Jpn. J. Cancer Res. 83, 288–293, March 1992

Histopathological and Immunohistochemical Study of Atypical Lymphoid Hyperplasia and Benign Lymphoid Hyperplasia of the Stomach

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We propose dividing histologically reactive lymphoid hyperplasia (RLH) of the stomach into two categories: atypical lymphoid hyperplasia (ALH), characterized by abnormal follicular architecture and infiltration of atypical lymphoid cells, and benign lymphoid hyperplasia (BLH), where normal follicular patterns are preserved and no atypical cells are found. Using twenty RLH cases (10 ALHs and 10 BLHs), both categories were compared from histopathological and immunohistochemical aspects. Macroscopic division into superficial, thick-walled and nodular types revealed most ALH (80%) to be superficial in character whereas in BLH superficial and thick-walled types were almost equally represented. Lesion size tended to be larger in ALH (>6.1 cm) than in BLH (6.0 cm>) cases. As for prognosis after gastrectomy, both categories were favorable. On immunohistochemistry, ALH tended to be strongly labeled by B lymphocyte markers as compared to BLH, where only a weakly positive reaction was expressed. Infiltration of cells labeled by T lymphocyte markers was less conspicuous in ALH than in BLH. From these data, we concluded that ALH and BLH are two distinctive categories of lesion and that ALH has characteristics similar to these demonstrated by malignant lymphoma.

Key words: Reactive lymphoid hyperplasia — Atypical lymphoid hyperplasia — Benign lymphoid hyperplasia — Immunohistochemistry — Stomach

The diagnostic term "reactive lymphoid hyperplasia (RLH[2])" to indicate lympho-proliferative lesions distinct from malignant lymphoma was first proposed by Smith and Helwig[1] for benign gastric lesions characterized by varying degrees of abnormal lymphoid hyperplasia. Nakamura *et al.*[2] advocated the use of the term "reactive lympho-reticular hyperplasia," their conclusion being that the lesions were exclusively benign, reactive conditions associated with chronic erosive gastritis or gastric ulcer but not including prelymphomatous states. On the other hand, Faris and Saltzstein[3] reported some cases of gastric lymphoid hyperplasia developing into malignant lymphoma, suggesting a slightly more extended usage of the term. The term pseudolymphoma has also been employed in the gastrointestinal tract for conditions like RLH. Whilst some investigators are in favor of usage limited only to benign lesions,[4,5] others consider transformation to overt malignant lymphoma possible, making analogy with similar conditions of the orbit and salivary gland.[6,7] Thus, gastric RLH or pseudolymphoma may imply not only benign lymphoid hyperplasia, but also a prelymphomatous state.[8,9] This confusing situation is clearly undesirable for both pathologists and clinicians.

While immunohistochemical approaches have proved quite useful in diagnosing malignant lymphoma,[10,11] there are very few data available for RLH. In this investigation we therefore first divided RLH into two subtypes, atypical lymphoid hyperplasia (ALH) and benign lymphoid hyperplasia (BLH), on the basis of detailed observation of hematoxylin and eosin-stained sections, and then evaluated immunohistochemical utilization of anti T and anti B lymphocyte monoclonal antibodies for differentiating between the two subtypes.

MATERIALS AND METHODS

Twenty resected cases of gastric RLH were collected from the file of the Department of Pathology, at the Cancer Institute, Tokyo, dated between 1965 and 1983. All the stomachs were fixed with 15% formalin, and the whole specimen was cut stepwise to determine the precise extension of the lesions, embedded in paraffin and stained with hematoxylin and eosin. Criteria applied for diagnosis of RLH were basically the same as those for pseudolymphomas described by Saraga *et al.*[11] and Brooks and Enterline.[9] Briefly, RLH is a condition obviously different from malignant lymphoma or gastritis follicularis since it has no unequivocal malignant cells and no unequivocal benign follicles in areas with abnor-

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[2] Abbreviations: RLH, reactive lymphoid hyperplasia; ALH, atypical lymphoid hyperplasia; BLH, benign lymphoid hyperplasia.

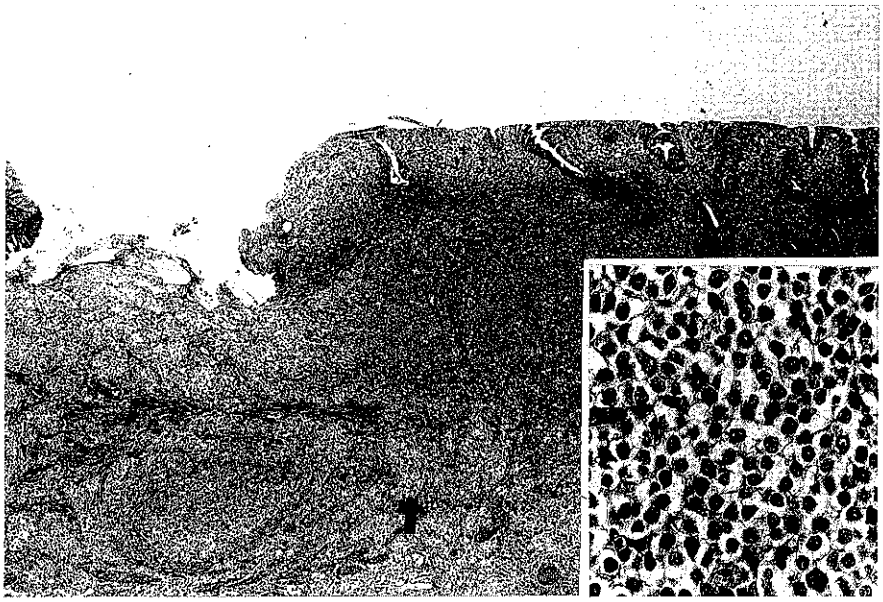

Fig. 1. ALH: Irregularly shaped lymph follicle composed of relatively uniform atypical lymphocytes with slightly bizarre nuclei and inconspicuous nucleoli. Atypical lymphocytes infiltrate the mucosa and submucosa, partly destroying the muscularis mucosae and normal mucosal structure. ×40. Arrow: Inset details uniform atypical lymphocytes. ×1000.

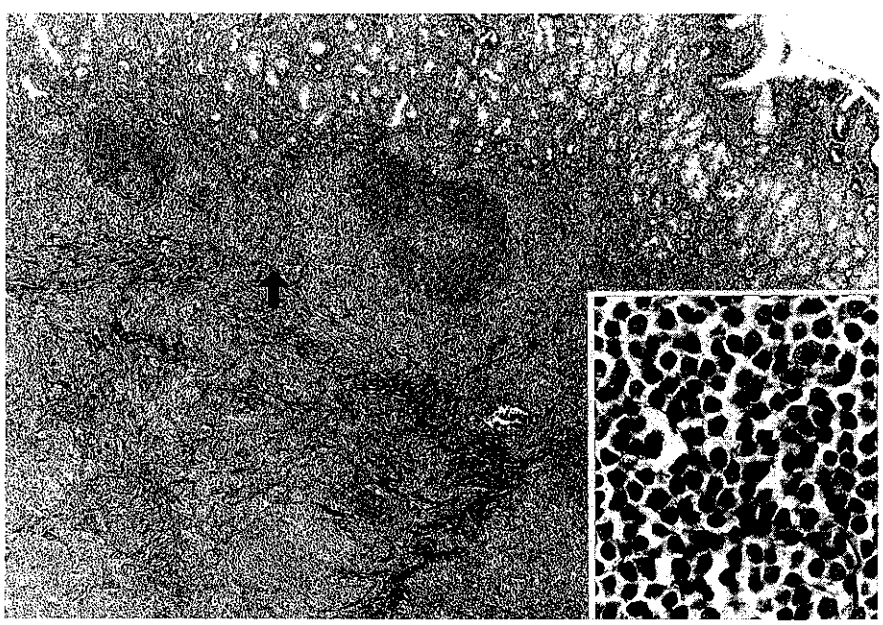

Fig. 2. ALH: Various sized and irregularly shaped lymph follicles constituted by wide mantle zone in the mucosa and submucosa. A small number of large to medium-sized atypical cells with large and polygonal nuclei and conspicuous nucleoli can be seen present in the hyalinized fibrotic interfollicular zone. × 40. Arrow: Inset details large atypical lymphocytes. ×1000.

mal lymphoid hyperplasia. Further, no case had either lymph node metastasis or peritoneal involvement.

RLHs were classified histologically into ALH or BLH according to the following criteria. ALH: lymphoproliferative lesion characterized by varying degrees of abnormality in follicular structure and lymphoid cells infiltrating; (1) irregularly shaped, partly lost mantle zone; or (2) intricate adhesions of lymph follicles and infiltrations of small numbers of atypical lymphoid cells with enlarged, irregular shaped nuclei; or (3) diffuse and monotonous assembly of slightly enlarged uniform cells with small, round or oval nuclei in a mantle zone or an interfollicular space (Figs. 1 and 2). BLH: lymphoproliferative lesion with prominent follicular structure and no

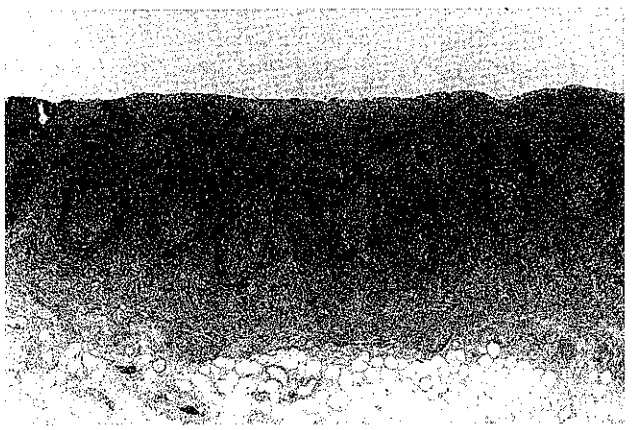

Fig. 3. BLH: A part of aggregates of distinct follicular structure. No abnormal lymphoid cell is visible. ×20.

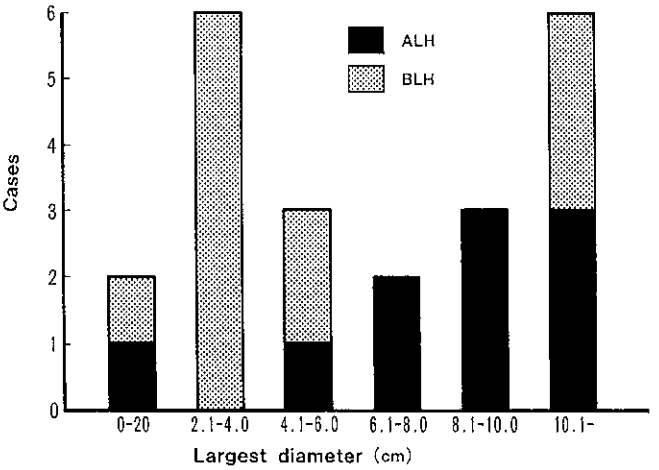

Fig. 4. Largest-diameter distribution of ALH and BLH.

infiltration of abnormal lymphoid cells; aggregating cells predominantly mature lymphocytes with a variable mixture of plasma cells, macrophages and eosinophiles; often associated with long-standing peptic or callosal ulcer (Fig. 3).

In addition to routine hematoxylin and eosin staining, immunohistochemical staining (avidin-biotin complex method) was applied to selected sections of all cases. The antibodies used were MT1 (Bio-Science, EmmenBrucke, Switzerland) and UCHL1 (Dakopatts, Glostrup, Denmark) against T cell antigens and MB1 (Bio-Science) and L26 (Dakopatts) against B cell antigens. Where necessary, anti-immunoglobulin antibodies (anti-kappa and anti-lambda light chain and anti-G, M and A heavy chain antibodies, BioGenex Laboratories, Dublin, CA), LeuM1 (Becton Dickinson Immunocytometry Systems, Mountain View, CA), anti-alpha-1-antitrypsin antibody (Dakopatts) and anti-lysozyme antibody (Dakopatts) were also applied. Avidin-biotin complex was obtained from Becton Dickinson.

Patient's charts were reviewed from clinical data, and information on prognosis was collected through the Follow-up Center of the Cancer Institute.

RESULTS

Pathological study Twenty RLH cases were classified histopathologically into 10 ALHs and 10 BLHs according to our criteria mentioned above.

Age and sex: The average age for all RLH patients was 50.8 years and for ALH and BLH cases, 51.5 years and 50.2 years, respectively. Male/female ratio was 0.54 (7/13) for all patients, 1 (5/5) for ALH and 0.25 (2/8) for BLH.

Prognosis: All BLH cases survived more than ten years. However, one ALH died of acute myelogenous leukemia following breast carcinoma three years after the gastrectomy, and another ALH case died of liver cirrhosis, two years after the gastrectomy.

Macroscopic and histologic findings: Lesion size ranged from 14×8 mm to 180×130 mm. We defined 2 categories of lesion by maximum diameter (A) smaller (0–6.0 cm) and (B) larger (6.1 cm<) (Fig. 4). Whilst most ALH were distributed in the larger group, the majority of BLH were in the smaller group. As for lesion locus, 11, 9 and 2 cases of RLH were found in the corpus, antrum and corpus-antrum, respectively. The respective figures were 6, 3 and 1 for ALH and 5, 6 and 1 for BLH. No significant difference in lesion locus was thus apparent between ALH and BLH.

On the basis of appearance RLH is generally classified as one of the following three types; a) superficial, b) thick-walled and c) nodular type. The superficial type is a diffuse, erosive or shallow ulcerative lesion without thickening of the gastric wall. (Fig. 5a). The thick-walled type arises because of prominent submucosal fibrosis or edema sometimes involving the proper muscle layer (Fig. 5b). The nodular type appears as a well-circumscribed elevated tumor which histologically comprises a densely packed lymphocytic mass accompanied by lymph follicular hyperplasia (Fig. 5c). The incidence of the superficial type for all RLHs was 64% (14/22 cases), for ALH 80% (8/10) and for BLH 50% (6/12). The thick-walled in the present series amounted to 32% (7/22), 20% (2/10) and 42% (5/12). Only one case of BLH presented as a nodular type. Thus, whereas most ALH were superficial in character the BLH were more evenly distributed between superficial and thick-walled types.

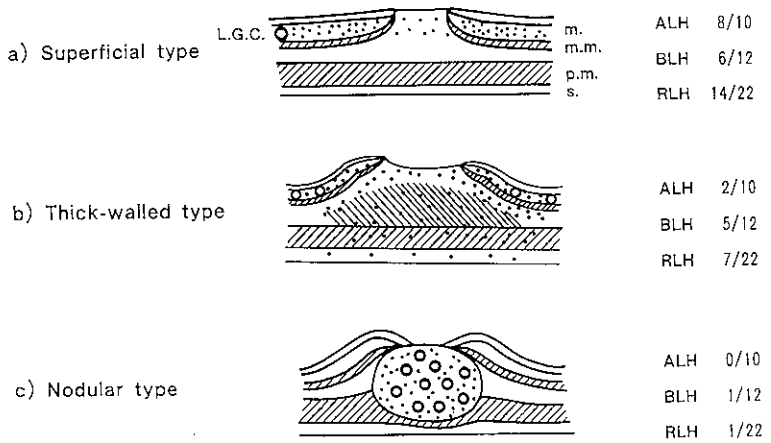

Fig. 5. Schematic representation of the three macroscopic types of RLH. L.G.C., lymphoid germinal center; m., mucosa; m.m., muscularis mucosae; p.m., proper muscle; s., serosa.

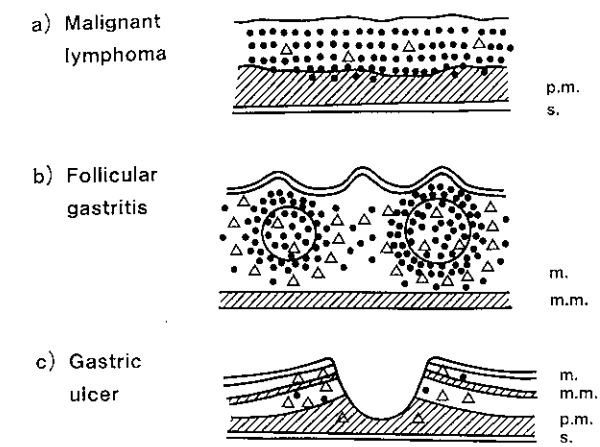

Fig. 6. Schematic representation of T and B lymphocyte distribution in gastric malignant lymphoma, follicular gastritis and gastric ulcer. Closed circles, lymphocytes positive for L26 and/or MB1 (cor Bs); open triangles, lymphocytes positive for UCHL1 and/or MT1 (cor Ts). a, Malignant lymphoma shows predominant population of cor Bs. b, Follicular gastritis demonstrates cor Bs in germinal centers and cor Ts in the interfollicular zones. c, In gastric ulcers cor Ts predominate cor Ts over cor Bs.

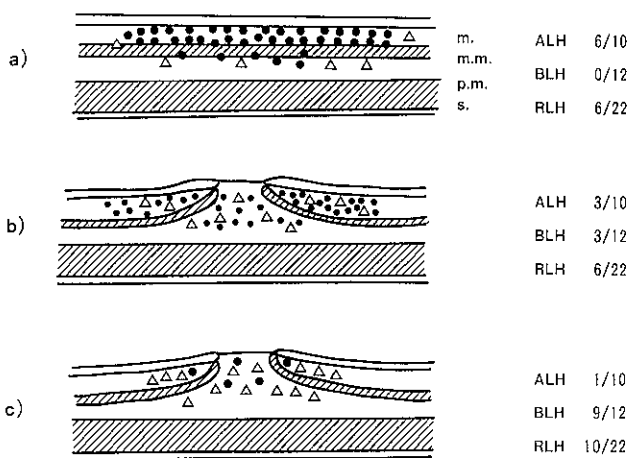

Fig. 7. Schematic representation of the three prototypes of T and B lymphocyte distribution in gastric RLH. a: Cor Bs densely predominate in the lesion. Cor Ts are present only around the mass of Cor Bs. b: Whilst cor Bs are predominant, the admixture of cor Ts within the lesion is more prominent than in a. c: Only few cor Bs and far more cor Ts are present. The incidences of each prototype in each histological type of RLH are shown on the right.

Immunohistochemical study Before examining the cell surface characteristics of the RLHs, 10 cases of obviously malignant gastric lymphoma, 10 cases of unequivocal benign follicular gastritis and 10 cases of gastric ulcer were examined as controls, utilizing anti-B cell and anti-T cell antibodies.

In the malignant lymphomas, cells positive for anti-B cell antibodies, which were considered to correspond to B cells (cor Bs), were predominant, but lymphocytes positive for anti-T cell antibodies (cor Ts) were also sparsely present (Fig. 6a). In follicular gastritis, cor Bs was expressed densely in germinal centers and rather sporadically in interfollicular zones, while in contrast cor Ts appeared mainly in interfollicular zones and sparsely in germinal centers (Fig. 6b). In gastric ulcers, cor Ts predominated over cor Bs, basically associated with granulation tissue (Fig. 6c).

In RLHs, three types of cor T and cor B distribution in the lesions were evident as illustrated in Fig. 7. In type a), cor B existed massively and densely throughout the

lesion and cor T was only sporadically demonstrated. Admixture of cor T in the cor B aggregates was rather rare. In type b), cor B similarly predominated with a greater cor T admixture. In type c), cor B was sparse and cor T populations denser, the cells sometimes aggregating in the reverse fashion to follicular gastritis. Six out of the 10 ALH lesions were of type a), three of 10 ALH and three of 12 BLH were of type b) and the remaining one ALH and 9 BLHs showed the type c) pattern. Thus, a definite tendency for cor B to predominate in ALH and for cor T to be more prominent in BLH was indicated. No obvious differences in lymph follicle cor T and B distribution among ALH, BLH, gastritis follicularis and gastric ulcer cases were evident.

Examination of immunoglobulin monoclonality in aggregates of atypical lymphocytes and/or atypical plasma cells, revealed no definite monoclonality in spite of intensive study. In some ALH cases, one light chain was apparently predominant in limited parts of lesions, but no monoclonal heavy chain staining was detected since Ig G and Ig M evenly appeared.

Large atypical cells were negative for LeuM1, anti-alpha-1 antitrypsin antibody and anti-lysozyme antibody, suggesting a non-histiocytic series character.

DISCUSSION

In common lymphoid hyperplasia, coalescence of three or more follicles or disorganizations of follicular structure such as vaguely demarcated germinal centers or loss of the mantle zone are not visible. Further, large atypical lymphoid cells with swollen or bizarre nuclei are not present. When such features are encountered, the possibility of malignant lymphoma should therefore be considered. However the actual findings are sometimes not conspicuously different from those of benign hyperplasia, and it is quite difficult to determine the nature of the disease or the level of atypia which should be assigned. Thus, the concept of RLH or pseudolymphoma has been well justified. Many pathologists, including specialists in the lymphoma field, are cautious in diagnosing malignant lymphoma, even in cases for which monoclonality of immunoglobulin expression is observed, because the lesion is minimal.[12] Therefore, for what is called RLH or pseudolymphoma there seems to be a spectrum ranging from lesions very close to completely benign lymphoid hyperplasia almost to malignant lymphomas. For this reason we think it is necessary and significant to divide RLH or pseudolymphoma into ALH and BLH categories, although by our criteria monoclonality of immunoglobulin corresponds to malignant lymphoma.

A number of investigators have reported a relationship between RLH and gastric lymphoma. According to Mohri,[13] co-existence of RLH was found in 40% of all gastric lymphomas and in more than 80% (32/38) of early cases. He therefore considered that RLH might be a prelymphomatous state. Furthermore, there have been several reports of malignant lymphomas developing from RLH during long-term follow-up,[14] a latent period of approximately five years being indicated. Isaacson *et al.*[15] also concluded RLH to be an early stage of malignant lymphoma on the basis that all their cases exhibited immunoglobulin light chain restriction and clonally rearranged gene fragments of immunoglobulin. It is not certain which type of RLH, i.e., ALH or BLH, was discussed in these studies, but most of them resembled ALH according to the histological figures presented.

The macroscopic types of RLH have been divided into three groups by Tokunaga *et al.*[16]: nodular, ulcerative and erosive, the nodular type being considered a precursor lesion for malignant lymphoma and the latter two as reactive states caused by gastric ulcer or inflammation. On the other hand, Mohri[13] reported that lesions closely related to the prelymphomatous state were of flat morphology, corresponding to the superficial type in our study. Since 80% of our ALH cases were of this type, our results are in agreement with those of Mohri. The one case of nodular type in our study was thought to be BLH. The discrepancy between our report and Tokunaga's findings may be partly due to the limited number of cases of nodular type in our series and differences in histological criteria for diagnosis of RLH. In our study there was a tendency for ALH to be larger than BLH, which could be used as one criterion in dividing RLH into ALH and BLH. It is speculated that ALH may be generally asymptomatic until it becomes large, while BLH is symptomatic and easily diagnosed at a smaller size because of the chronic presence of gastric ulceration and erosion.

Our immunohistochemical results demonstrated an explicit tendency for cor B to be more prominent and more diffusely distributed in ALH than in BLH and for cor T to be more prominent in BLH. According to our pilot study findings, cor B is exceedingly prominent in malignant lymphoma whereas cor T predominates in gastric ulcers, although numbers of lymphocytes are quite small in the latter case. These results thus indicated that the distribution of cor T and B in ALH is close to that in malignant lymphoma and that in BLH is similar to that in the gastric ulcer cases. However, we could not find any particular differences among ALH, BLH and gastritis follicularis in cor T and B distribution within lymph follicles, suggesting that these follicles are a completely benign component. Two cases of ALH showed the predominance of a single Ig light chain but a double clone in Ig heavy chain. We considered them as a stage in the prelymphomatous state that would represent a continuous and progressive B-cell neoplasia.

Most of the large atypical cells often observed in ALH were determined to be immunoblasts since they were negative for Leu M 1, anti-alpha-1 antitrypsin antibody and anti-lysozyme antibody. Nathwani and Brynes[17] proposed criteria for distinguishing between benign and malignant immunoblasts, pointing out that the benign variety have small nucleoli and do not show any marked variation in their size and shape, whereas malignant immunoblasts have prominent nucleoli and a broader spectrum of size and shape. The immunoblasts found in the present study more closely resembled the benign type. The remainder of the large atypical cells were of indeterminate type since informative results could not be obtained by immunohistology.

In conclusion, we propose a differentiation between ALH and BLH, the first being closely related to the prelymphomatous state and requiring removal by surgical operation, whilst the second is a variant of chronic gastric ulcer scarring which may not require operation. Both pathologists and clinicians should be aware that RLH is a heterogenous entity and not all RLHs are prelymphomatous.

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare.

(Received September 13, 1991/Accepted December 13, 1991)

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