Crohn disease lymph node homogenates produce murine lymphoma in athymic mice

(chronic inflammatory bowel disease/transmissible agent/immunofluorescence)

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ABSTRACT To study the putative agent(s) related to Crohn disease, we intraperitoneally injected mesenteric lymph node homogenates from four patients with active Crohn disease into 10-week-old athymic (nu/nu) mice. Control mice (nu/nu) were injected with homogenates of mesenteric lymph nodes from two patients with ulcerative colitis and four patients undergoing elective colectectomy, and with a homogenate of a cervical lymph node containing sarcoid granuloma. Thirty-four mice received filtered or unfiltered homogenates from Crohn disease lymph nodes. Thirty-two mice received homogenates or filtrates from lymph nodes of control patients. Four mice from the group injected with Crohn disease homogenates from four different patients developed generalized lymphadenopathy due to lymphoma 10–28 weeks after the injection. Two additional mice developed lymphadenopathy due to plasma cell hyperplasia. None of the control mice developed lymphomas or lymphadenopathy. Two lymphomas were homogenized, filtered, and injected intraperitoneally into a second group of nu/nu mice, which also developed lymphoma within 8 weeks of injection. Two lymphomas were cultured in vitro and B cell surface markers were identified. Indirect immunofluorescence studies in two lymphomas showed cytoplasmic staining of lymphoma cells with sera from 10 patients with active Crohn disease but not with sera from 13 control subjects, including 6 with ulcerative colitis and 7 with other gastrointestinal disorders. These results suggest that a transmissible factor present in Crohn disease lymph nodes produces lymphoma in nu/nu mice. Furthermore, sera of Crohn disease patients contain an antibody that recognizes an "antigen(s)" in the murine lymphoma.

Crohn disease is a common chronic inflammatory bowel disease of the small and large intestine; its etiology is unknown. There is no specific medical therapy, and surgical resection of the diseased segment is commonly followed by recurrence. The nature of the anatomic lesions and the clinical course of disease suggest an infectious etiology. A variety of infectious agents, including viruses, L-forms of bacteria, and mycobacteria have been inconsistently isolated from tissues of patients with Crohn disease (1–9).

The disease does not occur spontaneously in animals. Injection of mice and rabbits with homogenates of diseased intestine and lymph nodes from patients with Crohn disease produced granulomas in the intestine and at the injection sites. The lesions occurred several months after injection.

Uniqueness of athymic T cell-deficient (nu/nu) mice for the study of various infectious agents, including parasites, bacteria, and viruses, and of various neoplastic agents has been demonstrated (10–18). For example, Epstein–Barr virus, influenza virus, *Pneumocystis carinii*, *Listeria monocytogenes*, and *Mycobacterium leprae* could not be grown by conventional techniques but were identified after injection in *nu/nu* mice (10–14, 16, 19–21). Therefore, we used *nu*/*nu* mice (nu/nu) in a search for a disease-specific transmissible agent of Crohn disease.

MATERIALS AND METHODS

*Nude Mouse Colony.* A *nu/nu* mouse colony on a BALB/c background was established from nine male *nu/nu* and nine female *nu/+* mice obtained as a gift from C. T. Hansen, National Institutes of Health. Heterozygous females were mated with homozygous males to give a progeny approximately half homozygous and half heterozygous. Homozygous female mice were used in our experiments. Heterozygous females and homozygous males were used for breeding. Breeding and experimental animals were kept in germfree isolators.

The mice were separated as follows: Group I, 34 *nu/nu* mice received filtered or unfiltered homogenates of mesenteric lymph nodes obtained from four patients with active Crohn disease who were undergoing intestinal resection. At least 8 mice received homogenate of lymph nodes from each patient. Thirty-two mice received homogenates of (*i*) normal mesenteric lymph nodes from four patients who underwent elective colectomy (group II); (*ii*) mesenteric lymph nodes adjacent to diseased colon obtained from two patients with ulcerative colitis while they had total colectomy (group III); and (*iii*) a cervical lymph node from a patient with sarcoidosis (group IV). Eighteen and six mice were injected with material obtained from patients of group II and III respectively. Eight mice received filtrate from the patient of group IV. The number of mice injected with the material from each group depended on the amount of protein present in each filtrate.

*Conventional Mice.* In addition to *nu/nu* mice, conventional BALB/c female mice 10–12 weeks old were purchased from the Jackson Laboratory. Sixteen mice were injected intraperitoneally with Crohn disease lymph node homogenates from 2 other patients (5 mice per lymph node homogenate) and 6 received similar material from a control normal mesenteric lymph node. Four mice were sacrificed after 4 months and the internal organs were examined histologically.

*Lymph Node Homogenates.* The lymph nodes were cleaned of adherent adipose tissue with sterile techniques and then stored at −80°C. For experiments the lymph nodes were thawed, minced, suspended in double the volume of sterile Hanks’ balanced salt solution, and homogenized in a Potter-Elvehjem homogenizer for 1–2 min. Half of each homogenate was filtered on 0.45-μm pore diameter Millipore filters. Homogenates and filtrates were separately injected intraperitoneally in 10- to 16-week-old *nu/nu* and normal mice (BALB/c). Each mouse received 0.2–0.4 mg of protein (0.1–0.2 ml).

All mice were examined regularly for weight loss, development of diarrhea, occurrence of skin lesions, or changes in appearance for a period up to 12 months. Fifteen *nu/nu* mice died unexpectedly during the first few months of the study. Eleven were in the Crohn disease group (group I), 1 in group

Abbreviations: FITC, fluorescein isothiocyanate; P1/NaCl, phosphate-buffered saline (0.02 M sodium phosphate, pH 7.2/0.15 M NaCl); SRBC, sheep erythrocytes.
II, and 3 in group IV. Necropsy of 5 mice revealed no histological changes in the internal organs. The rest were badly autolysed when discovered. Eleven nu/nu mice, 7 from the Crohn disease group and 4 from the other three groups, were killed electively after 4–6 months and the internal organs were examined histologically.

Six of 23 surviving nu/nu mice that were injected with Crohn disease lymph node homogenates from four different patients developed generalized lymphadenopathy within 12–26 weeks. Lymphadenopathy was due to plasma cell hyperplasia in 2 and malignant lymphoma in 4. The mice were killed, internal organs were examined, and additional studies of the enlarged lymph nodes included histology, serial passage, tissue culture, and indirect immunofluorescence with patients’ sera.

**Passage Studies.** To investigate transmissibility, two of the four lymphomas were homogenized and the homogenates were filtered through 0.45-μm Millipore filters. Filtrates (0.2 ml containing 0.2–0.3 mg of protein) were injected intra peritoneally into five nu/nu mice.

**Establishment and Characterization of Two Lymphoma Cell Lines.** Two of the four lymphomas from nu/nu mice were cultured and have been maintained for 12 and 8 months, respectively. Cell culture conditions were similar to those for BALB/c B-cell lymphoma lines (21). The medium was RPMI 1640 (GIBCO) supplemented with 20% heat-inactivated fetal calf serum, 2 mM glutamine, 100 μg of streptomycin, 100 units of penicillin G, and 2.5 μg of amphotericin B per ml of medium, and 50 μM 2-mercaptoethanol. The pH of the medium was adjusted to 7.3.

**Fc Rosetting.** This rosetting was performed by adding equal volumes of an optimal dilution of anti-sheep erythrocytes (SRBC) antisera and a 5% suspension of washed SRBC followed by 2.5% suspension of sensitized cells in 10% fetal calf serum/phosphate-buffered saline (P/NaCl; 0.02 M sodium phosphate, pH 7.2/0.15 M NaCl). Rosetting was performed in small tubes, using 0.15-ml suspensions of lymphoma lines containing 2×10⁶ cells/ml of P/NaCl/10% fetal calf serum and an equal volume of sensitized cell suspensions. Insensitized SRBC were used as controls. The tubes were gently shaken in a rotator for 15 min at 37°C; 0.1 ml was removed, diluted with an equal volume of cold P/NaCl, and counted for rosettes. Lymphocytes were stained with 2% crystal violet prior to counting, and lymphoid cells attached to four or more SRBC were considered as positive rosettes. A minimum of 200 cells were scored.

**Complement Rosetting.** Sensitization of SRBC was performed as with Fc rosetting with an optimally diluted anti-SRBC antisera (IgM) (23). A 5% suspension of sensitized cells was incubated at 37°C for 20 min with fresh normal mouse serum diluted 1:8 as a source of complement. The cells were centrifuged, washed twice with cold P/NaCl, and resuspended in P/NaCl/10% fetal calf serum to prepare a 2.5% suspension. The method for rosetting lymphoid cell lines was similar to that used for Fc rosetting.

**Immunofluorescent Studies of the nu/nu Lymphoma. Antiserum.** Fluorescein isothiocyanate (FITC)-conjugated anti-human immunoglobulins [IgG and IgM F(ab′)₂], antihuman mouse serum, anti-mouse thymocytes, and anti-goat and antirabbit IgG F(ab′)₂ fragments were obtained from Cappel Laboratories (Cochranville, PA). Monospecificity of antiserum was screened by immunoelectrophoresis. Appropriate positive and negative controls were used to examine specificity of different antiseras as indicated in the description of each experiment. All sera were ultracentrifuged at 105,000 × g for 2 hr immediately prior to each experiment to remove any aggregates.

**Immunofluorescence Studies of Lymphomas by Indirect Technique.** To detect specific antigen(s) in nu/nu lymphomas (tumor tissues and lymphoma cell lines) indirect immunofluorescence studies were performed by using sera from patients with Crohn disease or ulcerative colitis and from normal control subjects.

Sera from 10 patients with active Crohn disease, 6 patients with active ulcerative colitis, 2 patients with other diarrheal disease, and 5 control subjects were used for immunofluorescence studies. None of the patients were treated with corticosteroids. The sera were absorbed twice with nu/nu spleen cells prior to use. Spleens from normal nu/nu mice were separated and gently homogenized in a glass homogenizer. The cells were separated from the supernatant by centrifugation at 675.6 × g for 10 min and washed twice with Hanks’ balanced salt solution. Cell pellets (4 × 10⁹ cells) were mixed with 250 μl of patients’ sera and kept on ice for 30–60 min. Absorbed sera were obtained by centrifugation at 675.6 × g for 10 min. Sera were ultracentrifuged at 105,000 × g for 2 hours prior to each experiment to remove aggregates and then were coded.

Cryostat sections (4 μm) of nu/nu tissues were incubated in a moist chamber for 45 min with appropriately absorbed deabsorbed sera and were washed two times in P/NaCl/5% bovine serum albumin. Sections were incubated with FITC-conjugated anti-human IgG and IgM. These antisera were also absorbed with nu/nu normal spleen cells prior to use as described above. Specimens were washed with P/NaCl and mounted. Dilutions of conjugates that yielded maximal sensitivity with minimal background were selected for indirect immunofluorescence. As a control for nonspecific fluorescence, an additional section was incubated with conjugated antisera of the same dilution during each experiment. Uninjected nu/nu lymph nodes and the lymph nodes obtained from the nude mice that were injected with the materials from groups II, III, and IV served as the controls.

Sections were examined by two experienced observers without prior knowledge of the source of patients’ sera. The intensity of fluorescence was scored on 0 to +++ scale: 0, no fluorescence; +, minimal fluorescence detected only after close observation; ++, clear-cut fluorescent pattern of cellular structures; ++++, diffuse intense band of fluorescence of the lymphoid cells. The distribution and pattern of staining were carefully examined at X400 and X630.

nu/nu lymphomas were also examined by indirect immunofluorescence for human reovirus 3 with anti-reovirus 3 (obtained from M. Horwitz, Albert Einstein College of Medicine). Cryostat sections were incubated with these sera (diluted 1:2 and 1:4) followed by incubation with FITC-conjugated antirabbit F(a-b)2 fragments.

**Immunofluorescent Studies with the Lymphoid Cell Lines.** Approximately 10⁶ lymphoid cells were washed twice in P/NaCl/5% bovine serum albumin and 0.1% sodium azide and centrifuged at 675.6 × g for 10 min. The cells were incubated on ice for 30–60 min with 50 μl of FITC-conjugated anti-mouse IgG (fluorochrome-to-protein ratio = 3.4) or anti-mouse thymocytes (fluorochrome-to-protein ratio 2.8), washed twice with P/NaCl containing bovine serum albumin and sodium azide, and examined with a fluorescence microscope. Mouse myeloma cell lines N-1 (non-Ig-producer), MPOC-104 (μ and δ producer) and S107 (α and κ producer) were used as controls. These cell lines were obtained from M. D. Scharff, Department of Cell Biology, Albert Einstein College of Medicine. For indirect immunofluorescence with patients’ sera, cells were initially incubated with the absorbed sera as described above, followed by incubation with FITC-conjugated anti-human IgG.

**Fluorescence Microscopy.** A Zeiss microscope equipped with...
vertical illuminator HB 200-W mercury lamp, FITC, KP-600 exciter filters, FL-400 reflector, and 50 barrier filter were used for FITC staining. Photographs were taken with Kodak Ektachrome ASA 400 film.

RESULTS

Table 1 summarizes the clinicopathologic findings in 66 animals injected with lymph node homogenates obtained from four patients with active Crohn disease who underwent intestinal resections, four individuals who had cholecystectomy, two patients with active ulcerative colitis who required total colectomy, and one patient with sarcoidosis.

Morbidity of nu/nu mice was generally low except among the animals injected with either Crohn disease material or filtrates of a lymph node from a patient with sarcoid granuloma. Autopsy examination was possible only in 5 of the 15 mice that died unexpectedly during the initial few months after injection. No obvious cause of death was found in them.

Six nu/nu mice that received Crohn disease homogenates from four different Crohn disease patients developed clinically significant lymphadenopathy (Fig. 1). In four animals, lymphadenopathy was associated with splenomegaly and was due to diffuse lymphocytic lymphoma (Fig. 2). In two other animals, lymphadenopathy was due to benign reactive changes characterized by pronounced plasma cell proliferation with no obliteration of normal architecture or invasion of surrounding tissues. Injection of homogenates from each of the four patients with Crohn disease resulted in the development of lymphoma in one of the injected animals. Two lymphoma-bearing animals received unfiltered lymph node homogenates and developed lymphomas 12 and 16 weeks after injection. The other two animals received filtered homogenates and developed the tumor 22 and 28 weeks after injection.

To investigate the presence of a possible tumor-associated transmissible agent, two of the tumors were homogenized and cell-free supernatants were injected into a group of five nu/nu mice (two with one tumor homogenate and three with the other). Three of these animals died within four days after injection. The remaining two injected with two tumor filtrates developed lymphomas 8 and 10 weeks after the injection. In each case tumor growth was predominantly intraabdominal. These tumors were histologically similar to those from which the extracts were prepared.

nu/nu mice that were injected with homogenates of lymph nodes obtained from two patients with ulcerative colitis, one patient with sarcoidosis, or four with cholecystitis did not develop lymphoma during the subsequent 12 months (Table 1). Additional control mice consisted of 10 nu/nu that were injected with the same volume of Hanks’ balanced salt solution and did not develop any lymphadenopathy. Conventional BALB/c mice that were injected with either lymph node homogenates from two patients with Crohn disease and one patient with ulcerative colitis also did not show any clinical changes during a 6-month follow-up. Four control nu/nu mice and seven nu/nu mice that were injected with Crohn disease lymph node homogenates were killed electively 4–6 months after injection. Autopsy showed no significant gross or microscopic abnormalities. BALB/c mice injected with Crohn disease control tissue homogenates were also killed 4 months after injection and autopsy showed no abnormalities.

One nu/nu lymphoma was examined by Microbiological Associates (Bethesda, MD) for the following murine viruses: pneumonia virus, rheovirus 3, Sendai, Theilers encephalomyelitis, polyoma, minute virus, mouse adenoma, myocarditis-

**Table 1. Clinical and histological changes in nu/nu mice**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Patients</th>
<th>Mice injected</th>
<th>Mortality (during 0–4 mo)</th>
<th>Malignant lymphoma</th>
<th>Reactive lymphadenopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crohn disease</td>
<td>4</td>
<td>24</td>
<td>11</td>
<td>4*</td>
<td>2</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cholecystitis (normal mesenteric lymph nodes)</td>
<td>4</td>
<td>18</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Each patient’s lymph node homogenate produced one lymphoma.

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**FIG. 1.** A nude mouse (nu/nu) with generalized lymphadenopathy in axilla and neck. (A) Axillary lymphadenopathy viewed dorsally; (B) enlarged lymph nodes seen in axilla and neck after dissection.

**FIG. 2.** Primary lymphoma in an animal injected with Crohn disease lymph node homogenate. Tumor cells are small and uniform in size and some show plasmacytoid features. The large clear cells are histiocytes, some with intracytoplasmic nuclear debris. (Paraffin embedding and hematoxylin and eosin staining; ×45.)
hepatitis, lymphocytic choriomeningitis, ectromelia, and lactic dehydrogenase virus. Screening was performed by using the mouse antibody production test in which tumor tissue is injected into mice and development of specific viral antibodies is examined immunologically. The same tumor was also examined by Microbiological Associates for mycoplasma by using the Hoechst stain. Each of these studies gave negative results.

Table 2 summarizes the surface markers of the two lymphoma cell lines being maintained for 12 and 8 months. Both lymphoid cell lines showed surface fluorescence when stained with polyvalent anti-mouse immunoglobulins. To confirm that the fluorescence staining with anti-Ig was not due to Fc receptors on cell membranes, F(ab')2 antisera were used; the results were similar to those with intact antibody. Seventy to 80% of both lymphoid cell lines were positive for Fc receptors and negative for complement receptors as tested by rosetting techniques. Immunofluorescent staining for a T cell specific marker, Thy-1.2 antigen, was negative. These results indicate that the lymphomas are of murine B cell origin.

All Crohn disease sera obtained from ten additional patients in 1:4 and 1:8 dilutions showed intense fluorescence (++) when incubated with sections of lymph node tumor.

Two patterns were observed: (i) uniform cytoplasmic staining with intense fluorescence of lymphoid cells present in clusters (Fig. 3B), and (ii) scattered intracytoplasmic dense staining in speckles or “inclusions.”

Sera from the control subjects including six patients with ulcerative colitis, two with nonspecific diarrhea, and five normal subjects did not show any such fluorescence (Fig. 3A and Table 3). Sera of neither Crohn disease nor control patients showed any staining when incubated with a normal lymph node from a nu/nu mouse or lymph nodes obtained from the mice injected with material from non-Crohn disease patients. In addition, sera from three patients with Crohn disease were absorbed with diseased ileum from a different patient with Crohn disease. Indirect immunofluorescence studies of the lymphoma were performed with these pre- and postabsorbed sera. Absorption with Crohn disease tissue reduced the staining significantly.

Immunofluorescent staining with anti-human reovirus 3 was negative.

**DISCUSSION**

Recent investigations suggest that Crohn disease may be caused by a transmissible agent(s) (1–9). After injection of homogenates of intestine or lymph node from Crohn disease patients into normal mice, a granulomatous reaction occurred at the injection site and in the intestine in 3–17 months. Both local and systemic granulomata developed in normal and thymectomized CBA mice after repeated inoculation with Crohn tissue (4). Several laboratories described a virus (30–90 nm) in tissue culture monolayers that were inoculated with filtrates of Crohn tissue homogenates (5–8). An RNA virus was suggested because growth was not inhibited by 5-iodo-2'-deoxyuridine (6).

In other laboratories, cell wall-deficient bacterial variants like those of *Pseudomonas* group Va have been found in the small intestine and in mesenteric nodes from some patients with Crohn disease but not in any patients with ulcerative colitis (9). It is unclear whether virus or cell wall-deficient bacterial variants are etiologic agents in Crohn disease or whether they preferentially invade tissues affected by this disease. The organisms that have been isolated have not been tested in serial passage and none of the implicated organisms has been recovered from animals that received Crohn disease tissue homogenates.

Congenitally athymic nude mice (nu/nu) do not develop typical granulomatous lesions because they lack T cells. Injection with agents that produce granulomas in normal mice may result in atypical lesions in nude mice (15, 16, 24). Virus replication may be blocked by immunological reactions in normal mice, whereas it may be expressed in nu/nu mice (19, 25).

The development of lymphoma after injection of mesenteric lymph node homogenates from patients with Crohn disease was unexpected and raises several questions. The possibility of spontaneous lymphoma is unlikely because lymphoma was produced by injecting homogenates from four patients with Crohn disease. Cell-free filtrates of two tumors produced intraperitoneal lymphoma in a second group of mice. Two of the four lymphomas developed in mice less than 6 months old, an age at which spontaneous lymphoma in nu/nu mice is extremely rare (26, 27).

The development of lymphoma may be related to a virus in the lymph node homogenates of patients with Crohn disease causing transformation of lymphoid cells in nu/nu mice. It may also result from a “factor,” infectious or chemical, that stimu-

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**Table 2.** Summary of surface markers of two lymphomas to determine whether the lymphoma is of T or B cell origin

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>Surface</th>
<th>Ig</th>
<th>Fc</th>
<th>Complement</th>
<th>Thy-1.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1-1</td>
<td>Ig+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K13-1</td>
<td>Ig+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 3.** Indirect immunofluorescent staining of two nu/nu lymphomas with patients’ sera

<table>
<thead>
<tr>
<th>Patients</th>
<th>Fluorescence at serum dilution</th>
<th>Distribution of immunofluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crohn disease</td>
<td>10 1:4 + + +</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>6 1:8 + +</td>
<td>Connective tissue</td>
</tr>
<tr>
<td>Other gastrointestinal disorders</td>
<td>2 0-+ 0</td>
<td>Connective tissue</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>5 0-+ 0</td>
<td>Connective tissue</td>
</tr>
</tbody>
</table>

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**FIG. 3.** Indirect immunofluorescence studies for antigenic recognition of the nu/nu lymphoma by the patients' sera. The first antibody was patients' sera (diluted 1:8) after absorption with nu/nu mouse spleen cells. The second antibody was FITC-conjugated anti-human IgG F(ab')2 fragment. (A) Control serum from a patient with ulcerative colitis did not stain the nu/nu lymphoma. (B) Homogenous intense cytoplasmic staining can be seen around the nuclei of certain groups of lymphocytes when the identical section of the lymphoma was stained with Crohn disease serum.
lates a latent murine oncogenic virus. In favor of the first possibility is the strongly positive cytoplasmic immunofluorescence of the mouse lymphoma by all Crohn disease sera and not by control sera.

There are several possible explanations for induction of lymphoma in only 16% of nu/nu mice injected with Crohn disease homogenates. The "viral" titer in Crohn disease filtrates may be low. During the second passage, two of the five nu/nu mice injected with lymphoma filtrates developed lymphoma, which suggests amplification of "viral" titer. "Virus particles" may coprecipitate with organelles during homogenization and centrifugation and may not have been injected into nu/nu mice. If a virus is present in the Crohn disease filtrates that were injected into nu/nu mice, a natural killer cell response may eliminate the virus, as was demonstrated after injection of measles- and mumps-infected cell lines into nu/nu mice (28).

Certain lymphocytes of nu/nu mice infected with Crohn disease "agent" may escape the immune mechanism and produce lymphoma. Suppression of natural killer response by irradiation or injection of anti-lymphocyte serum (29) may enhance the incidence of lymphoma after injection of Crohn disease homogenates.

The surface markers of the two lymphoid cell lines suggest that they are murine B cells and have positive surface Ig and Fc receptor without complement receptor and Thy-1.2 antigen, a T cell marker. Production of B cell lymphoma may reflect the intact B cell system of the nude mice, which could be stimulated by Crohn disease tissue homogenates. However, selective B lymphocyte tumors in conventional mice have been induced experimentally by using carcinogens or Abelson virus (30–33). Whether such a specific stimulation of B cells occurs by the putative agent related to Crohn disease is not clear.

The number of surviving mice that received control tissue homogenates was 25 as opposed to 2 for the Crohn disease group. None of the mice injected with control lymph node homogenates from patients with sarcoidosis or ulcerative colitis or with normal mesenteric nodes from patients with cholecystitis developed lymphoma. The validity of these results may be argued because of the small number of animals. However, production of lymphoma in 4 of 23 mice injected with materials from four patients with Crohn disease and the recognition of the tumor tissue by the sera from patients with Crohn disease support a direct relationship between production of murine lymphoma and Crohn disease.

Sera from patients with active Crohn disease apparently contain antibodies to a factor present in the induced lymphomas. The likely factor would be a virus or virus-related protein(s). Amplification of this agent may be unique in nu/nu mice due to its enhanced growth and expression. This putative agent related to Crohn disease has yet to be isolated and characterized.

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