

CYTOLOGY AND CYTOCHEMISTRY OF THE LEUKEMIC CELL

Beckmann, H.¹, Neth, R.¹, Gaedicke, G.¹,
Landbeck, G.², Schöch, G.¹, Wiegers, U.³, Winkler, K.²

- ¹ Molekularbiologisch-hämatologische Arbeitsgruppe und
² Abteilung für Gerinnungsforschung und Onkologie
Universitätskinderklinik, Hamburg
³ Physiologisch-chemisches Institut
Universität Hamburg

In contrast to the modern science of molecular biology, cytology and cytochemistry of human blood cells have a long history. Some of the principal mechanisms of protein biosynthesis were already discovered with cytological methods in the 19th century. In 1899, a young French anatomist, GANIER (8), demonstrated in his thesis the relationship between specific morphological changes in the cytoplasm of pancreatic glands and the secret production of these glands. During the synthesis of this specific pancreatic secret, the cytoplasm becomes basophilic. GANIER named this active cytoplasm: ergastoplasm, meaning that this cytoplasm produces something, however, we know today that this "something" is actually protein. With the lightmicroscope GARNIER has demonstrated in his experiments a relationship between acid groups in the cytoplasm and protein synthesis.

Forty years later BRACHET (3) and CASPERSON (4) demonstrated by cytochemical methods that this acid groups consisted of RNA and they found the dependent relationship between RNA synthesis and protein synthesis. Today we know that the cause of GANIER's basophilia is the high RNA content of the ribosomes, which are the centre of protein synthesis (5)

This short historical introduction has shown us that lightmicroscopy is necessary and not a hopeless method in modern biological research.

Studying the cytology and cytochemistry of the human leukemic cell, we can prove how valuable the lightmicroscope is. Therefore, we must ask ourselves the following questions:

1. What do we know about cytological and cytochemical markers in normal and leukemic cell differentiation?
2. Do we have specific markers which show us the leukemic transformation of a single blood cell?
3. Which of these markers can give us information for our diagnostic and therapeutic behavior with the leukemic patient?

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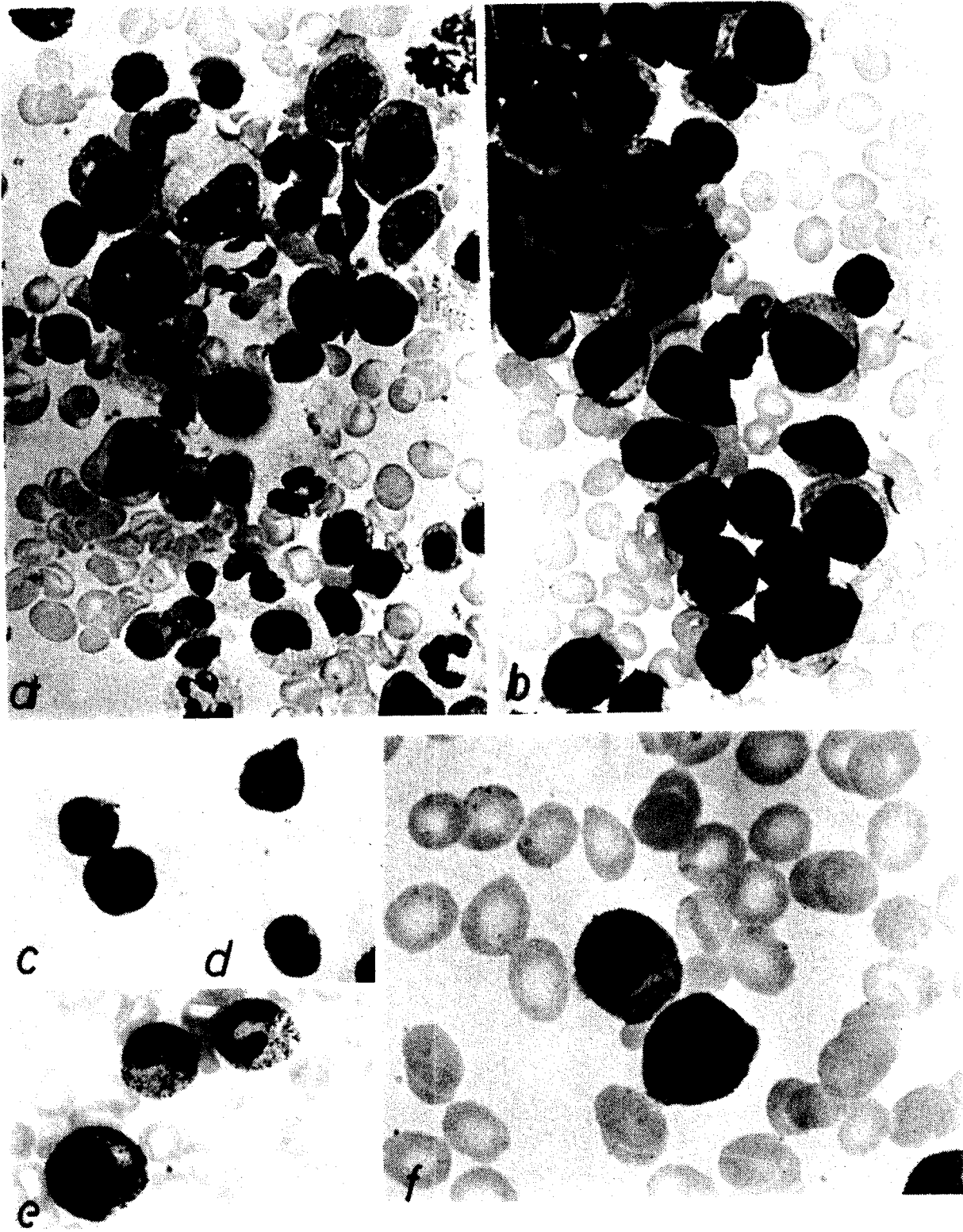

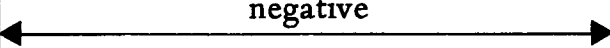

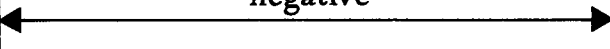

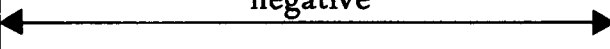
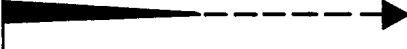

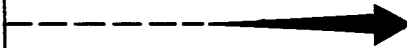




Fig. 1

- a) normal bone marrow, various stages of maturation of erythropoietic and leukopoietic cells,
 - b) leukemic bone marrow, blast cells, showing the same differentiation,
 - c+d) blasts with nuclear cleavage of the Rieder cell type,
 - e) pseudo pelger cell between a promyelocyte and a metamyelocyte,
 - f) Auer rods in the cytoplasm of a parpromyelocyte.
- a-e) 700 x; f): 1,500 x

CYTOCHEMICAL REACTIONS IN BLOOD AND BONE MARROW CELLS

Reaction	Promyeloc.—Neut. P. M. N.	Monocyte	Lymphocyte	Plasma Cell	Pronormbl.	Normobl.	Erythrocyte
PAS		weak diffus positive peripheral granules	negative, positive granules	negative			
PEROXY- DASE		negative, weak positive	negative	negative			
N-AS-D-CL- ESTERASE		negative, weak positive	negative	negative			
ALPHA-N- ESTERASE		positive, strong positive	negative, positive granules	weak positive	paranuclear positive	perinuclear positive	negative, positive granules
ACID PHOSPHA- TASE		positive, strong positive	negative, positive granules	diffus positive, positive granules	paranuclear positive	paranuclear positive, negative	negative
ALKALINE PHOSPHA- TASE		negative	negative	negative	negative		
SULFIDE SILVER		negative	negative	negative			

Tab. 1 methods for cytochemical reactions in blood and bone marrow cells: PAS: Mc MANUS and HOTCHKISS mod. (30), peroxydase: GRAHAM-KNOLL (30), naphthol-AS-D-chloroacetate-esterase: LEDER (17), alpha-naphthyl-acetate-esterase: LEDER (17), acid phosphatase: GOLDBERG and BARKA mod. (17), alkaline phosphatase: KAPLOW mod. (23), sulfide-silver: NETH et al. (24).

1. What do we know about cytological and cytochemical markers in normal and leukemic cell differentiation?

Besides the well known cytology of the different blood cell lines of granulopoiesis, erythropoiesis, lymphopoiesis and thrombopoiesis (Fig. 1 a), the specific cytochemical markers in each cell line are also well known (Tab. 1). Leukemic cells show abnormalities in the differentiation between the nucleus and cytoplasm (Fig. 1 b). In addition, these cells may show deformities of the nucleus, such as nuclear cleavage of the Rieder cell type (Fig. 1 c-d) or such as the acquired Pelger-Huet anomaly of neutrophils (Fig. 1 e). Abnormalities are also shown by azurophil granules, called Auer rods (Fig. 1 f) or by defective granularity in the eosinophils.

A cytological classification of the undifferentiated leukemic blast cells is mostly impossible (Fig. 2 a-h), however, leukemic cells can be classified with the aid of specific cytochemical markers based on the reactions in normal blood cells (19, 7, 10, 22, 1, 20, 27, 12, 18, 21). HAYHOE and his coworkers (10) used periodic acid Schiff (PAS)-, peroxidase- and sudan black reaction to classify the leukemic blast cells in lymphoblastic (Fig. 2 i), myeloblastic (Fig. 2 j), monocytic (Fig. 2 k) and erythroblastic cells. In addition to the above mentioned reactions we used: Naphtol-AS-D-chloroacetate-esterase (N-AS-D-Cl-esterase), alphanaphthylacetate-esterase (alpha-N-Esterase), acid and alkaline phosphatase and sulfide-silver reaction (Tab. 2).

To know how far cytochemical markers in leukemic blast cells might give genetic parameters we need, in addition to the cytochemical reactions, the biochemical analysis of the specific products in the blood cell lines. We already know that hemoglobin is a specific cell product in the erythropoietic cell line, therefore, in this cell line we could have a good chance to compare cytochemical markers with biochemical analysis.

LEDER (15, 16) and DRESCHER (6) have demonstrated with cytochemical methods that in some undifferentiated leukemic cells there is an erythropoietic specific acid phosphatase reaction. We found, in addition to the specific acid phosphatase reaction non-hem iron with the sulfide-silver-reaction (SSR) (29) in this undifferentiated leukemic cells (2) (Fig. 4 a-c). Until now we could not demonstrate the presence of hemoglobin in these leukemic cells, but we could demonstrate that the polysomes in these leukemic cells have messenger RNA with the information for a protein which coeluates with the added carrier Alpha- and Beta-globin chains (Fig. 4 d). The messenger RNA of these leukemic cells was tested in the *Xenopus* oocyte system (14) and the synthesized labelled product was analysed on carboxymethylcellulose-urea columns. We are now hypothesizing that the coeluated activities are Alpha- and Beta-globin chains. Experiments to prove this by fingerprints are in progress. We have to look for similar experiments to find out how far more cytochemical markers (proved by biochemical methods) can be used to demonstrate specific steps in normal and leukemic cell differentiation.

2. Do we have specific markers which show us the leukemic transformation of a single blood cell?

Until now, most of the cytochemical markers have not been specific for the detection of a single leukemic cell in normal appearing blood and bone marrow smears. In a few cases some markers, such as PAS-positive-blocks or peroxidase-positive Auer rods give us evidence of leukemic transformation. It is very difficult,



Fig. 2

a-h) Various morphological aspects of leukemic cells differentiated by cytochemical reactions, May-Grünwald-Giemsa-stain.

a) undifferentiated blasts, b) and c) paralympoblasts, d) and e) paramyeloblasts, f) and g) paraproerythroblasts, h) paramonocytes.

i-k) cytochemical differentiation of leukemic cells: i) PAS positive blocks and granules in the cytoplasm of blast cells, paralympoblastic leukemia, j) peroxidase: faint positivity or negative reaction in the cytoplasm of paramyeloblasts and parapromyelocytes, k) alpha-naphthylacetate-esterase: varying intensity of reaction in the cytoplasm of paramonocytes.

a-k) 700 x

CYTOCHEMICAL DIFFERENTIATION OF STEM CELL LEUKEMIAS (N=259)

TYPE	PAS	PEROXYDASE	N-AS-D-CL ESTERASE	α-N-ESTERASE	ACID PHOSPHATASE
1. NON-DIFFERENTIATED LEUKEMIA N=47	negative (positive fine granules in a few cells)	negative	negative	negative	negative (pos. diffuse tinge, pos. fine granules in a few cells)
2. PARALYMPHOBLASTIC LEUKEMIA*) N=149	positive coarse granules blocks	negative	negative	negative (positive granules in a few cells)	negative (pos. diffuse tinge in a few cells)
3. PARAMYELOBLASTIC LEUKEMIA N=46	positive diffuse tinge, fine granules	positive up to 3 %	positive up to 1 %	negative (positive granules in a few cells)	negative
4. STEM CELL**) LEUKEMIA WITH RED CELL LIKE CYTOCHEMICAL PATTERN N=17	negative (positive granules and blocks in a few cells)	negative (weak positive in a few cells)	negative (weak positive in a few cells)	positive paranuclear up to 61 %	positive paranuclear up to 100 %

*) further investigations have shown that this group is not only of one genetic origin (18)

**) further investigations have shown that in this group leukemias with Thymus Tumors

Tab. 2

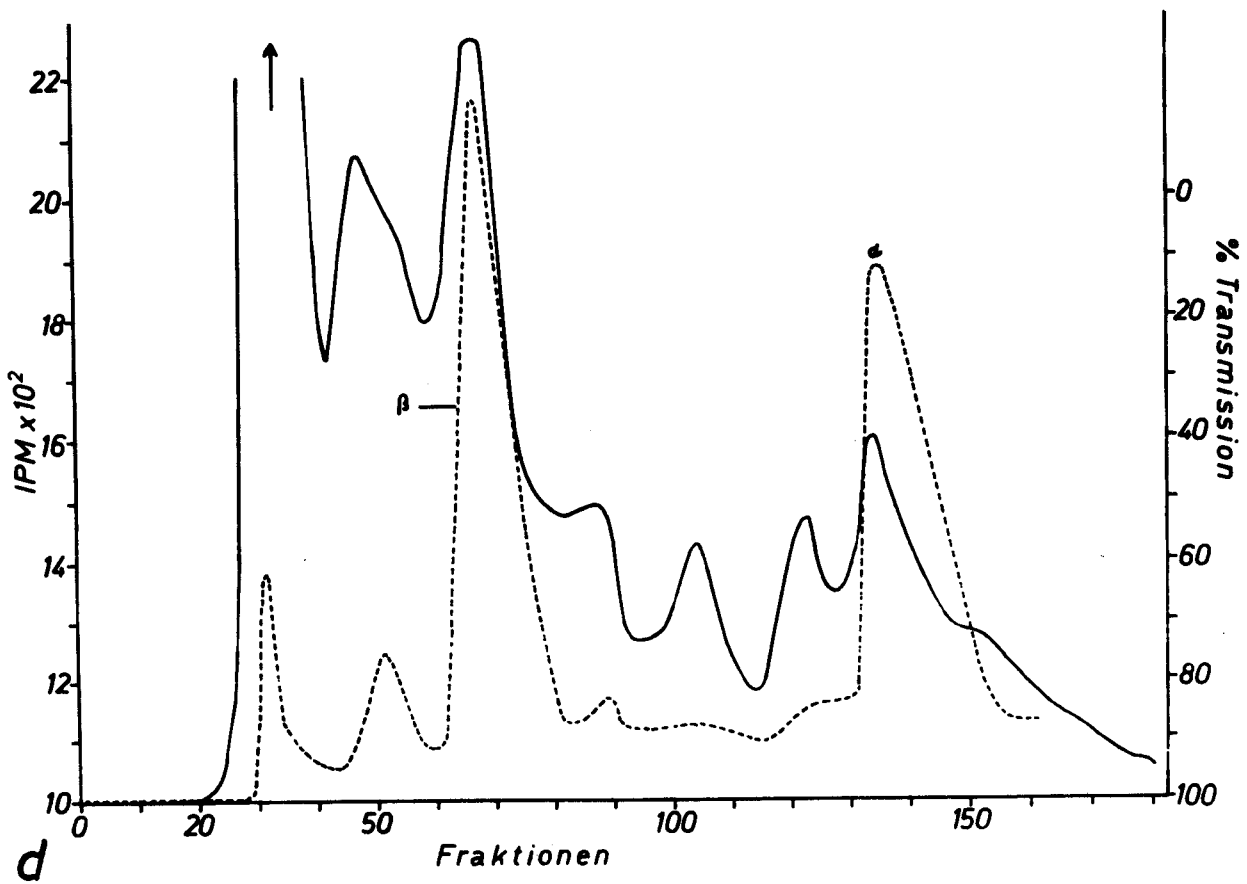
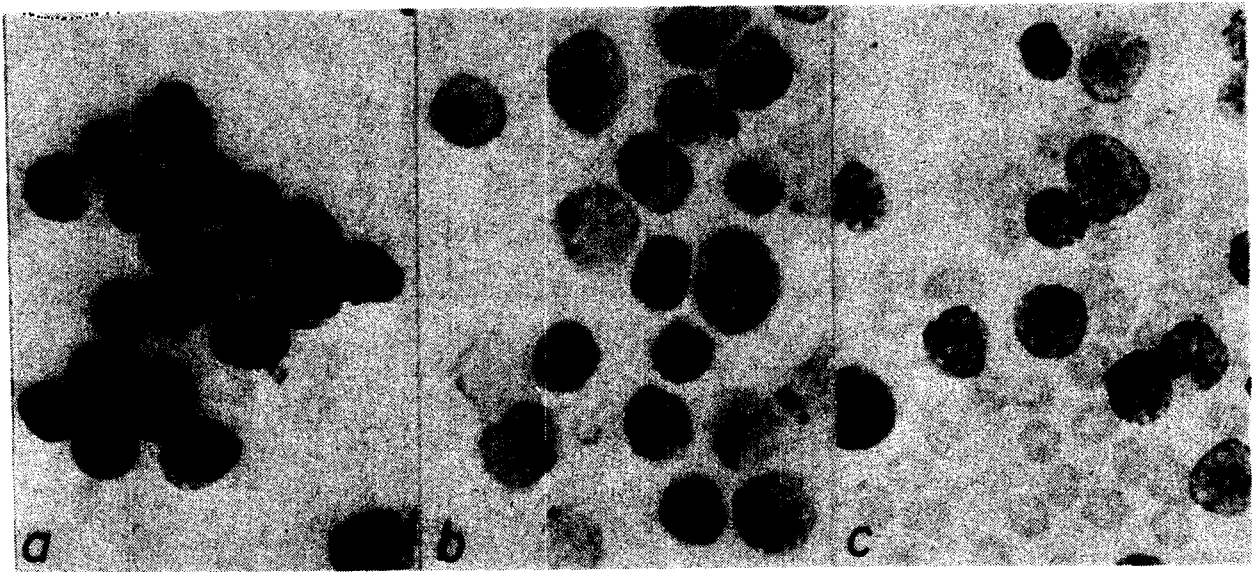


Fig. 4

Blasts of acute leukemia with red cell like cytochemical pattern.

a) May-Grünwald-Giemsa stain, b) acid phosphatase: paranuclear localized red reaction product, c) staining for non-hem-iron: black granules and brown diffuse tinge in the cytoplasm.

a-c) 700 x

d) Globin synthesized in frog oocytes in response to '8-16 S' RNA extracted from polyribosomes of the above leukemic blood cells.

however, to demonstrate single leukemic cells between normal blood and bone marrow cells with PAS or peroxidase. Because of this difficulty, we need more specific methods for recognizing a single leukemic cell.

As we know, the transformation of a normal cell to a malignant cell is generally accompanied by varying alterations in the cell membrane. One of the specific alterations on the cell surface, that can be made visible by morphological methods, is the appearance of new antigens. These antigens can be seen on DNA virus as well as on RNA virus transformed cells. BAUER and his group, using different techniques, demonstrated tumor specific surface antigens on cells of chicken, mice and hamsters which were transformed by avian tumor virus (9, 13). There is increasing evidence that human leukemic cells could possibly have tumor associated antigens similar to those shown in studies with virus induced leukemia in animals (11, 13). To visualize human leukemic cells tumor specific surface antigens are the most important markers. One of the possibilities to make the tumor specific antigens visible on the cell surface is to use the immunoferritin method (9). Until now the immunoferritin method has been most successful only with an electronmicroscope. Because the electronmicroscope is difficult to operate, this method has proven too complicated for marking a single leukemic cell.

Therefore, we applied the immunoferritin method to the lightmicroscope, using the sulfide silver reaction (SSR) (Fig. 3 a) (29). With the SSR one can make a ferritin solution visible in the lightmicroscope, because iron particles can be magnified to more than 100.000 : 1 (24). We showed with the SSR a specific antigen binding on the surface of rabbit lymphocytes immunized with apoferritin (Fig. 3 b–d) (25). Using the same method we also demonstrated a specific binding of ferritin labelled antigens on the cell surface after immunization with human γ -globulin.

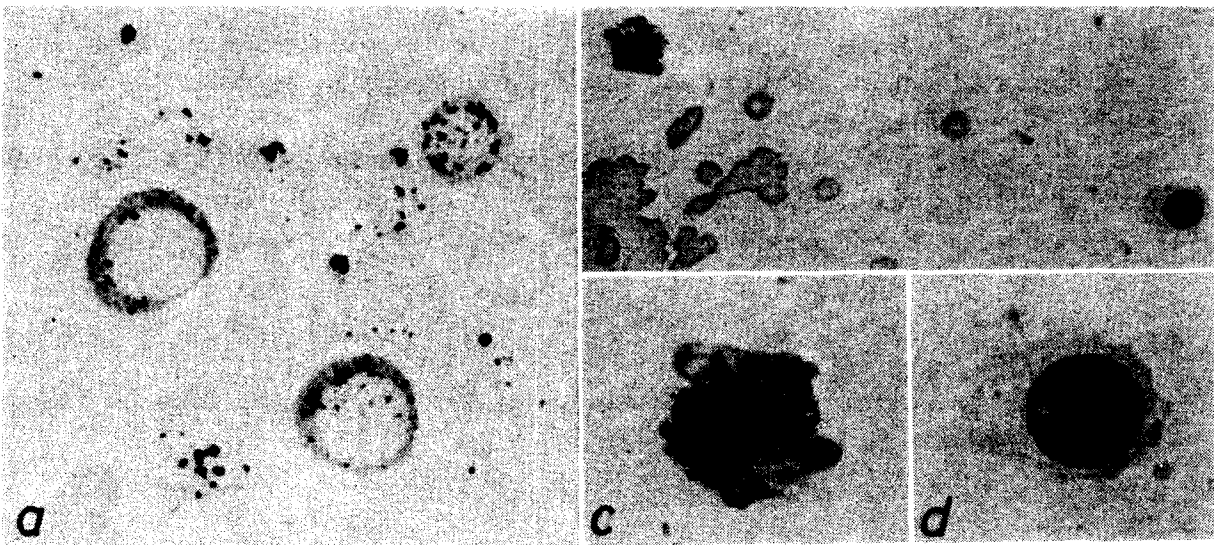


Fig. 3

- a) staining for non-hem-iron : diffuse brown tinge and black granules in the cytoplasm of erythroblasts and erythrocytes of a child with rh-erythroblastosis: 1.000 x
- b–d) demonstrating specific binding of ferritin on blood lymphocytes after immunisation with apoferritin. Normal lymphocyte with negative reaction right and left a stimulated lymphocyte with basophilic cytoplasm and positive reaction.
b): 648 x; c) and d): 1,200 x (25)

If one could find tumor specific surface antigens in human leukemic cells, then it might be possible to mark antibodies against these antigens with ferritin, peroxidase or immunofluorescence to make the specific binding of these antibodies visible. Then we might have the opportunity to detect single leukemic cells in the blood and bone marrow smears at a very early stage of leukemia.

3. Which of these markers can give us information for our diagnostic and therapeutic behavior with the leukemic patient?

HAYHOE and his coworkers demonstrated nearly ten years ago a relationship between cytological and cytochemical markers in leukemic cells and therapeutic results (10). All further investigations in the past few years have been concerned with proving HAYHOE's results. We found in 300 acute leukemia cases in children a much better prognosis for the undifferentiated or paralympoblastic types of leukemias than for the myeloblastic types. The therapeutic concept of PINKEL and his group (28) contributed much to therapy in leukemic children. According to the therapeutic results of this group it must be proven again how far cytological and cytochemical methods may be helpful as prognostic factors in leukemia.

Summary

Cytological and cytochemical methods offer additional items concerning the classification of leukemia with regard to prognosis and therapeutic consequences.

The morphologic demonstration of a single leukemic cell is impossible until now. But there is increasing evidence that human leukemic cells could have tumor associated antigens. Based on the assumption that tumor specific surface antigens exist in human leukemia, one can speculate about an early diagnosis by using the sulfide-silver immunoferritin method or other techniques for visualizing these antigens. Such an early detection of single leukemic cells can have therapeutic consequences to the patient.

Comparing cytochemical and biochemical investigations stemcell leukemias show that it is possible to demonstrate specific steps of normal and pathologic cell differentiation by cytochemical methods.

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