# IMMUNOGLOBULIN-PRODUCTION AND SURFACE-BOUND IMMUNOGLOBULINS IN CHRONIC LYMPHOCYTIC LEUKEMIA, LYMPHOSARCOMA, AND SO-CALLED RETICULUM CELL SARCOMA

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Conventional light and electron microscopic methods do not allow a differentiation of T- and B-cells. A classification of neoplastic lymphatic cells into one of these subpopulations is therefore impossible with common morphological methods. It was not until specific cell funktions, i. e. immunoglobulin(IG)-production and the formation of surface-Ig(S-Ig)-receptors, were demonstrable that one could identify B-cells and thereby distinguish them from T-cells. The demonstration of Ig-production therefore represents a method which reveals more information about the nature of malignant lymphoma cells.

## Results

We have studied the Ig-content of the tumor tissue homogenates from 18 cases of chronic lymphocytic leukemia (CLL), 11 cases of lymphocytic lymphosarcoma, and 18 cases of reticulum cell sarcoma (RCS). These Ig-determinations were performed with radial immunodiffusion. Fig. 1 shows the results. Ten of the 18 CLL, 7 of the 11 lymphocytic lymphosarcomas, and 14 of the 18 RCS revealed an increased IgM-content. In contrast, the serum-IgM-level was above the normal range only once in CLL, twice in lymphocytic lymphosarcoma, and 3 times in RCS. There was no correlation between the tissue-IgM-content and the serum-IgM-level. These findings indicate that the produced IgM was in most cases not released but instead retained in the tumor tissues. Other Ig-classes were seldom detected in the tumor tissues: IgG was increased in 2 CLL and IgA in one RCS.

In order to compare the results of our tissue-Ig-determinations with the data from S-Ig-labeling reported by other authors we have started parallel investigations of S-Ig and tissue homogenate-Ig. We used horse-raddish-peroxidase-labeled antibodies to demonstrate S-Ig, since this technique allows a relatively satisfactory cytological judgement of the labeled cells. Light microscopically the labeled antibodies are represented by a brown peroxidase reaction product, which due to its electron density also allows an electron microscopic demonstration of the antibodies.

Fig. 2 shows a representative of the most common type of lymphocytic lymphosarcoma in our material. This type is characterized by a uniform proliferation of small to mediumsized cells, whose nuclei are very irregular in shape and often notched.

With peroxidase-labeled antibodies 85–90 % of the cells of this lymphocytic lymphosarcoma reacted strongly positive for S-IgM/lambda (Fig. 3 and 4). Kappa-chains were not detectable. In the tissue homogenate the tumor revealed a slight, but highly significant increase in the amount of IgM. The serum-IgM-level, on the other hand, had decreased to below the norm, although the tumor had affected almost all of the lymphatic tissue.

A further case of this type of lymphocytic lymphosarcoma showed a very dense and strong labeling for S-IgM/kappa on nearly all of the cells. There was no increase in the tissue-IgM.
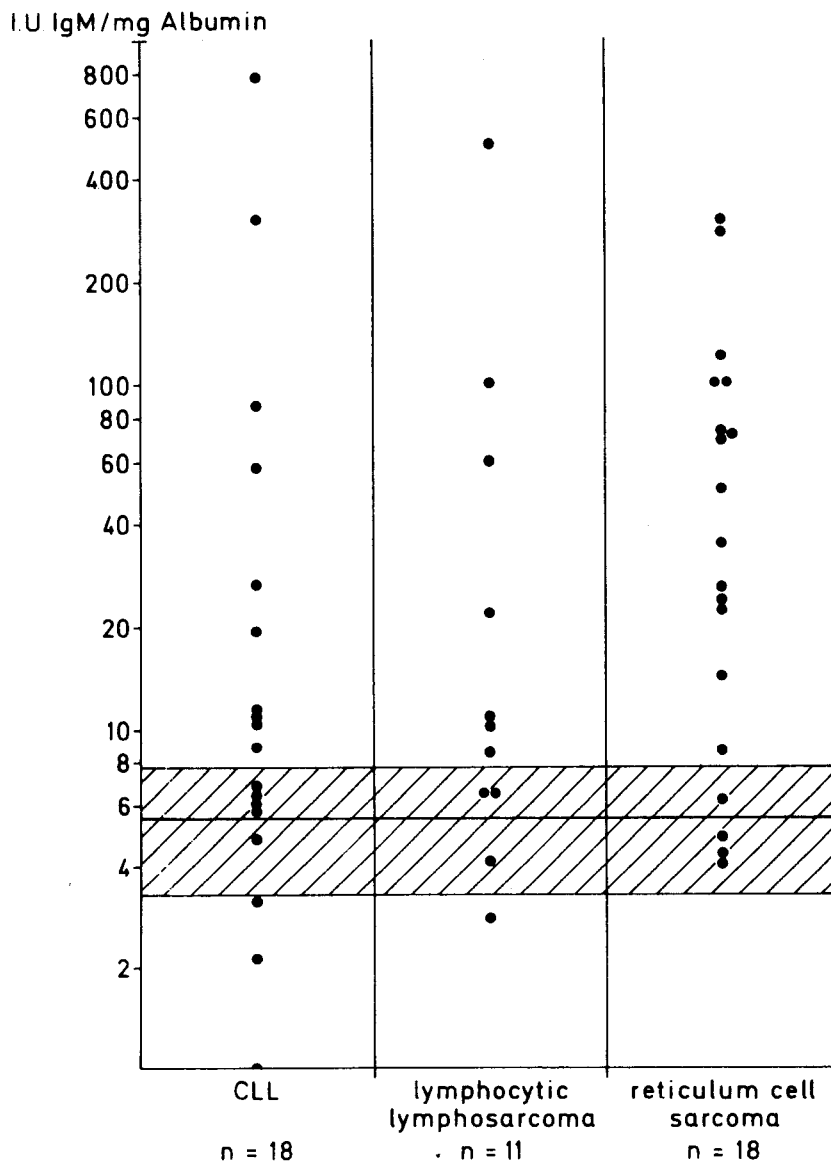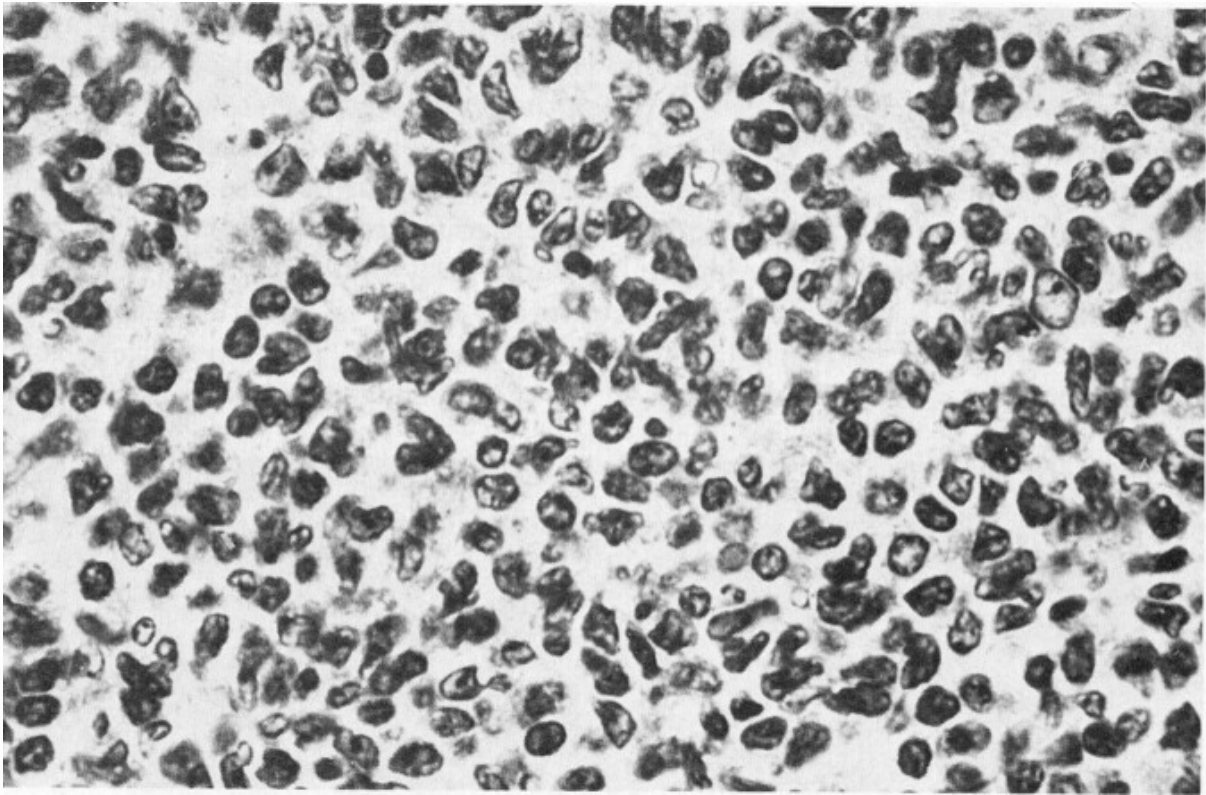


Fig. 1: Tissue homogenate-Ig-content of 47 lymphomas.

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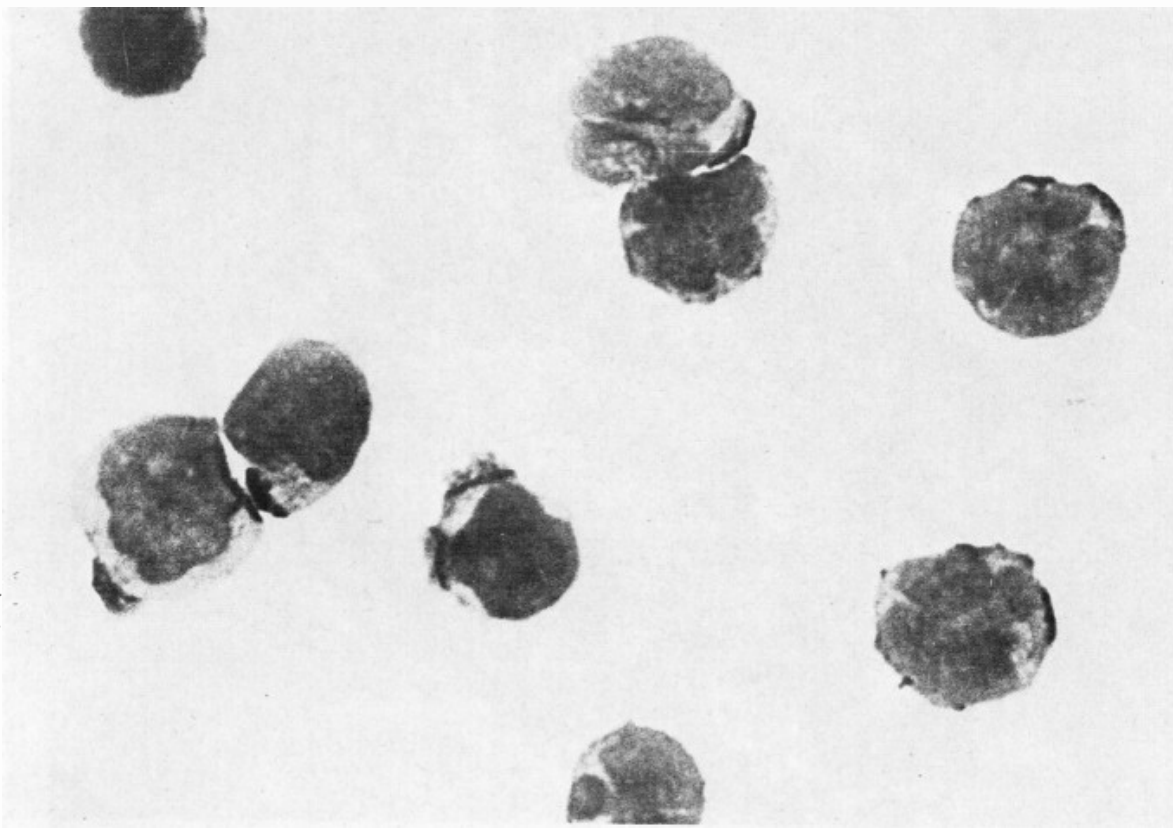Fig. 2: Lymphocytic lymphosarcoma. GIEMSA-stain. 880 x



Fig. 3: Lymphosarcoma cells from the case shown in Fig. 2 labeled with horse-raddish-perox-idase-coupled antibodies. The labeled Ig is visible as black bands or spots on the cell surface. Nearly 85—90 % of the cells reacted positively for IgM/lambda. Note the distinct cap-formation. Haemalaun counterstain. 1,400 x

Histologically the CLL in our series was distinctly different from the lymphocytic lymphosarcoma. The CLL never showed a uniform proliferation, but instead a so-called pseudofollicular pattern: between the lymphocytes there were areas which appeared lighter in the GIEMSA-stain and which consisted of cells clearly larger than the lymphocytes. The most mitoses seemed to occur in these areas. The nuclei of the lymphocytes and of the larger cells were round to oval and never as irregular in shape as those of the cells in the lymphocytic lymphosarcoma type described above. In two CLL we found S-IgM and in one case S-IgG on the leukemic cells. The cells of one of the two S-IgM-positive CLL revealed a patchy pattern for the labeling product (Fig. 5). Although all of the labeling procedures were done at
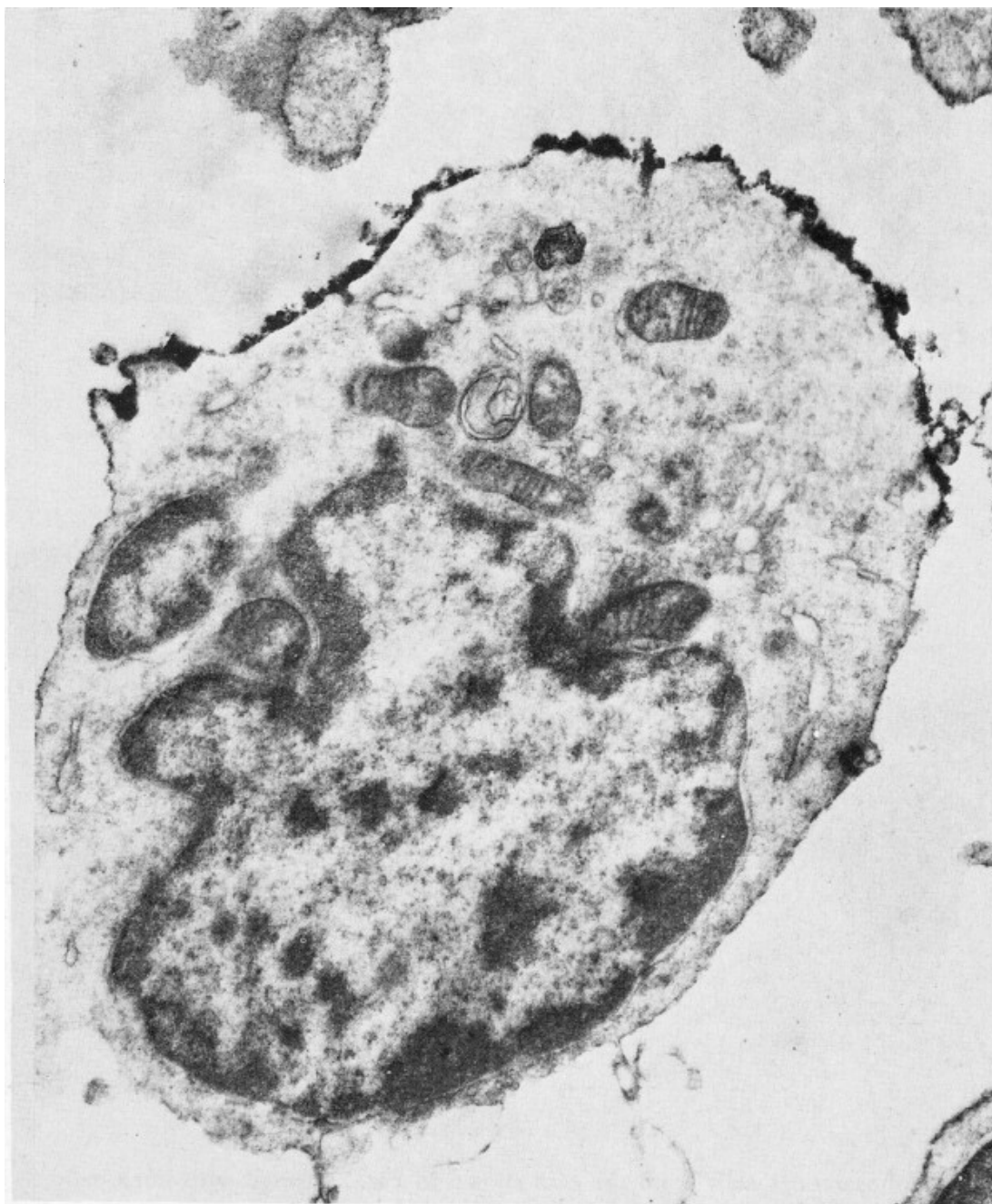


Fig. 4: Electron microscopic demonstration of S-IgM on the same lymphosarcoma cells with peroxidase-labeled antibodies. 17,000 x
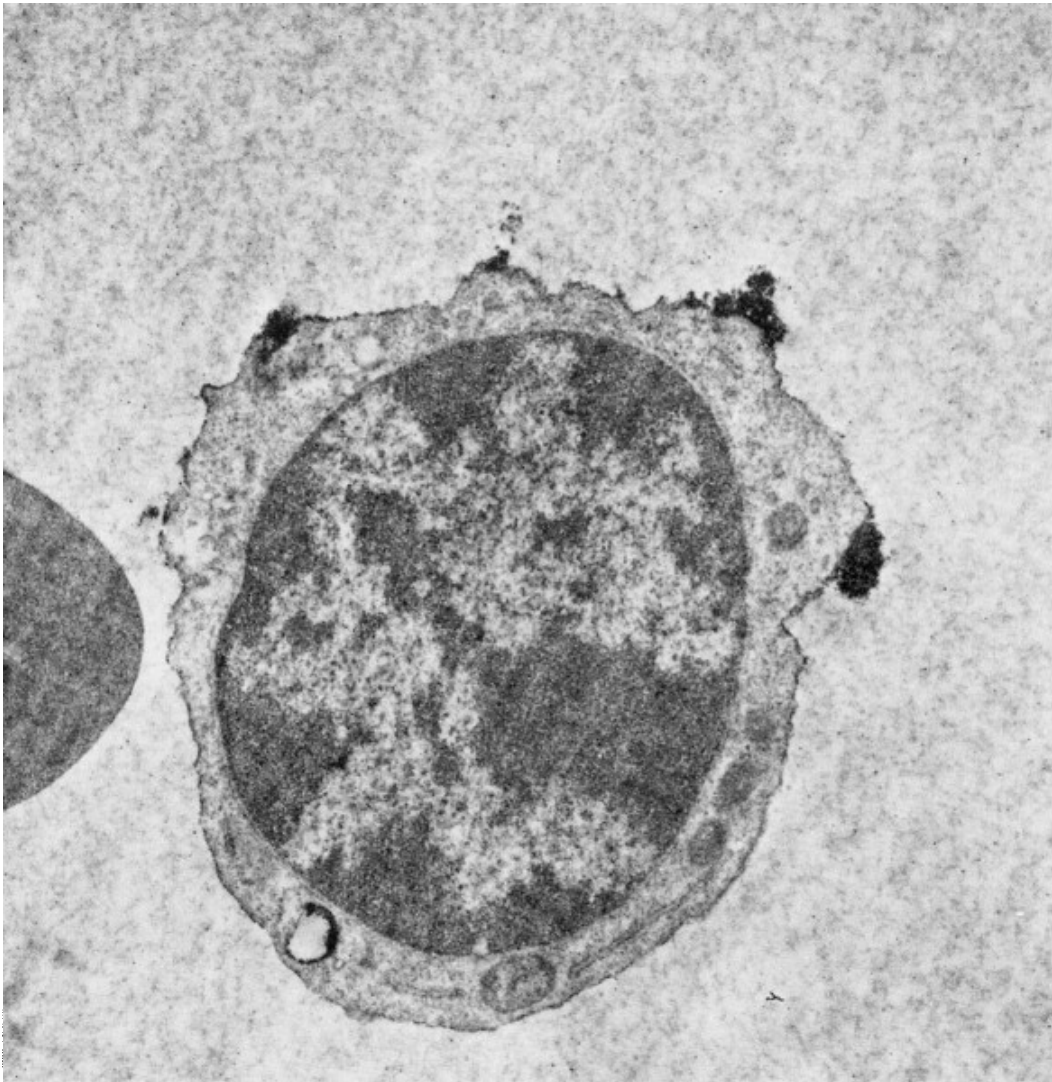
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Fig. 5: Tumor cell from a CLL labeled for S-IgM. 15,000 x

25 °C, there was no cap-formation. Of two nearly equally densely labeled CLL one case revealed a highly increased and the other a slightly increased tissue-IgM-content. In the S-IgG-positive CLL we could demonstrate that the tissue-IgG was disc-electrophoretically monoclonal, whereas the serum-IgG was polyclonal (Fig. 6).

A strong S-Ig-labeling (Fig. 7 and 8) was revealed by a special type of chronic leukemia: leukemic reticuloendotheliosis or hairy cell leukemia, which is characterized by a chronic development, anemia, frequent leukopenia, extreme splenomegaly, absence of significant lymph node swelling, and the presence of so-called hairy cells in the blood and bone marrow, in the BILLROTH pulp cords of the spleen and in the intermediary sinus of the liver. The electron microscope showed that the S-Ig was often located at the hair-like projections. Under prolonged cap-formation conditions these hair-like projections bearing the S-Ig migrated to one pole of the cell (Fig. 8). The tissue homogenate of the spleen, which consisted almost only of hairy cells, showed an IgM-concentration within the normal range. However, despite this finding, we were able to extract significant amounts of IgM from a homogenate prepared from a cell suspension consisting of 98 % hairy cells isolated from the spleen and washed 4 times.

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We were expecially struck by the results from the labeling of the RCS, or at least what has been collected under this term. We consider the following as the typical morphological characteristics of this lymphoma group. The RCS-cells have large nuclei with sparse nuclear chromatin and distinct nucleoli. The cytoplasm is clearly darker than the nucleus and hard to define (Fig. 9). Both of the RCS we had the chance to label reacted strongly for S-IgM. The tissue-IgM-content was also highly increased, whereas the serum-IgM-level lay within the normal range.
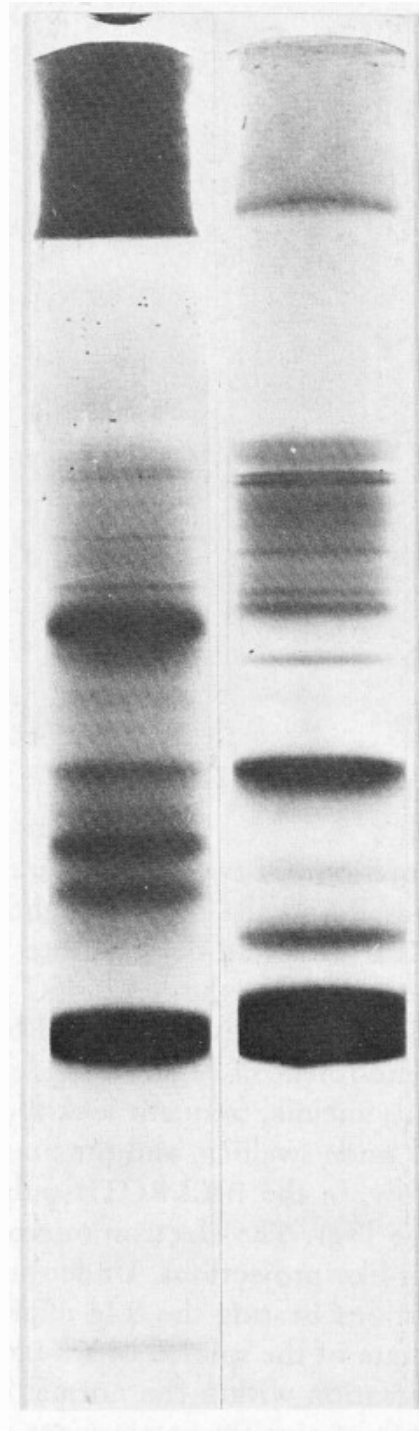


Fig. 6: Disc-electrophorogram of the lymph node homogenate (left) and the serum (right) of a CLL whose cells bear S-IgG. Note the monoclonal IgG-migration of the lymph node extract and the polyclonal IgG-migration of the corresponding serum.
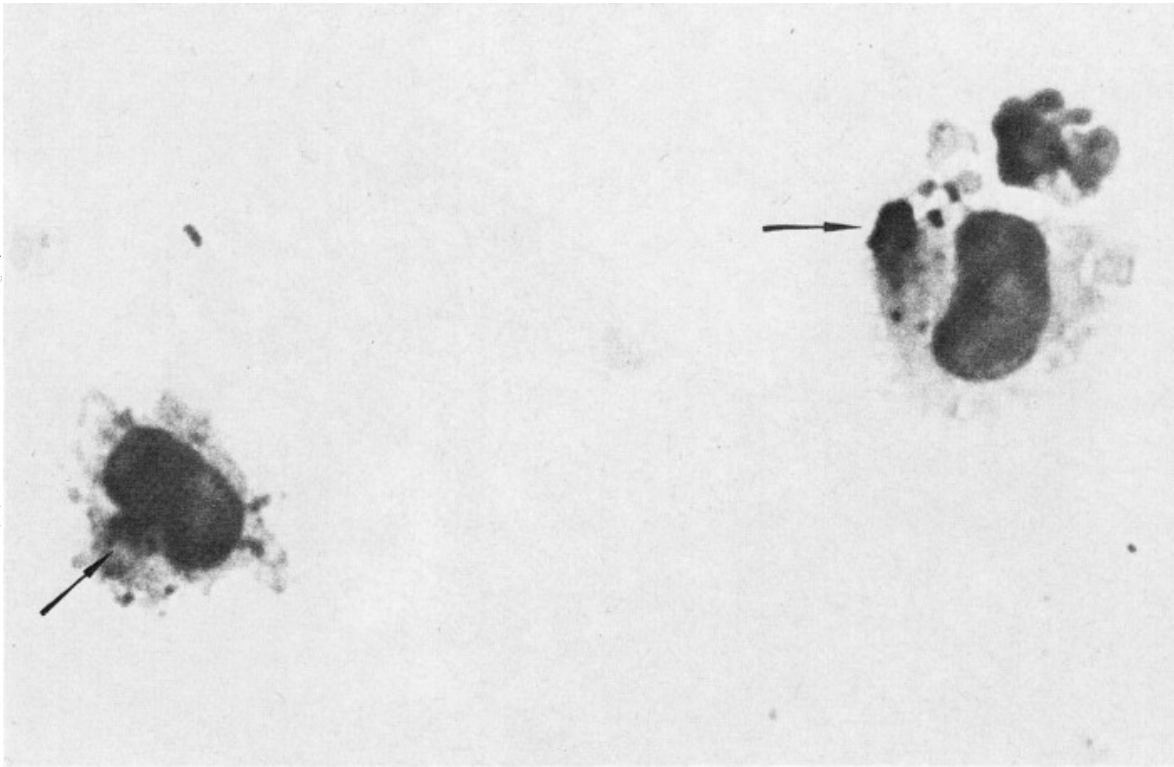
Fig. 7: "Hairy cells" labeled for S-IgM. The S-Ig-labeling (arrows) is collected at one pole of the cytoplasm. In the original slides the brown color of the S-Ig-labeling contrasted well with the blue-greyish counterstain of the cytoplasm. Haemalaun counterstain. 1,000 x

## Discussion

2/3 of the lymphomas we investigated contained significantly increased amounts of IgM in the tumor tissue homogenates. However, the question as to the cytological localisation of the Ig could not be answered by these analyses alone. Besides a negligible blood fluid contamination there are only two possibilities for the origin of the tissue-Ig:

1. The tissue-Ig was mainly derived from the Ig bound to the cell membranes.
2. The tissue-Ig was mainly derived from the interior of the cells.

With surface-bound-Ig there remains the question as to whether it was merely passively taken up by the cell membranes or whether it was produced by the cells themselves. To answer these questions we needed the help of a direct visualization of the Ig with labeled antibodies at the cellular level. The data presented prove that IgM sat on the surface membrane of many tumor cells. In contrast, intracytoplasmic Ig was not detectable with labeled anti-Ig-antibodies. This finding is consistent with those of PREUD'HOMME and SELIGMANN (1972) and others. However, since we found no correlation between the density of S-Ig and the amount of extracted tissue-Ig, we believe that some of the tissue-Ig must have originated from the surface membranes of the homogenized cells, but that most of the tissue-Ig originated from the cell sap. This assumption is supported by the experimental work
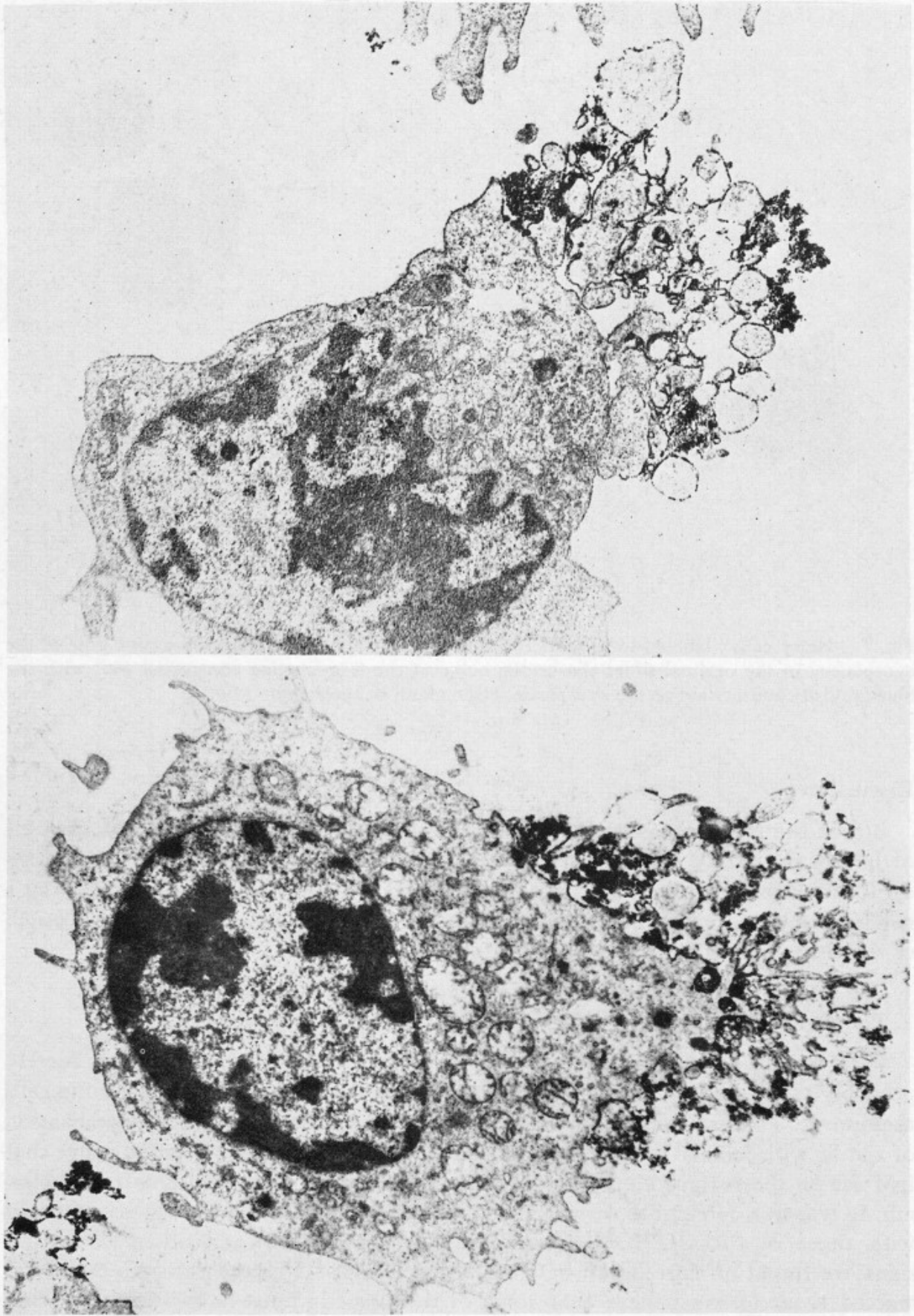
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Fig. 8 a and b: Electron micrographs of two cells from a hairy cell leukemia which were labeled with peroxidase-coupled antibodies for S-Ig. a 11,000 x b 13,000 x
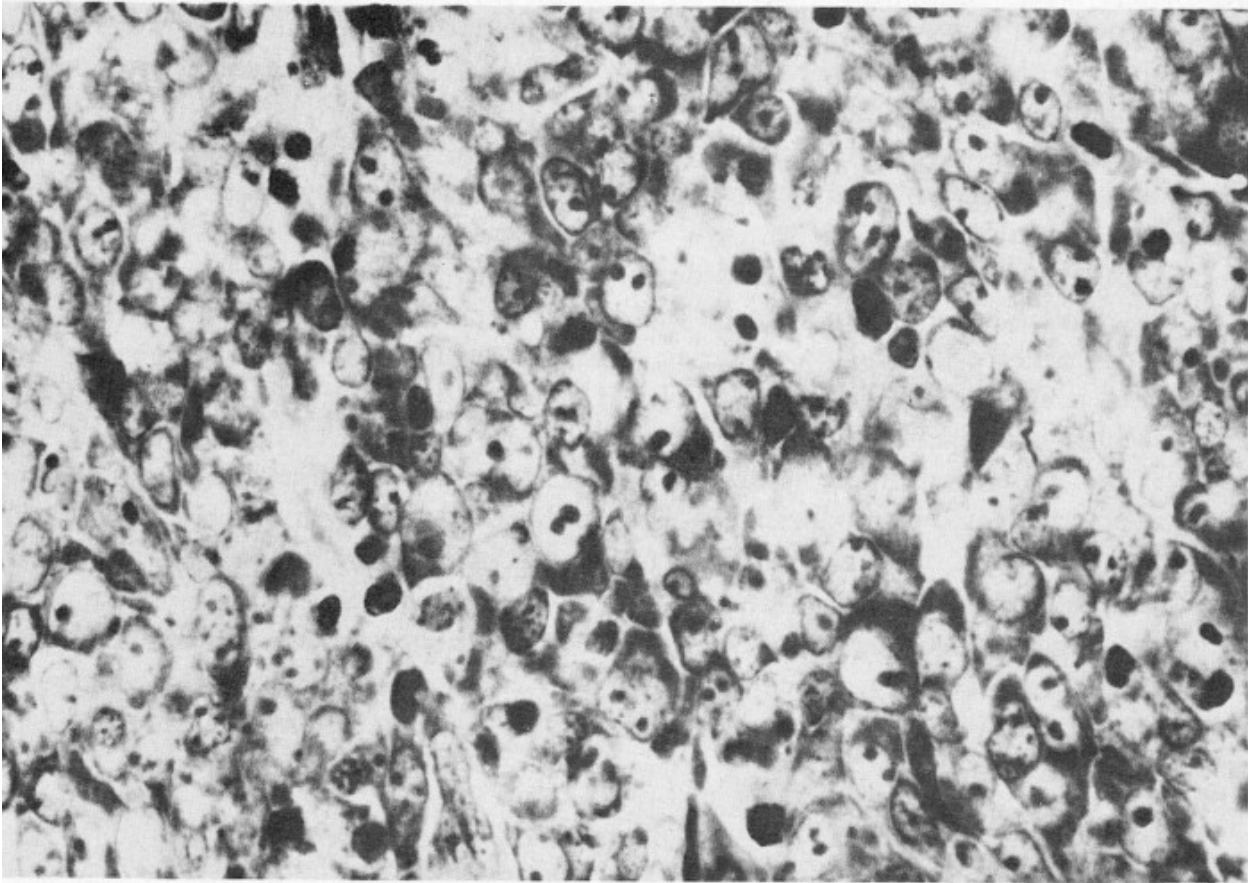
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Fig. 9: Reticulum cell sarcoma. Note the contrast between the transparent nuclei and the basophilic cytoplasm. GIEMSA-stain. 880 x

of SHERR et al. (1972). They reported that the amount of intracytoplasmic Ig far exceeded that of S-Ig in a BURKITT-lymphoma-cell-line when the two Ig's were measured separately with the coprecipitation technique. These findings indicate that the tissue-Ig in most of our cases was derived from the cell sap and therefore represented a synthesis product of the tumor cells themselves.

The direct demonstration of intracytoplasmic Ig with fluorescin or peroxidase labeled antibodies failed probably because of a low affinity of the labeled antibodies to the intracytoplasmic Ig of the fixed or frozen cells and not because of a total absence of intracytoplasmic Ig.

We conclude that not only S-Ig, but also tissue-Ig can be used a reliable marker for B-cell lymphomas, even though the latter marker is not as sensitive as the former. The main advantage of the tissue-Ig-marker is that tissue-Ig-determination is not bound to viable cells. The tissue-Ig-content can also be determined in deep frozen or lyophilized material.

It is striking that the increased tissue-Ig belonged to the IgM-class in all but 3 of our cases: 2 CLL produced IgG and 1 RCS produced IgA. Also interesting were the results of the S-Ig-labeling and the homogenate-Ig-determination for the case of hairy cell leukemia. Until now it was not clear whether hairy cells are derived from reticular or from lymphatic cells. There have been many arguments in favor of both alternatives. However, the positive Ig-labeling results and the detection of IgM in

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the homogenate of washed, purified hairy cells weight the arguments in favor of the lymphatic hypothesis. We therefore believe that hairy cell leukemia represents an irreversible proliferation of a special type of B-cells.

Ig-production was most constantly demonstrable in the so-called RCS-group. This finding is especially important since it sets the previously accepted nature of RCS-cells in doubt.

In 1930 ROULET described and defined „Retothelsarkom", which is now generally called RCS, as a tumor of cells of the reticulo-endothelial system with inclusion of the other phagocytic cell series. This conception was accepted all over the world and is still held valid today (BERNHARD and LEPLUS 1964; RAPPAPORT 1966; MORI and LENNERT 1969; MATHE et al. 1970; SCHNITZER and KASS 1973). The high accumulation of IgM in RCS and the demonstration of S-Ig on RCS-cells, however, speak decisively against a reticulocytogenous origin. Then, as far as we know, no one has been able to convincingly demonstrate Ig-synthesis in reticulum cells. We have also found no S-Ig-labeling of real reticulum cells, i. e. cells which are light microscopically characterized by strong non-specific esterase and acid phosphatase activity. Since Ig-production is a specific function of lymphatic cells, we believe that RCS, or at least those cases presented here, are of lymphatic origin.


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