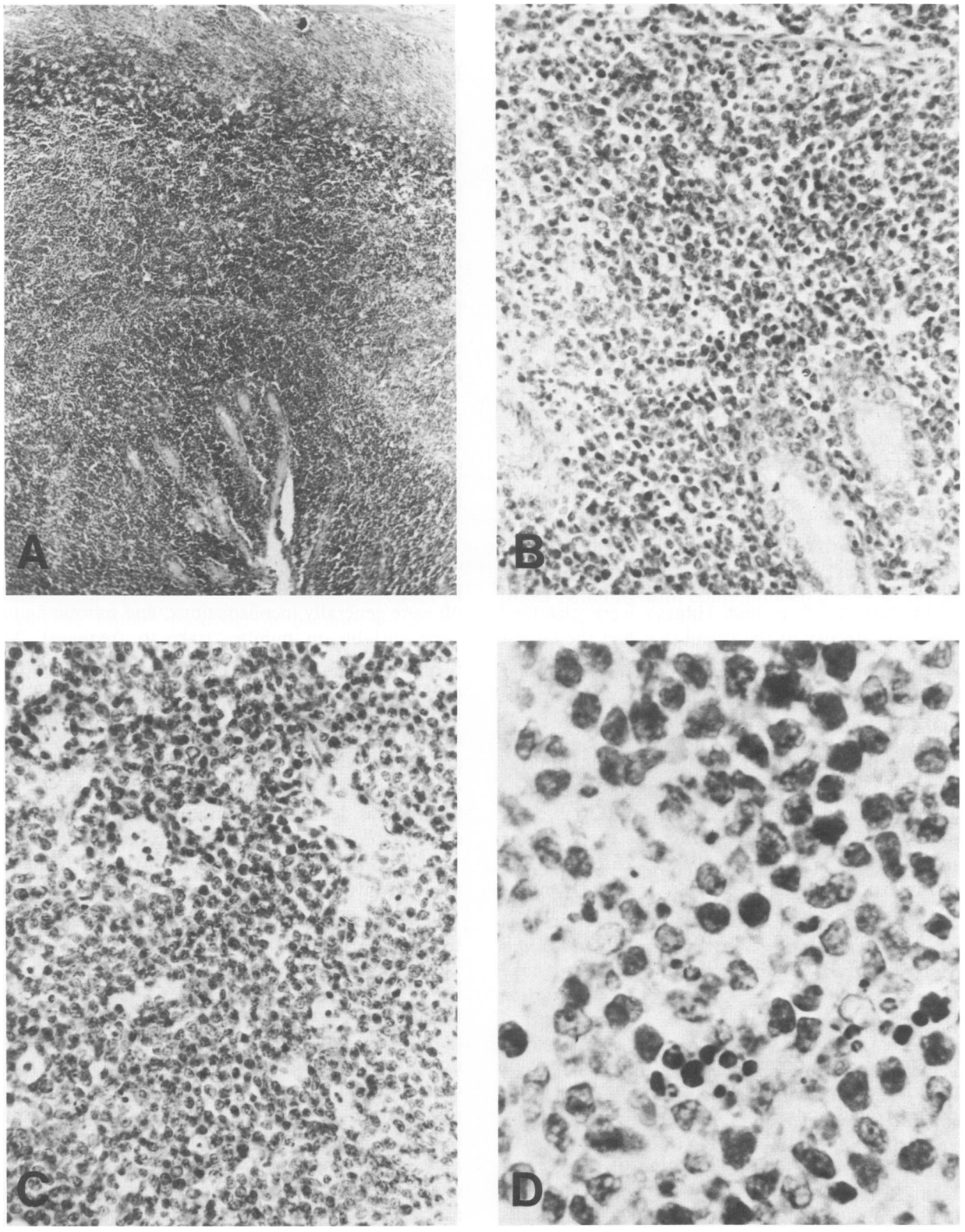

Figure 1A — Intestinal lymphoma with involvement of mucosa, lamina propria and muscularis. (H&E, × 40) **B** — Intestinal lymphoma involving mucosa. (H&E, × 200) **C** — Intestinal lymphoma with macrophages and tingible bodies producing a "starry sky" pattern. (H&E, × 200) **D** — Intestinal lymphoma with karyorrhectic debris. (H&E, × 1000) (With a photographic reduction of 9%)

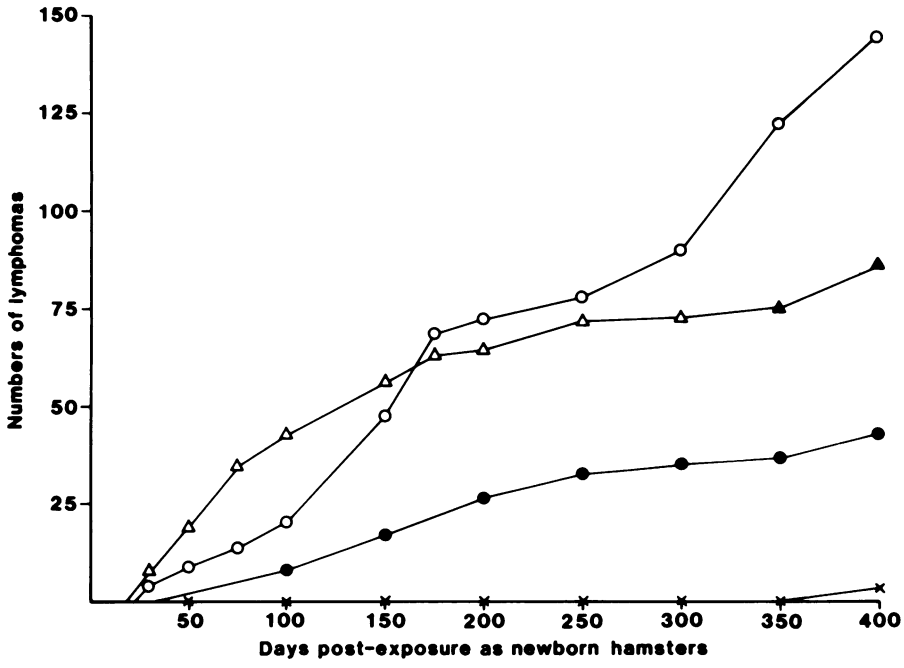

Figure 2—Appearance of lymphomas in LVG hamsters at various sites following exposure of neonates to agent-contaminated facility. O, Intestinal bowel associated lymphomas. ▲, Liver-associated lymphomas. ●, All other combined sites. ×, Spontaneous lymphoma occurring in normal LVG hamsters maintained outside the lymphoma facility.

Chromosome studies conducted on 12 T- and B-cell lymphomas from males and females that developed among room-exposed neonates revealed a modal chromosome number of 43 (normal hamster, 44) with a range of 41 to 51 chromosomes. No definitive chromosome abnormality was noted in 28 different spontaneous tumors examined.

Time Course of Lymphoma Appearance

The typical time course of lymphoma appearance of intestinal, hepatic lymphomas and neoplasms of other body sites following exposure of the neonate LVG or LSH hamsters to agent in the contaminated area is given in Figure 2. Notice that in normal hamsters housed in agent-free control facilities there occurred only one lymphoma (cervical), in a 29-month-old hamster among 1280 hamsters observed for 1 year. This finding contrasts with the detection of 262 lymphomas confirmed by gross and histopathologic examination among 773 hamsters housed in one agent-contaminated area during the same period.

Electron Microscopy

Typical micrographs of an intestinal, hepatic, and thymic lymphoma are shown in Figure 3. Electron microscopy of the hamster lymphomas demonstrated a spectrum of lymphocytic differentiation that included well-differentiated lymphocytes, poorly differentiated lymphocytes, immunoblasts, and plasma cells. The lymphomas were frequently composed of mixtures of closely related lymphocytic forms. Extreme ranges in differentiation were not present.

In 5 years of studies involving three research groups (Oak Ridge National Laboratories, Frederick Cancer Center, University of South Alabama) we have examined several thousand sections from hundreds of spontaneous and hamster-passaged lymphomas and have consistently failed to observe the reliable appearance of mature virions or viruslike particles in any of the lymphomas. Only rarely could budding Type C particles be demonstrated, and these are also detected in supplier hamsters never exposed to the facility. Acanthosomes, spokelike spiney structures, were initially confused with virus-like particles. These cellular structures were identified in lymphatic tissue of nondiseased hamster as well as in animals with lymphoma.

Other Lesions Associated With Epizootic Lymphoma

Several other specific pathologic conditions prevailed in the diseased colonies over a 6-year period. These included a nonbacterial enteritis, intussusception, pyelonephritis, hepatic disorders of a mixed variety, and warts caused by a papillomavirus. A brief description of each is included because these conditions have not been adequately described in our previous reports.[6-9] Any or all of these associated symptoms are characteristic of an epizootic and have

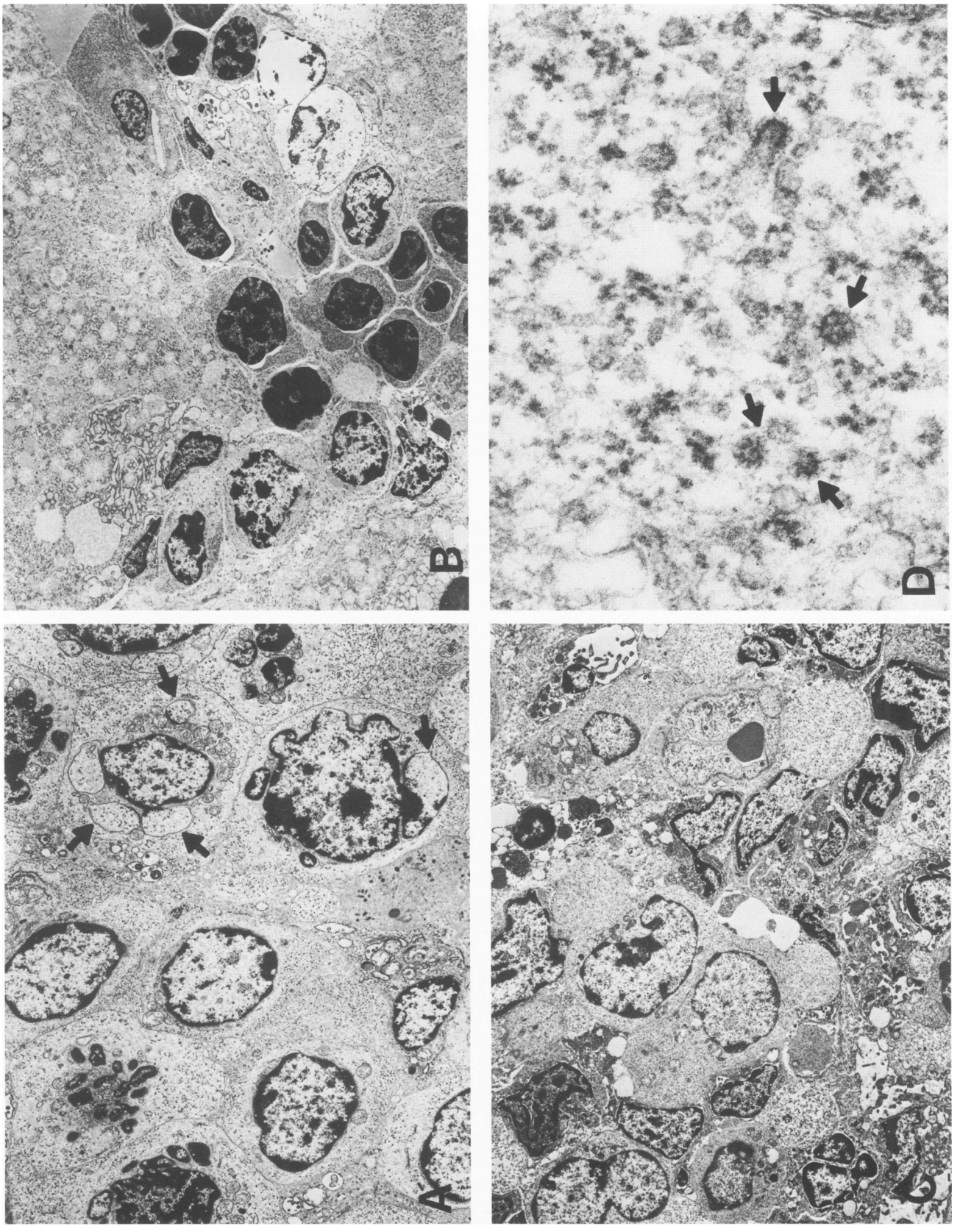

Figure 3 – Electron micrographs of lymphoma cells from various tumor sites. A – Intestinal lymphoma cells showing typical blebbing of the nuclei and parts of the peripheral heterochromatin absent. (×3000) B – Hepatic lymphoma. Parenchyma infiltrated by tumor cells. (×3000) C – Thymoma. Overview of the pattern of tumor cell arrangement. (×3500) D – Acantasome structure in an intestinal lymphoma. (×100,000) (With a photographic reduction of 10%)

heralded a similar outbreak at other major hamster facilities.

Enteritis and Intussuceptions

Enteritis outbreaks have preceded or occurred along with the appearance of lymphomas in the agent-contaminated facility. Two distinct types have occurred, which vary clinically and morphologically. One type was a nonhemorrhagic enteritis characterized by sudden onset, fetid diarrhea with yellow, mucus-filled intestine resembling "wet tail."[17] Peyer's patches were very prominent, with red halos and yellow–white centers. Occasional ileal intussusceptions and gastric ulcers were associated with this form of enteritis. This occurred in 5–25% of young hamsters housed in the lymphoma colony over four distinct epizootics. When death occurred, these young hamsters (< 10–15 weeks of age) did not have histopathologic evidence of active lymphoma.

Another, more severe form of enteritis involved significant internal hemorrhage with massive intussusceptions. These pathologic changes did not occur in the "wet tail" condition described above, and the two types of enteritis did not occur simultaneously in a given holding room. The hamsters with intussusceptions huddled with the onset of disease, were extremely irritable and dehydrated, and had bloody mouths and perianal areas. Blood was found in the gastrointestinal tract, including the esophagus and the mouth. The cecum was a purple color and firm because of extensive intussusception of the ileum and jejunum or was completely flaccid and blood-filled. Intussusceptions occurred in the descending colon and rectum. Again, Peyer's patches were swollen with reddish halos and whitish centers. Other major organs showed evidence of blood loss. The liver was variegated (yellow/red), the spleen discolored (pale red), and the kidneys an unusual dark brown. The uninvolved ileum was not thickened, and no gastric ulcers were detected. The only microorganism that could be cultured from the intestine of these hamsters was *Escherichia coli*. The condition afflicted primarily young hamsters within 45 days of exposure to the facility. When ocasional bloody diarrhea occurred in older hamsters, no intussusceptions occurred.

Renal Lesions

Renal disease accounted for only 1–2% of nontumor-related deaths among animals less than 1 year of age; however, pyelonephritis was observed in the majority of animals on which necropsy was performed in the lymphomatous colony. Pyelonephritis ranged from subacute to acute to chronic and was a common finding among most hamsters with evidence of dehydration but free of detectable lymphoma. Microscopically, necrosis of tubules and fibrosis with inflammatory involvement of the parenchyma were observed. Glomeruli were uninvolved except in cases where extensive scarring caused severe distortion of the renal cortex. It appeared often in hamsters less than 40–60 days of age in the lymphoma facility and was not observed in hamsters in our normal colony in age-matched controls. In a typical sample of 130 kidneys obtained at necropsy from sick hamsters that had no gross evidence of lymphoma, 98 had some degree of pyelonephritis and only 7 were found to contain actual lymphoma by histologic examination. When cellular infiltrates occurred, they varied from a few foci of small lymphocytes, plasma cells, and neutrophils to a dense, cellular infiltrate exhibiting pleomorphism of cell types and frank lymphoma. Lymphoma was not detected histologically in kidneys with chronic pyelonephritis.

Agent-exposed hamsters with pyelonephritis had abdominal organs smaller and more pale than age-matched, normal controls. The diseased kidneys often had pitting due to scarification, which ranged from one to two cortical indentations to complete destruction of the kidneys, leaving them as small, yellow, firm masses with complete fibrous replacement of renal structure. Severe renal disease was associated with a very mild enteritis (distinct from the severe enteritis previously described), characterized by intestinal and cecal hyperemia without heavy mucus content. The spleens of hamsters with pyelonephritis were small, pale, and fibrotic.

Liver

Primary hepatic lymphoma accounted for one-third of the lesions in one series. Cholangitic disease of a nonlymphoma type accounted for another 45–50% with benign cysts, other tumors (benign hemangiomas) and cirrhosis being responsible for the remainder. Liver lymphomas were distinctive lesions at necropsy, appearing as whitish foci with the hepatic parenchyma. Focal infiltration of normal-appearing lymphocytes into the portal triads and stasis of bile were considered suggestive of cholangitis rather than lymphoma, the two being readily distinguishable on histologic examination. Since liver was not routinely evaluated microscopically and only when gross lesions were observed, accurate statements about the extensiveness or the magnitude of liver disorders cannot be given.

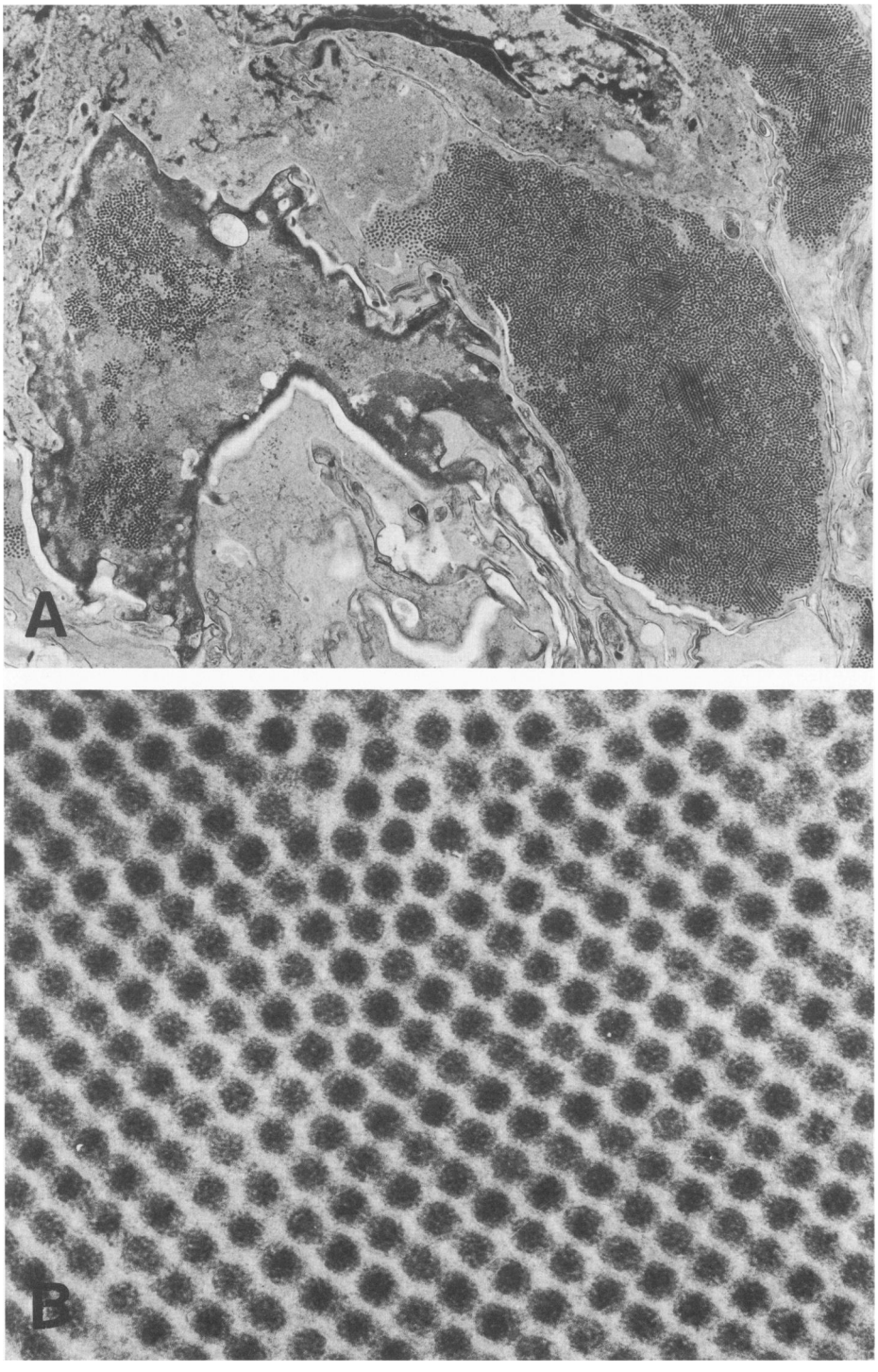

Figure 4—Hamster papilloma virus in warts. A—Intranuclear virus particles and a large inclusion with crystalline arrangements of hamster papilloma virus. (×13,650) B—Magnification of crystalline virus in wart cells. (×191,000)

Cutaneous Lesions

Wartlike lesions described as papillomas, fibromesothelial nodules, trichoepitheliomas, verrucae, and pilosebaceous cysts were observed with 5–10% frequency. Grossly, these lesions were either pigmented (melanotic) or clear, wartlike, raised structures about the eyes, mouth or perianal areas primarily. During early epizootics, warts were not noted but may have occurred. Microscopically, these warts were proliferations of the pilosebaceous apparatus and were most consistently described as fibromesothelial nodules. Electron micrographs of these wartlike structures revealed crystalline arrays of a

papilloma virus (Figure 4) which originated in the nucleus and were later "pocketed" in intracellular spaces. The papilloma virus could be readily extracted from a spontaneous wart in saline by grinding in a chilled mortar and pestle. A filterable papilloma virus was obtained which produced warts in LVG and LSH hamsters (< 50% transmission rate) when scratched into abraded skin or when injected intradermally. Among more than 200 hamsters given injections of or abraded with the papilloma virus extracted from warts, none ever developed lymphoma when housed outside the contaminated facility.

B-Cell and T-Cell Lymphomas

Most intraperitoneal lymphomas tested carried surface immunoglobulin (SIg) and failed to produce rosettes with sheep red blood cells, as we have reported hamster T cells to be capable of doing.[12] These lymphomas were not susceptible to cytotoxic antiserum[12] prepared against hamster brain in rabbits, whereas this serum would kill greater than 90% thymus cells. Lymphomas appearing as primary tumors in thymus were found to bear no SIg detectable by immunofluorescence with the use of anti-IgM or anti-IgG antibody; but they rosetted significantly with sheep red blood cells, as we have observed normal hamster thymocytes (T cells) to do.[12] These thymic lymphoma cells were destroyed by anti-T-cell serum prepared in rabbits.

Hematology

Total white and erythrocyte counts as well as differential counts were performed with EDTA-treated cardiac blood collected from 10 randomly sampled hamsters residing in the lymphoma facility born to mothers from large groups exposed to the lymphoma facility at 10 days of pregnancy. Similar samples were collected from control hamster groups born in adjacent, lymphoma-free "normal" areas served by a common corridor or in another disease-free animal facility (Medical Science Building, MSB) several blocks away. Camco Quik stain or Wright's stain (Cambridge Chemical Products, Inc., Lauderdale, Fla) were used. The results, which were confirmed in a repeat trial started 6 months later, are shown in Figure 5. Total leukocyte counts were consistently higher ($P < 0.05$ to 0.01) in hamsters housed within the lymphoma facility area (active lymphoma colony *per se* or "normal" colony lymphoma facility).

In a repeat trial 6 months later (October 28, 1981), the mean value for the total leukocyte count for the lymphoma area was somewhat lower than that detected in the first trial but within the standard error of the mean for that trial. Surprisingly, a vastly

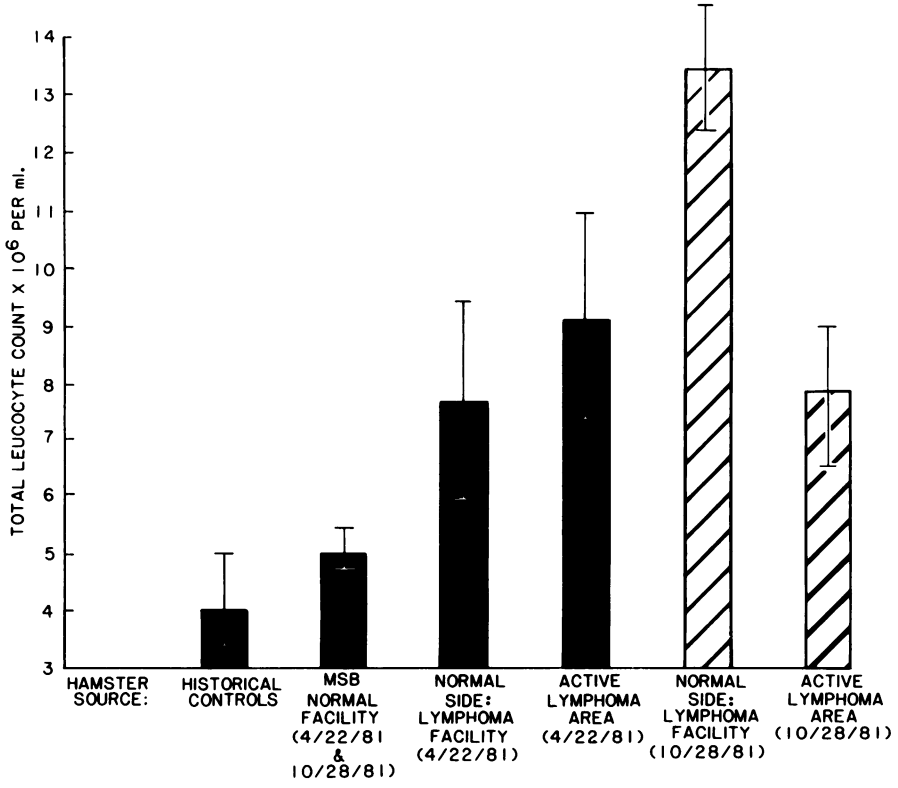

Figure 5—Total leukocyte counts for hamsters housed in lymphoma area or Medical Science Building (MSB) "normal" facility.

higher leukocyte count ($P < 0.001$) was detected for apparently nondiseased 5-week-old hamsters born in the "normal" area of the lymphoma facility. After 10 weeks, hamster breeding stock in this "normal" colony of hamsters adjacent to the active lymphoma epizootic area on a common corridor showed a significant outbreak of lymphoma. Hamsters in this "normal" area have since experienced a high incidence of lymphoma, and contamination of the area by the agent has been established. Importantly, no significant differences in total leukocyte count were noted at 10 or 25 weeks after exposure in any groups. Differential analysis confirmed that hamsters showing elevated leukocyte counts 5 weeks after exposure in the lymphoma facility had only 30.5% (active lymphoma area) or 48.3% ("normal" area) lymphocytes, whereas nonexposed hamsters in another building showed the usual 57% lymphocyte count typical of well hamsters. In contrast, neutrophils were significantly elevated ($P < 0.01$) in hamsters exposed to the facility (58% and 52%, respectively), compared with the normal 30% detected in MSB disease-free hamsters. This was a consistent finding. No other significant differential count differences were noted.

Discussion

The natural transmission of a filterable (< 0.22 μ porosity), oncogenic agent responsible for the lymphomas described here has been shown to be a highly infectious, non-capsid-encased, horizontally transmitted entity that is quite resistant to many virucidal agents, including UV. The agent is suspected to be directly or indirectly associated with a spectrum of other disorders described here. It should be emphasized that the basic lumping of this disease into a single nosologic category with a presumed common cause is still presumptive, though clearly indicated. In future studies it will be necessary to stringently fulfill Koch's postulates for each associated disease category, including the enteritis condition and other associated syndromes.

The pathologic features of these lymphomas were remarkably stable. A comparison of fixed preparations from four different epizootics that occurred over a 5-year period demonstrated no obvious differences in the histopathology of the tumor types appearing in each eipzootic. Hence, the oncogenic agent seems stable in its transforming, tumor-inducing property. The possibility that the causative agent is a transfecting viroid DNA and that similar tumors or other lesions in man and other animals may be mediated by viroids not detectable by standard virologic or immunologic procedures makes the hamster infectious lymphoma model potentially valuable for understanding of the host–viroid relationship in mammals. The methods developed in this model may aide in kindling a pragmatic search for similar human "viroidlike" agents. Another important consideration in being able to characterize this disease pathologically is that it may pose a continuing threat to hamster research facilities, as two significant outbreaks elsewhere suggest.

The classification of lymphomas is a complex issue.[13-14] Modern procedures have turned lymphoma classification away from strictly morphologic procedures to now include immunglobulin markers at the cell surface or intracellularly, a variety of histochemical procedures, rosetting and the susceptibility of T or B cell types to antiserums prepared against SIg-bearing or theta-like antigens. The majority of lymphomas induced by the agent described above are of the B-cell lymphoepitheloid group, as opposed to histiocytic lymphomas reported by other investigators in hamsters.[16-19] The observation that intestinal lymphomas selected randomly all were SIg^+, $theta^-$, $rosetting^-$ clearly suggests a true B-cell origin for these lymphomas. A detailed study of a large number of those lymphomas for T- or B-cell phenotype, combined with a detailed histologic evaluation, will be necessary to properly classify these lymphomas pathologically. The lymphoma cells appeared to be of the small lymphocyte series, and many contained cells of the plasmacytoid type. The observation that a significant number of lymphomas of apparent thymic origin (SIg^-, $theta^+$, $rosetting^+$) were found in the epizootics indicates that the thymus-derived target cell was also susceptible to the oncogenic agent. The general pathologic features of this epidemic lymphoma did not provide any evidence regarding a common tissue of origin in which the oncogenic agent initiates primary transformation. Only a rare intestinal lymphoma appeared to be of the immunoblastic sarcoma type.

The various pathologic conditions described here, involving kidney, liver, spleen, lymph node, skin, and the gastrointestinal tract, were all curious correlates to epidemic lymphoma, found only in the diseased colony. It is extremely difficult to make meaningful statements about the cause-and-effect relationship between these syndromes and the actual induction of lymphoma, but their occurrence in all epizootics in our facility and elsewhere points to their clear correlation with the disease, even if they are effects rather than causally related.

The disease may have occurred elsewhere. Graffi et al[15-16] reported that lymphomas appeared in a special strain of hamsters following injection with hamster

papilloma virus, papillomavirus DNA, human wart virus, and some human tumor DNA, as well as other solutions. This is a curious finding and difficult to reproduce in normal hamsters. Indirect evidence was employed to propose that the DNA of papilloma virus might activate a retrovirus which caused lymphocytic lymphoma in hamsters.[16] Spontaneous thymic lymphomas in hamsters are extremely rare.[17] Although Graffi[15,16] did not actually report horizontal transmission of lymphoma, we have to wonder whether Graffi's colony might have been experiencing an unrecognized epizootic caused by the same agent active in our hamster colony which was ongoing during the screening of his papilloma virus preparations. Several lines of evidence support this contention. First, uninoculated controls were not described for the Graffi study.[15,16] Had they been tested, we would possibly have an answer. In our experience, the number of agent-induced lymphomas (facility exposed) that appear within a group of hamsters simply exposed to the agent-contaminated facility could vary from 0, in hamsters exposed at 2–3 months of age, to 90% in facility-exposed newborns. The 90% value is derived from the introduction of 200 neonates into a given area where lymphoma disease is active. Fifty-three animals died within 3–6 weeks of exposure, in the main, from enteritis and intussusception disease but with no physical evidence of lymphoma. Of the 147, 132 developed pathologically confirmed lymphomas within the next 18 months. The rate of naturally transmitted lymphomas was dependent on 1) age of exposure and 2) incidence of disease ongoing in the exposure area. An average incidence of ~50% for all groups evaluated was noted. Injection of lymphoma or tumor extracts not stabilized with protamine sulfate can suppress the background incidence of lymphomas (caused by natural agent transmission) to 0–10% in a given cage of hamsters and by 40–50% overall in an experiment.[8,9] Thus, an illusion of variable oncogenicity among the different preparations Graffi tested could be created, particularly since uninoculated control animals were not evaluated.[15,16]

C-type virus particles were noted infrequently by electron microscopy in the epizootic lymphomas we reported.[8,9] Similar particles were observed in nonexposed, normal hamster tissues as well; p30 and gp69 were not consistently found in lymphoma-bearing hamsters. Kistler experienced an outbreak of lymphoma similar to ours in 1976 in Switzerland, and he reported C-type particles to be consistently present in these tumors (personal communication), but the description of such particles in univolved control groups was not given.

C-type particles were also reported by Graffi in lymphomas seemingly induced by DNA preparations of papilloma virus in hamsters[15,16]; however, it was never clear exactly what role these particles played in the lymphomagenic process. Gardner[21] attempted to repeat Graffi's observations in 1976. He observed that when Graffi stock hamsters were given injections of saline alone, lymphomas developed in 40%. Graffi did not see tumors of the peripheral lymph nodes very frequently, although they were not an uncommon finding in our facility-exposed hamsters. In the original report of 35 apparently spontaneous tumors in the colony which was the source of Graffi's hamsters,[16] there were 28 intraperitoneal lymphomas, two liver lymphomas, two axillary lymphomas, one cervical lymphoma, a leukemia with lymphatic and histiocytic elements, and several other nonlymphatic tumors. The distribution of these tumors was similar to that noted in our epizootics.

The papillomas we observed were similar to those Graffi reported in hamsters inoculated with hamster fetal tissue grown in culture.[18] Graffi[18] could not transmit papillomas with cell-free extracts, although they were naturally transmitted to uninoculated dams of offspring that received injections and to uninoculated cage mates.[18] We noted that homogenates of spontaneous papillomas occurring in both our inbred and random-bred strains were able to transmit wart production with cell-free filtrates. Unlike Graffi, however, our papilloma extracts had no detectable lymphomagenic activity. It is clear from electron-microscopic studies that no papilloma virus particles could be found in primary lymphoma[8,9] or in metastases or in passaged lymphoma cells produced in our epizootics or in the lymphomas Graffi observed.[15,18] We conducted extensive studies in the attempt to induce lymphomas in hamsters by inoculation (subcutaneous injection or via scarification) using papilloma virus isolated from wartlike lesions appearing among lymphomatous animals as well as virus from warts on lymphoma-free cage mates and consistently failed to induce a lymphoma. Perhaps the intact papilloma virus responsible for wart production in these hamsters does not induce lymphoma. However, it is important to determine whether papilloma virus or some other viroid agent might not be defective in lymphomatous tissue and cause persistent antigenic stimulation reflected by enteritis[19] and possibly the high incidence of pyelonephritis observed in the kidneys of hamsters (and neurophil increase detected in the afflicted animal colony.

The transient high leukocyte counts detected at 5 weeks of exposure in neonates were a reflection of greatly increased neutrophils and subnormal lymphocyte levels, suggesting a partial inflammatory re-

sponse with specific depression of lymphocyte production. These lymphomas might be induced by an indirect mechanism involving lymphoma clone production following persistent antigenic stimulation caused by a transfecting papilloma virus DNA or some other agent unable to produce mature virus particles. Such an immunologic mechanism has been previously proposed elsewhere.[20] The severe, lethal enteritis seen in a high percentage of hamsters may again be a reflection of hyperimmune stimulation disorder caused by a defective agent. Other possibilities cannot be excluded.[9] Further study of this interesting association is needed.

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