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Evidence for B-Cell Origin of Reticulum Cell Sarcoma

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Summary. Sixteen cases of so-called reticulum cell sarcoma (RCS) of the lymph node were systematically studied using histological, cytochemical, electron microscopic, and immunochemical methods. In 8 cases some of the tumor cells showed PAS-positive globular cytoplasmic inclusions. The reactions for non-specific esterase or other marker enzymes were not positive in any case. Electron microscopically the cytoplasm contained many free polyribosomes. There was little ergastoplasm in most cases, but a moderately to strongly increased amount in 3 cases. The tumor tissue homogenate contained significantly increased amounts of IgM in 12 cases and of IgA in one case. Of the 12 IgM-positive RCS 5 showed serum IgM-levels within, 3 below, and only 2 above the normal range. This indicates that most of the RCS produced but did not secrete IgM. 2 cases labeled for surface-IgM revealed a positive reaction on a large number of tumor cells. We conclude that at least most of the RCS in our study were not derived from reticulum cells or histiocytes, but instead from lymphatic cells of the B-cell series. The morphologic similarity of the RCS-cells to "antigen-induced blasts" (called immunoblasts) suggests that RCS are derived from such blasts. This interpretation is especially supported by one case whose cells resembled immunoblasts not only morphologically but also with respect to the site of Ig-production: the perinuclear space. In general we therefore call RCS immunoblastic sarcoma of B-cell type and specify the tumor as plasmoblastic sarcoma if a significant amount of ergastoplasm is electron microscopically detectable.

In discussing reticulum cell sarcoma (RCS) it is essential to know what a reticulum cell (RC) really is. If we understand the confusing data presented in the literature correctly, 2 main types of RC are generally distinguished: the nonphagocytic RC that capture antigen on the cell surface and phagocytic RC that are capable of phagocytosis to an appropriate challenge (Maruyama and Masuda, 1965; Nossal *et al.*, 1968; Mori and Lennert, 1969; Veldman, 1970). The latter type is therefore regarded by many authors as a fixed macrophage (Gall, 1958; Rappaport, 1966; Carr, 1973; Cline and Gold, 1973). According to most pathologists RCS is derived from these phagocytic RC (Bernhard and Leplus, 1964; Kellner *et al.*, 1966; Rappaport, 1966; Lukes, 1968; Mathé *et al.*, 1970; Berard, 1972; Schnitzer and Kass, 1973). These cells are called histiocytes by Gall (1958) and Rappaport (1966) and numerous American pathologists. For RCS they therefore use the term malignant lymphoma, histiocytic type.

Reports of the occasional occurrence of paraprotein-"emia" or monoclonal macroglobulinemia in cases of RCS (Schaub, 1952; Azar *et al.*, 1957; Dutcher and Fahey, 1959; Krauss and Sokal, 1966; Waldenström, 1968; Worlledge *et al.*, 1968; Moore *et al.*, 1970) as well as the finding that the cells of some RCS contain relatively well developed rough endoplasmic reticulum (Bernhard and Leplus, 1964; Lennert, 1966; Okano *et al.*, 1966; Mori and Lennert, 1969) led us of assume that RCS-cells are more closely related to immunoglobulin(Ig)-producing

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cells than to reticulum cells. For two years we have therefore been studying the Ig-content of malignant lymphoma tissue. In preliminary communications (Stein *et al.*, 1972 a; Stein *et al.*, 1972 b) we reported on 4 cases of RCS where we were able to extract highly increased amounts of IgM from the tumor tissues compared to the amounts found in normal lymph node tissue. In order to examine whether this increased tissue-IgM represented a relatively constant finding we have now analyzed a larger series of RCS for the tissue Ig-content. In addition, we have investigated the cells of 2 RCS for the occurence of surface-Ig (S-Ig).

Material and Methods

Source of Material

The following investigations were performed on 120 malignant lymphomas. Most of them were sent by mail to our institute in special containers from various hospitals and institutes of pathology in Germany and Austria. Before shipment the tumor tissues were cut into 3 portions and packed as follows: one portion was put into formalin, the second was fixed for 2 hours in glutaraldehyde and then put into cacodylate buffer, and the third (the largest) was placed in a thermos bottle and covered with dry ice. Imprints were made from the fresh cut surface of each tumor. Serum samples were also placed in the thermos bottle.

Light Microscopy

The part of the tumor tissue fixed in 10% formalin was embedded in paraffin. The sections were stained with the following methods: hematoxylin-eosin (H & E), Giemsa (Merck, Germany), PAS, and Ladewig (1938) and silver-impregnation (Gomori).

Enzyme-Cytochemistry

The activities of acid phosphatase, non-specific esterase, naphthol-AS-D-chloroacetate-esterase, and endogenous peroxidase were studied in the imprints of more than 30 RCS-cases. The enzyme reactions were performed according to the methods reported by Leder (1967).

Electron Microscopy

Samples from the lymph nodes were cut into 1 or 2 mm^2 pieces and placed in 5% glutaraldehyde (0.1 M cacodylate buffer, pH 7.3) for 2 hours. They were post-fixed in 1% OsO_4 for 2 hours at 4° C after washing in cacodylate buffer. Then they were gradually dehydrated in acetone and embedded in Araldite. Ultrathin sections were contrasted with uranyl acetate followed by lead citrate and examined with a Siemens Elmiskop I at 80 kV.

Determination of Tissue-Ig

Unfixed, deep-frozen biopsy material was lyophilized, re-suspended in saline solution, homogenized, and kept for 1 hour at 4° C. After centrifugation at 105000 g for 60 min at 4° C the supernatant was examined for its concentration of IgM, IgA, IgG, and albumin with radial immunodiffusion using Hyland-immunoplates (USA). Serum-Ig-analyses were done as well. The Ig-content of the lymphoma tissues was expressed as the ratio of I. U. Ig/mg albumin. An increase in this ratio in comparison to that for the serum meant a true Ig-increase and fairly excluded an Ig-increase in the tumor tissue caused merely by contamination with serum proteins.

Demonstration of Surface-Ig

Surface-Ig (S-Ig) was demonstrated with the indirect immunoperoxidase technique described previously (Stein and Drescher, 1973). Polyvalent goat-anti-human-Ig-serum (GaHIg-serum) and monospecific goat-anti-human-IgM-serum (GaHIgM-serum) were obtained from Hyland (USA). A Rabbit-anti-human-IgM-serum (RaHIgM-serum) was obtained

Table 1. Results of S-Ig-labeling with peroxidase-coupled antibodies (indirect procedure)

Experiment No.	1st reagent added	2nd reagent added	% of labeled cells Case 5	% of labeled cells Case 8
1.	GaHlgS[a]	RaGlgGa-HRP [f]	94	85
2.	GaHlgMS[b]	RaGlgGa-HRP [f]	96	77
3. control	GNS[c]	RaGlgGa-HRP [f]	2	5
4. control	TC-199	RaGlgGa-HRP [f]	—	—
5.	RaHlgMS[d]	SaRlgGa-HRP[g]	94	69
6. control	RNS [e]	SaRlgGa-HRP[g]	—	1
7. control	TC-199	SaRlgGa-HRP[g]	—	—

[a] GaGlgS = Goat-anti-human-Ig-serum.
[b] GaHigMS = Goat-anti-human-IgM-serum.
[c] GNS = Goat-normal-serum.
[d] RaHlgMS = Rabbit-anti-human-IgM-serum.
[e] RNS = Rabbit-normal-serum.
[f] RaGlgGa-HRP = Rabbit-anti-goat-IgG-antibody-horseradish-peroxidase.
[g] SaRlgGa-HRP = Sheep-anti-rabbit-IgG-antibody-horseradish-peroxidase.

from Behring, Germany. Rabbit-anti-goat-IgG-sera (RaGIgG-sera) were raised by immunizing rabbits with goat-IgG prepared using chromatography on DEAE-cellulose in 0.0175 M phosphate buffer, pH 6.5. Sheep-anti-rabbit-IgG-sera (SaRIgG-sera) were raised using purified rabbit-IgG, also obtained with DEAE-cellulose-chromatography. The RaGIgG- and SaRIgG-sera were exhaustively absorbed with insolubilized human serum proteins. Purified antibodies of these antisera were obtained using affinity chromatography on the corresponding insolubilized antigen according to the method described by Avrameas and Ternynck (1969). The SaGIgG- and SaRIgG-antibodies were coupled to horseradish peroxidase (HRP) Type VI (Sigma Chemical Co., USA) using the one-step procedure (Avrameas, 1969). Remaining active sites of the coupling agent, glutaraldehyde, which have aften led to nonspecific labeling results, were inactivated with dialysis against 0.2 M lysine buffer. The unconjugated HRP was removed with ammonium sulfate precipitation (50%) repeated 4 times.

All of the antisera used were found to be monospecific when tested with the double diffusion technique, with immuno-electrophoresis, and with the very sensitive reverse radial immunodiffusion test (Reimers *et al.*, 1970).

The cells were labeled using the following procedure. RCS-cells were passed through a stainless steel mesh, suspended in TC-199 containing 5% fetal-calf-serum (both from Difco, USA) and 0.005 M sodium-EDTA, washed 3 times, and incubated in anti-human-Ig-sera (first reagent), followed by 3 washings and incubation in the appropriate HRP-labeled antibodies (second reagent). The details of specific and control incubations are shown in Table 1. After incubation in the HRP-labelled antibodies the cells were washed 3 times and centrifuged onto glass slides with a cytocentrifuge. The slides were dried for 12 hours and then incubated in Graham and Karnovsky's medium (1966) for 20 min. After washing with tap water, the slides were counterstained with Haemalaun for 30 min and mounted with Eukitt (Merck, Germany).

Results

Of 120 malignant lymphomas 16 revealed a histological picture similar to that of large cell type RCS described by Lennert (1964) and malignant lymphoma, histiocytic type described by Rappaport (1966) and Lukes (1968). Figs. 1–3 show representatives of our RCS series in a hematoxylin-eosin and Giemsastain. The normal lymph node architecture is replaced by sheets of large cells varying from 12–50 μ in diameter, with large, well-defined, chromatin-poor nuclei containing one or more distinct nucleoli. The nuclei are mostly round to oval but

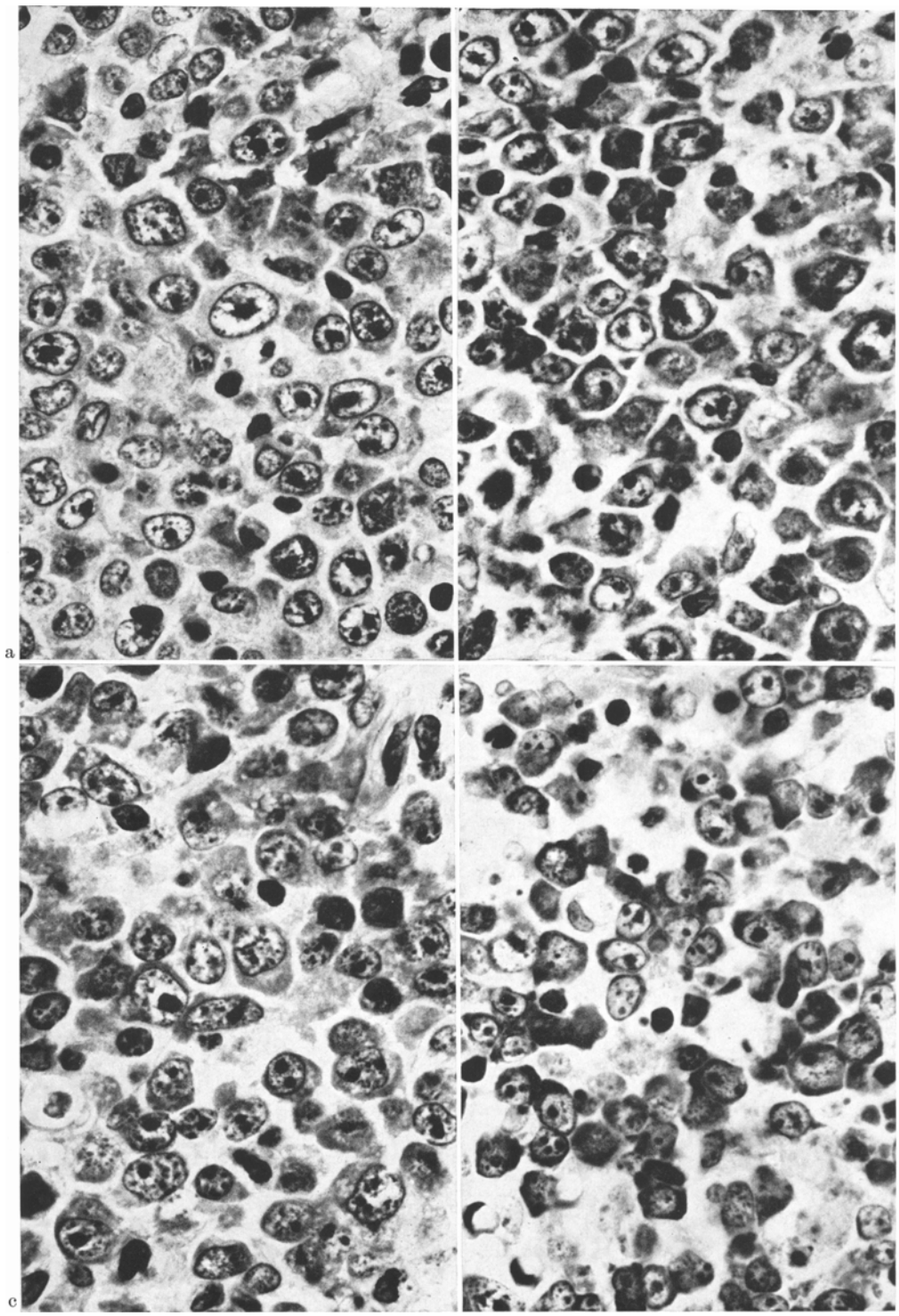


Fig. 1. a Reticulum cell sarcoma (RCS), Case 10 of Fig. 5. H & E-stain. ×840. The tissue homogenate of this RCS showed a 24-fold increase of IgM. b RCS, Case 10; Giemsa-stain, ×840. c RCS, Case 15; H & E-stain, ×840. The tissue IgM of this case lay within the lower half of the normal range. d RCS, Case 15; Giemsa-stain ×840

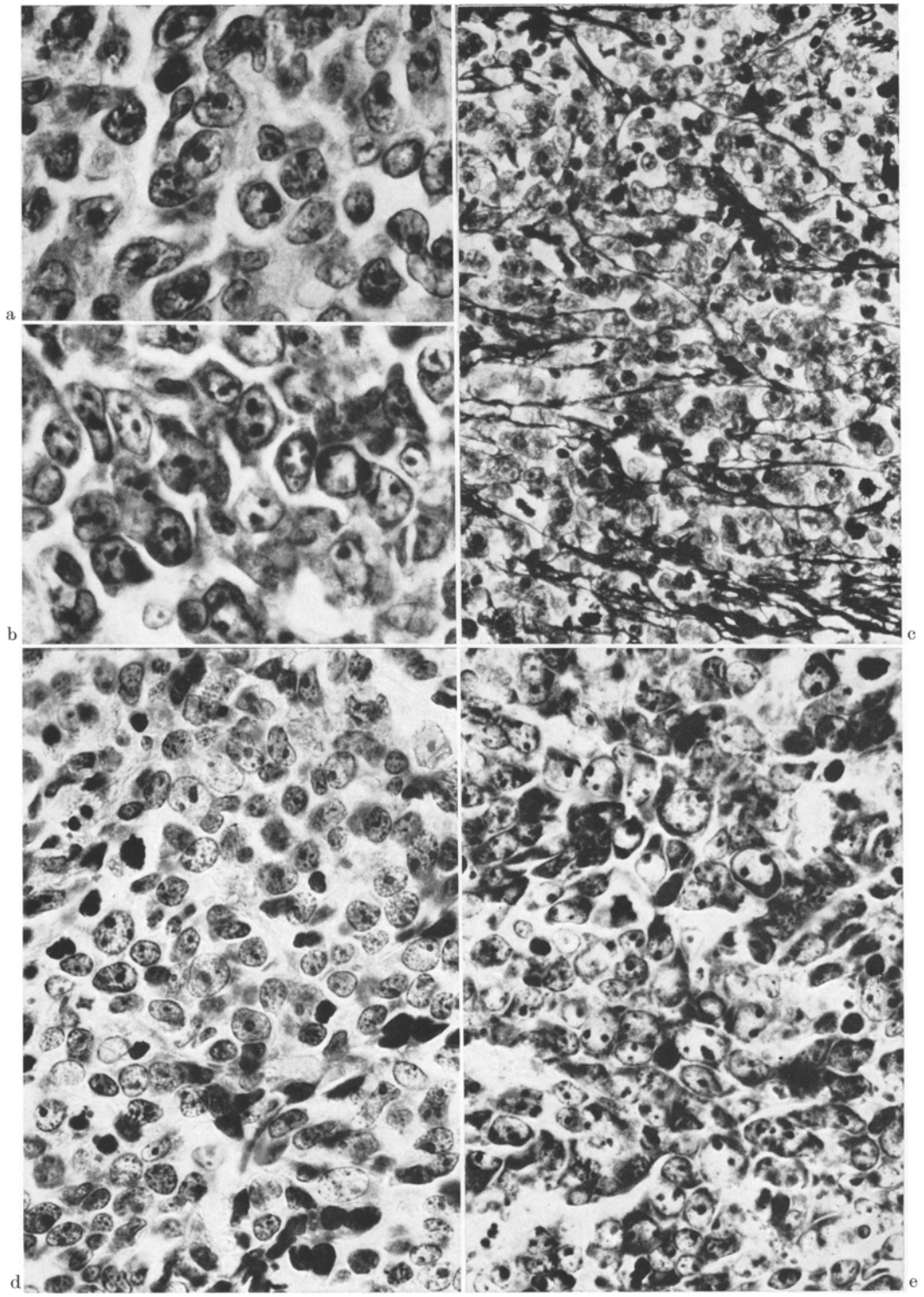
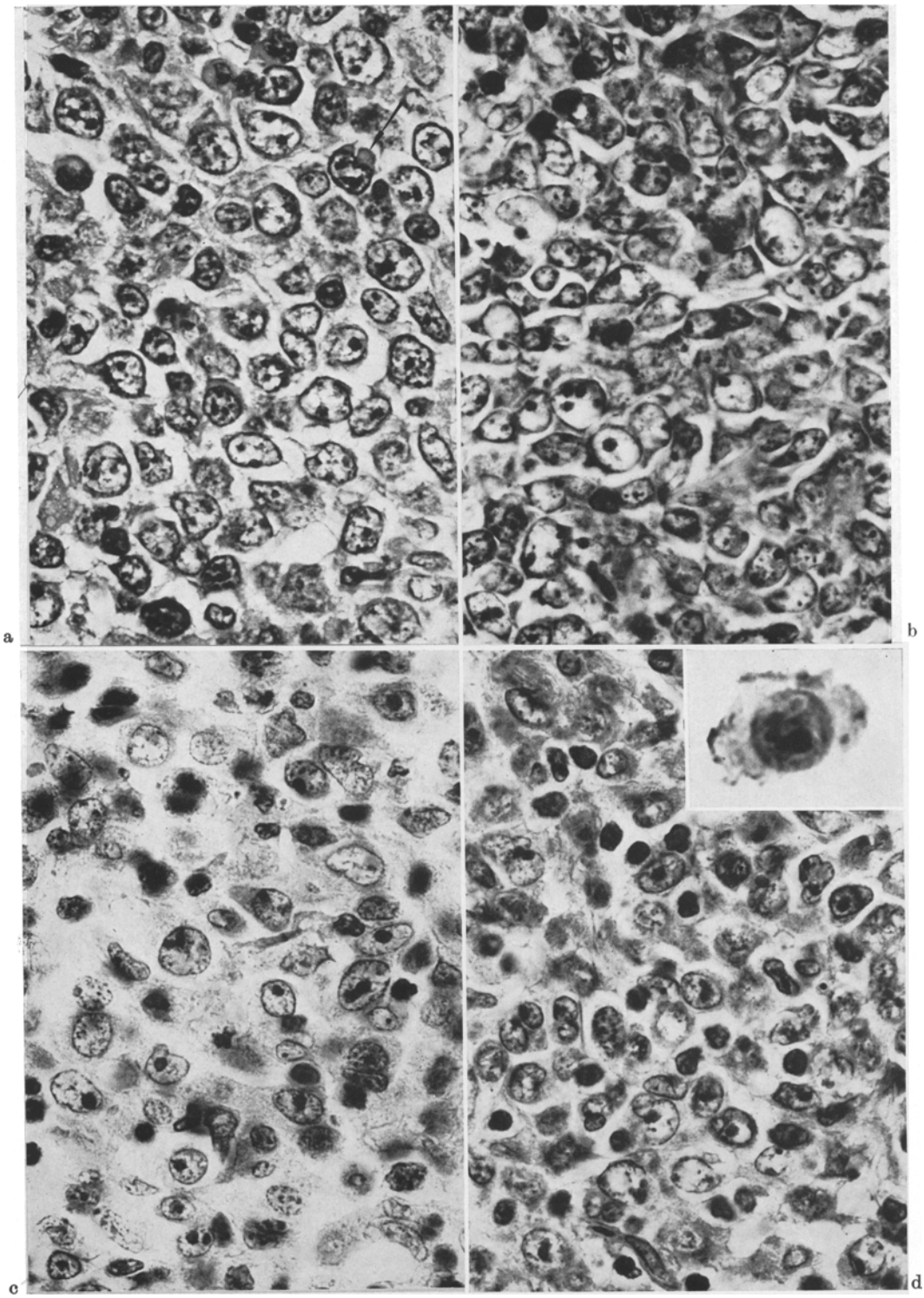


Fig. 2. a RCS, Case 2 of Fig. 5. H & E-stain, ×840. The tissue IgM was increased 47 times. b RCS, Case 2; Giemsa-stain, ×840. c RCS, Case 2; silver-stain, ×840. d RCS, Case 5; H & E-stain, ×560. The tissue IgM was increased 20 times. e RCS, Case 5; Giemsa-stain, ×560



Figs. 3a—d

sometimes irregular in shape or deeply indented. The cytoplasm varies in quantity. With the H & E stain it is often hard to define because of the pale staining with eosin.

With Giemsa the nuclei stain similar to those in the H & E stain. In contrast, the cytoplasm stains moderately to strongly basophilic and thereby becomes clearly visible. Beside the cell size, the light nuclear chromatin, and the prominent nucleoli, we consider this contrast between the light nuclear chromatin and the deep blue to violet staining of the cytoplasm as one of the most typical morphological characteristics of this tumor group. Beside this distinctly neoplastic cell type the tumors contained varying amounts of reticulum cells, macrophages and epithelioid cells. Some of the macrophages had phagocytized material within the cytoplasm. These cells showed neither in sections nor in imprints atypias characteristic of neoplastic proliferation.

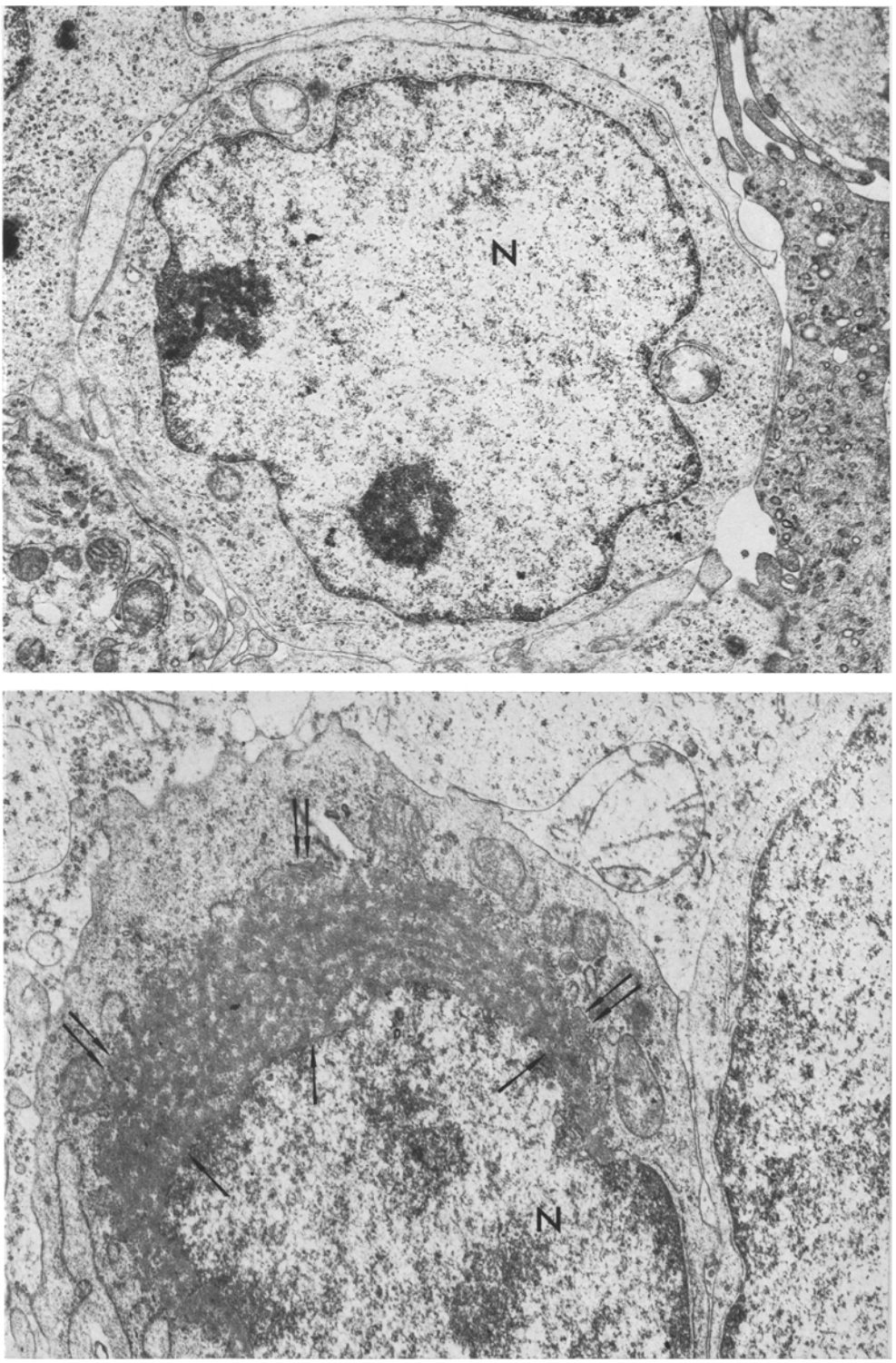
The fiber-content of RCS, which is thought by some authors to be of special diagnostic relevance (Warren and Picena, 1941) differed greatly in our RCS-series, even though the often described catkin-like accumulation of the tumor cells on reticulum fibers was often visible (Fig. 2c).

For more than 30 RCS the acid phosphatase, non-specific esterase, chloroacetate-esterase, and peroxidase reactions were performed on imprints. We did not find a reaction-pattern of the tumor cells similar to that of normal histiocytic reticulum cells, macrophages or monocytes. However, in some cases we found a large number of reticulum cells and macrophages with large amounts of acid phosphatase and non-specific esterase.

The PAS-reaction revealed globular PAS-positive cytoplasmic inclusions isomorphous with Russell bodies in the tumor cells of 8 cases after extensive study. These inclusions stained blue-greyish with Ladewigstain.

Electron microscopically the RCS of our series (Fig. 4a) consisted of cells which were in general characterized by a high nuclear-cytoplasmic ratio. The large nuclei were round to oval in shape and electron transparent. The heterochromatin was dispersed in small spots over the nuclei. The prominent nucleoli were sometimes centrally and sometimes marginally located. The cytoplasm contained monoribosomes, but mainly polyribosomes. With 3 exceptions, the cells of the RCS were poor in rough endoplasmatic reticulum (ER). However, after prolonged search, some ER-rich tumor cells could be found among the ER-poor ones, especially in those cases with PAS-positive inclusions. Interestingly, the cells of one RCS

Fig. 3. a RCS, Case 3; H & E-stain, ×840. The tissue IgM was increased 24 times. Note the eosin-stained material very close to the nucleus of one of the tumor cells (arrow) and compare with Fig. 4b. b RCS, Case 3; Giemsa-stain, ×840. c RCS, Case 16 of Fig. 5 H & E-stain, ×840. The tissue IgM was higly reduced. Instead IgA was increased 4 times in the tumor tissue homogenate. The cytoplasms of the tumor cells were as basophilic as in the other cases d RCS, Case 7 of Fig. 5. H & E-stain, ×560. This RCS represents the classical histiocytic subtype, c.f. Rappaport: Atlas of Tumor Pathology. Tumors of the hematopoietic system, Fig. 109. The tissue IgM was increased 9 times. Inset: Representative RCS-cell of Case 7 labeled for surface IgM with horseradish-peroxidase-coupled antibodies. Note the dark peroxidase-reaction-products which represent the labeled receptor-IgM-molecules on the cell surface membranes. In the original slide the brown peroxidase-reaction-products contrasted well to the blue-greyish counterstain of the nucleolus and cytoplasm of the labeled cells. Hemalaun-counterstain. ×1200



a

b

Fig. 4a and b

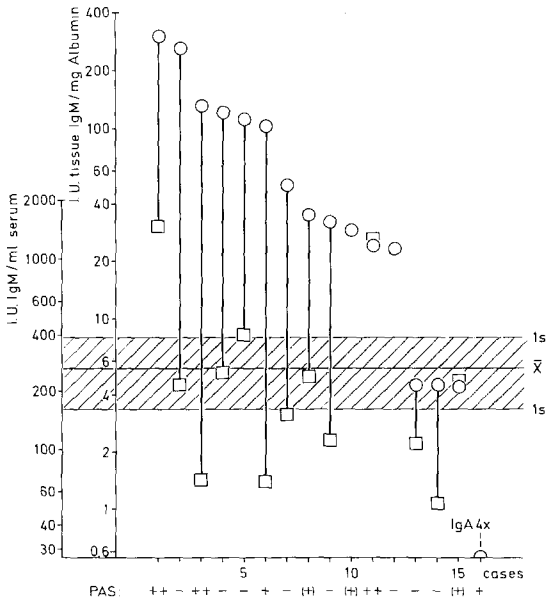


Fig. 5. Histologically typical RCS arranged according to their tissue-IgM-content (○). The corresponding serum-IgM-level is symbolized by □

showed electron dense inclusions located in the distended perinuclear space (Fig. 4 b), as well as typical polysome-rich and ER-poor cytoplasms.

We never found distinct morphological signs of fiber-production by the tumor cells. Phagolysosomal inclusions were not visible in the tumor cells. In contrast, these inclusions were more or less abundant in macrophages or phagocytic RC, which occurred in varying amounts in the individual RCS. Some RCS also contained cells which were cytologically similar to or isomorphous with epithelioid cells. Dendritic and interdigitating RC were not observed.

The results of the Ig-determination of the tumor tissues and corresponding sera of the 16 RCS are shown in Fig. 5. 12 of the 16 RCS revealed a significant and 7 of these a more than 10-fold increase of IgM in the tissue extracts. Normal amounts of IgM could be extracted from only 3 RCS. One case even showed a

Fig. 4. a Electron micrograph of RCS Case 3 of Fig. 5 (the light microscopic picture of these tumor cells is presented in Fig. 3 a and b). Note the high nuclear-cytoplasmic-ratio, the transparent heterochromatin, the two prominent nucleoli, and the cytoplasm rich in polyribosomes. ×14000. b RCS cell from the same case with a widely distended perinuclear space in which electron-dense thread-like material is embedded. The cytoplasm is rich in polyribosomes whereas a rough endoplasmatic reticulum is nearly completely absent. The large amount of IgM in the tumor tissue homogenate indicates that the deposits in the perinuclear space represent IgM. From these data it was concluded that these tumor cells had just shifted from the synthesis of nonsecretory IgM to the synthesis of secretory IgM, which regularly begins in the perinuclear space. 1 arrow = inner nuclear membrane, 2 arrows = outer nuclear membrane. N = nucleus. ×20000

nearly 10-fold reduction in the tissue-IgM and instead had a 4-fold increase of IgA. Fig. 5 also shows that the serum-IgM-levels did not correlate with the tissue-IgM-concentrations. Of the 12 IgM-positive RCS 2 revealed significantly increased serum-IgM-values, 5 had values in the normal range, and 3 reduced values. The serum-IgM-level of the 3 RCS with a normal tissue-IgM-content was reduced in 2 cases and lay within the normal range in the 3rd.

Notable is that there were no clear or constant histological differences between the RCS with a high amount of tissue-Ig and those with a normal or reduced tissue-Ig. The case with the IgA-increase also did not differ morphologically. Evaluating the amount of polysomes and rough ER in comparison to the tissue- and serum-Ig-concentrations, we had evidence for a striking correlation between these parameters. Details will be presented elsewhere.

The results of the labelling experiments are presented in Table 1. The cells of the two RCS (Case 5 and Case 7, see Figs. 2a, d and 3d) we labeled showed a specific labeling with the antisera directed against human Ig and human IgM. The anti-IgM-sera from different sources gave similar results. In Case 5, 94–96%, and in Case 7, 69–70% of the cells showed a positive reaction for μ-chains. In Case 7 the labeling with anti-Ig-serum directed against IgG, IgA, and IgM revealed a slightly larger number of positive cells than labeling with the anti-IgM-sera.

The controls, for which the first reagent, the anti-Ig-serum, was substituted with TC-199, were negative. The controls with normal goat serum or normal rabbit serum, when absorbed with insolubilized human serum proteins, showed only short and weak bands of peroxidase reaction product on 2–5% of the cells. In contrast, the specific labeling was represented by a strong, dense brown peroxidase reaction product on the cell membrane (Fig. 3d, inset).

Discussion

In 1930 Roulet described under the name "Retothelsarkom" a lymph node tumor with a histological picture similar to that of the cases described here. This type of tumor was quickly adopted under the name reticulum cell sarcoma by most pathologists as a new group of lymph node tumors outside the already recognized lymphosarcoma and Hodgkin's disease. While Oberling (1928) considered the cells of this tumor form to be reticulum cells, differentiated from lymphocytes, and Ewing (1928) regarded them as immature lymphocytes derived from "germinal follicle cells", Roulet (1930) believed that RCS-cells are derived from reticulum cells originating from mesenchyma or histiocytes. His concept was soon generally accepted and is still held valid today (Bernhard and Leplus, 1964; Kellner *et al.*, 1966; Rappaport, 1966; Lukes, 1968; Berard, 1972; Akazaki, 1973; Carr, 1973; Cline and Gold, 1973; Schnitzer and Kass, 1973). There have been repeated attempts to distinguish two histological subtypes of RCS: 1) undifferentiated RCS (Oberling, 1928; Bernhard and Leplus, 1964; Rappaport, 1966) Retothelsarcom, "unreife Form" (Roulet, 1930), stem cell sarcoma (Gall and Mallory, 1942) or histioblastic RCS (Mathé *et al.*, 1970); 2) differentiated RCS (Oberling, 1928; Bernhard and Leplus, 1964), Retothelsarcom, "reife Form" (Roulet, 1930), dictyocytic (Robb-Smith, 1938), clasmatocytic (Gall and Mallory, 1942) or histiocytic sarcoma (Mathé and Seman, 1963; Rappaport, 1966; Lukes, 1968).

Our series contained RCS of both subtypes; the histiocytic type seemed to predominate. However, due to the lack of hard and objective criteria we consider the classification of our cases into one of these subtypes as neither very informative nor useful.

Our findings do not support the reticulocytic origin of RCS. 75% (12/16) of the RCS investigated contained significantly increased amounts of IgM in the tumor tissue homogenates, compared with that in the homogenates of lymph node tissue showing slight nonspecific lymphadenitis. The Ig-contents of lymph node tissue were chosen as reference values because they represent the tissue-Ig-range under non-neoplastic conditions. Since non-neoplastic lymph nodes normally contain only one third B-cells (Raff *et al.*, 1970; Stein, 1973), we believe that most lymphomas composed only of B-cells should reveal a higher concentration of tissue-Ig than non-neoplastic lymph node tissue. However, one has to consider that the tissue-Ig-values also include the Ig's which are derived from the serum of the extracellular space of the tissue. For these reasons only those lymphomas were recognized as Ig-producers whose Ig-content exceeded that of normal lymph node tissue. Low Ig-producers could not be identified. The possibility that the IgM-increase in the tumor tissues was caused merely by blood fluid contamination can be disregarded for the following reasons:

a) The tissue homogenate-Ig was expressed as a ratio of Ig/albumin. Since albumin is synthesized only by liver cells, it represents a suitable marker for serum-protein contamination of organs other than the liver. Any measured increase in the Ig/albumin ratio therefore meant a true Ig-increase in the tissue homogenate; b) there were no correlations between the measured tissue-IgM and the corresponding serum-IgM-levels. As Fig. 5 shows, the serum-IgM-level was even often reduced in cases with high tissue-Ig-values.

Beside blood fluid contamination there are two further possibilities which should be discussed for the origin of the tissue-Ig:

a) The tissue-Ig originated mainly from the interior of the cells;

b) the tissue-Ig originated mainly from Ig bound to the cell membranes.

We had morphological evidence of intracytoplasmic Ig from the PAS-reaction and from the electron microscopic findings for some RCS. After prolonged search, globular, PAS-positive cytoplasmic inclusions were found in some tumor cells of 8 of the 16 RCS. For some cases it could be electron microscopically demonstrated that the inclusions were embedded in the cisternae of the rough ER of the tumor cells. With the Ladewig-stain, which stains IgG and IgA orange to red and IgM blue-greyish (Lennert, 1973), the PAS-inclusions in these tumors stained blue-greyish. For this reason it seems highly probable that the cytoplasmic inclusions represented IgM produced by the tumors cells themselves, and that the increased amounts of tissue-IgM originated from these cytoplasmic inclusions and therefore from the interior of the cells.

The remaining 5 RCS with increased tissue-IgM showed no such direct signs of Ig-production. Electron microscopically the cells of these RCS were particularly rich in polysomes and poor in rough ER. Attempts to localize the IgM at the cellular level with labeled antibodies in these cases were only partly successful Intracytoplasmic Ig is detectable only on fixed cells whereas S-Ig only on viable ones. Neither light microscopically nor electron microscopically were we able to

reliably demonstrate intracytoplasmic Ig using peroxidase-coupled antibodies prepared with the one-step-procedure described by Avrameas (1969). Even with smaller antibody-conjugates such as fluorescin-labeled antibodies the detection of intracytoplasmic Ig was often impossible in Ig-producing cells of the ribosomal (non-secretory) type, i.e. CLL-cells, Burkitt-lymphoma cells and others (Preud' Homme and Seligman, 1972; Knapp, 1973). This was probably due to the high molecular weight of the one-step conjugate ($5–9 \times 10^5$ daltons estimated with Sephadex-G-200-chromatography), the comparatively slight accumulation of Ig within the cell sap, and to the unavoidable background with fixed cells. We were therefore not convinced by the findings reported by Suchi *et al.* (1973) from labeling studies on RCS, which were done after the presentation of our preliminary results on increased IgM in RCS at the lymphoma meeting in Nagoya, 1971.

In contrast, S-IgM-molecules could be clearly demonstrated on a high percentage of the cells of two RCS of the ribosomal type which we had the chance to label. We assume that the other polyribosomal RCS with increased tissue-IgM also bore surface-IgM.

However, with surface-Ig there remains the question as to whether it was merely taken up by the cell membranes or whether it was produced by the cells themselves. At the present state of knowledge there are three possibilities for the origin of surface membrane-bound Ig:

a) Autoantibodies directed against the tumor cells and produced by the unaffected lymphatic tissue accumulated at the tumor cell surface membranes; b) Ig or immunocomplexes were attached to the cell membranes via Fc-receptors (Basten *et al.*, 1972; Dickler and Kunkel, 1972), or complement-receptors (Bianco *et al.*, 1970); c) antigen-IgM-complexes, which are now thought by some authors (Feldman, 1972; Gutman and Weissman, 1972) to be derived from the reaction of the antigen with T-cells, may have been bound to the surface of dendritic reticulum cells.

However, for the following reasons we believe that the surface and tissue-Ig of RCS represent rather a synthesis product of the same cells than picked-up Ig:

a) In all RCS only 1 Ig-class was found to be increased in the tissue homogenates. If all of the tissue-Ig in RCS were derived from autoantibodies on the cell membranes, one would expect an increase of more than one Ig-class in the tissue homogenates.

b) Ig bound to the surface membrane via Fc-receptors belonged to the IgG and not to the IgM or IgA class (Basten *et al.*, 1972). Besides the receptor-bound Ig is bound only in the form of immunocomplexes. Our extraction method does not split antigen-antibody bridges. Therefore such immunocomplexes, if present, should remain bound to the membranes and thus largely escape detection.

c) Surface-IgM-producing lymphatic cells predominate in man under normal conditions and in CLL and lymphosarcoma Pernis *et al.*, 1971; Siegal *et al.*, 1971; Grey *et al.*, 1971; Aisenbarg and Bloch, 1972; Preud'homme and Seligman, 1972; Stein *et al.*, 1972). The increase of the IgM-class in RCS is consistent with these findings.

d) For a large number of lymphomas the de novo synthesis of surface-Ig has been well established by several authors (Lerner *et al.*, 1972; Preud'Homme and Seligman, 1972; Sherr *et al.*, 1972).

e) Since we found no correlation between the density of surface-Ig-labeling and the amount of tissue-Ig in lymphatic neoplasias (Stein *et al.*, 1973), it seems rather unlikely that surface-Ig can alone give rise to such highly increased tissue-Ig as we found in RCS (up to 70×). This conclusion is supported by the experimental work of Sherr *et al.* (1972) who showed that the amount of intracytoplasmic Ig also far exceeded that of surface-Ig in polysome-rich and ergastoplasm-poor lymphoma cell line cells (Daudi cells).

Since, as far as we know, no one has been able to convincingly demonstrate Ig-synthesis in reticulum cells, the high incidence of tissue-Ig-increase in RCS-tissue suggests that RCS is in most cases lymphatic and not reticulocytic in origin.

However, the former concept of the reticulocytic origin of RCS is understandable if one considers that it was based mainly on the judgement of H & E stained sections (Roulet, 1930; Lumb, 1954; Rappaport, 1966). With the H & E-stain RCS-cells are often very similar to reticulum cells and macrophages, especially in the nuclear features. Immature myeloid sarcomas can also resemble RCS. One of the 120 cases in our series of malignant lymphomas was definitely indistinguishable from typical RCS in H & E stained sections and was first put in the RCS-group. However, positive reactions of the tumor cells for non-specific esterase, peroxidase, acid phosphatase and chloroacetate-esterase identified their origin as the myelopoietic cells of the bone marrow. The Ig-content of this tumor was extremely low.

The reticulocytogenetic concept of RCS is also understandable since this concept goes back to a time when it was believed that reticulum cells were the only potential cells from which a variety of other cells could originate. At that time the blast-transformation and the staining features of transformed lymphocytes were not known. Now, in view of the capacity of lymphatic cells to proliferate and transform, the lymphatic concept of RCS-origin fits well with our morphologic and immunochemical findings:

a) In sections and especially in imprints stained with Giemsa or Pappenheim the RCS-cells showed a remarkable cytological similarity to blast-transformed lymphocytes, which Damashek (1963) called immunoblasts.

b) The electron microscopic findings gave the same impression. The RCS-cells were most similar to immunoblasts and partly to plasmoblasts. It should be noted that we did not find a syncytium in any RCS-cases, which has been regarded by some authors as typical for the undifferentiated type of RCS (Bernhard and Leplus, 1964; Rappaport, 1966; Akazaki, 1973; Cline and Gold, 1973).

c) The high IgM contents of the RCS investigated conform well with the experimental data of Melchers (1973) who reported that mitogen-stimulated B-cells show a remarkably increased IgM-synthesis.

d) The tumor cells in RCS never looked like phagocytic, reticulin associated, interdigitating or dendritic reticulum cells. In addition we never found signs of phagocytosis or of fiber production by the tumor cells nor did we find desmosomes between the tumor cells, which are characteristic of dendritic reticulum cells.

The lymphatic concept is further supported by our enzyme cytochemical findings. All of the cells of the RCS investigated were devoid of non-specific esterase and poor in acid phosphatase. Both enzymes occur regularly in large amounts in normal histiocytic reticulum cells.

The large number of reticulum cells or macrophages demonstrated by Dorfman (1964) with the acid phosphatase and non-specific esterase reaction in some cases of RCS probably do not represent tumor cells, as the intensive study of imprints and electron microscopic sections showed. These cells also occur in lymphosarcoma and other lymphoproliferative disorders, which demonstrates that they have little significance for histiogenetic conclusions.

From a theoretical point of view and on the basis of our morphological and immunochemical findings there seem to be the following possibilities for the derivation of RCS-cells:

a) RCS-cells originate from plasmocytic cells, which dedifferentiate to large RCS-like cells during malignant transformation and proliferation. This view is supported by the transplantation studies of Osserman (1964) with oil-induced plasmocytomas in mice. After several transplantations the original plasmocytoma had morphologically transformed into a typical RCS. RCS-like dedifferentiation of cases of Waldenström's disease and other malignant lymphomas has been reported in the literature (Klemm *et al.*, 1968; Österberg and Räusing, 1970). We have made some similar observations.

b) The other alternative is that the tumor cells originate directly from B-cells which are in (=immunoblasts) or close after the transformation into ER-developing cells (=plasmoblasts). The greatly varying amount of ER between the cells of some individual tumors shows that some reveal the whole spectrum of this transformation cell series. This means that some sarcoma cells have not lost the ability, although limited, to transform and mature. Based on the predominating kind of proliferated cell one can separate RCS into 2 types: those consisting mainly of immunoblasts (hardly any ER) and those with plasmoblasts (some ER).

The direct immunoblastic derivation of RCS is supported especially by one case of RCS whose cells resembled immunoblasts not only morphologically but also with respect to the site of Ig-production. The cytoplasm was abundant in polysomes and nearly completely devoid of ER. Only the distended perinuclear space of 20% of the tumor cells contained electron dense inclusions. The experimental work of Avrameas *et al.* (1970) has shown that the site of production of secretory Ig is at first the perinuclear space of the immunoblasts. Later, with the transformation of these cells into plasma cells, the secretory Ig-production shifts to the cytoplasmic ER. Biochemical studies using cultured rabbit lymphatic cells immunized with $\Phi \times 174$ produced the same findings (Panijel *et al.*, 1971). In the cells of the case under discussion only the polyribosomes bound to the cell membranes showed secretory Ig-production, as do immunoblasts with incipient secretory Ig-synthesis. These data suggest that the cells of this tumor were derived from immunoblasts which were in the process of shifting from the synthesis of nonsecretory IgM to the synthesis of secretory IgM.

The question still remains as to whether there is also an immunoblastic sarcoma derived from T-cells. Based on the known morphology of T-cells and their derivatives (Veldman, 1970) we assume that immunoblastic sarcoma of the T-cell type is probably morphologically very similar to that of the B-cell type, except that it should lack cells containing globular PAS-positive cytoplasmic inclusions and/or significant amounts of ER. It is possible that the 3 RCS in our series with normal or reduced Ig-values were derived from T-cells. However, further investigation is required before this question can be resolved.

From the data presented we conclude that at least most RCS are lymphatic in nature and apparently originate from B-cell-derived immunoblasts or—if more differentiated—from plasmoblasts. T-cell immunoblastic sarcoma and true reticulum cell sarcoma probably exist but seem to be rare. We therefore propose the term immunoblastic sarcoma of B-cell type for this morphological tumor entity instead of the former terms "reticulum cell sarcoma" or "malignant lymphoma, histiocytic type". With additional electron microscopic study immunoblastic sarcomas may be separated into:

a) immunoblastic sarcoma, without plasmoblastic differentiation, if the tumor consists mainly of immunoblast-like cells which are rich in polysomes and poor in ER, and

b) immunoblastic sarcoma with plasmoblastic differentiation, if the tumor contains ER-developing cells in significant amounts.

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