Table III. Inhibition of frog egg agglutinin by monosaccharides, oligosaccharides and glycolipids

Minimum amount of inhibitor (in μmole) to inhibit hemagglutination caused by 50 μg of *Rana japonica* agglutinin	
	μmole per 0.1 ml
D-Glc; D-Gal; D-Mann; D-Xyl; L-Fuc; L-Arab; D-GlcNAc; D-GalNAc; N-acetylneuraminic acid; lactose; melibiose; globoside	All these sugars and glycolipids do not inhibit even at 10–20 μg
Erythrocyte ganglioside[a]	1
Desialylated erythrocyte ganglioside	3–5
Ovomucoid; bovine albumin; egg albumin	Do not inhibit even at 50 μg

[a] A gift from Professor S. HAKOMORI; structure and properties, see[7,8].

was not inhibited by N-acetylgalactosamine, which will be described in detail elsewhere. Normal cultured cells showed some agglutination by as much as 250–1000 μg of the agglutinin, whereas the transformed counterpart was agglutinated by as little as 5–10 μg of the agglutinin (Table II).

Inhibition of tumor cell agglutination by simple monosaccharides or by oligosaccharides have been unsuccessful so far (see Table III). This is in rather striking contrast to the fact that a number of agglutinations caused by plant agglutinins were inhibited by simple sugars. Hemagglutination caused by anti-carbohydrate antibodies is, however, difficult to inhibit by simple sugars or oligosaccharides. It may be that the frog egg agglutinin differs from lectins but rather resembles the antibodies directed to carbohydrates. It is noteworthy that a ganglioside fraction of human erythrocyte membrane[7,8] was capable of inhibiting the agglutination.

In preliminary analysis of the agglutinin fraction, all proteins showed cathodic migration on cellulose acetate electrophoresis at various pH's, including pH 9, indicating that the agglutinin seems to be classidied as a basic protein or proteins[9].

Zusammenfassung. Nachweis, dass der Auszug einer basischen Eiweissfraktion aus Eiern verschiedener Froscharten (*Rana japonica* Guenther, *Rana nigromaculata nigromaculata* Hallowell) mit isotonischer Salzlösung bei diversen Tumorzellen zu besonderer Agglutination führt, während diese Wirkung auf transformierte, normale Zellen oder Erythrozyten ausbleibt. Mit Gangliosiden menschlicher Erythrozyten konnte die Agglutination gehemmt werden, nicht aber mit den bisher geprüften Zuckern.

H. KAWAUCHI, F. SAKAKIBARA and K. WATANABE

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Ultrastructure of Cytoplasmic Hemosiderin Inclusion Bodies in Malignant Phagocytic Lymphocytes

During 15 years of routine observations on various lymphoma-leukemia group blood samples, a patient in whom cytoplasmic iron-containing inclusions could be detected in about 20% of the peripheral lymphoid cells, was found for the first time. An earlier study of KOZEWSKI in 1955[1] on the storage of hemosiderin in normal lymphocytes and monocytes of anemic patients suffering from spontaneous hemochromatosis led us to study similar inclusions in malignant lymphoid cells. An ultrastructural analysis of previously unstudied inclusion material was also carried out.

Whole blood from patients diagnosed as having chronic lymphatic leukemia and lymphosarcoma was processed for cytochemical and electron microscopic observations. Several staining procedures employed included reactions for phospholipids[2], alkaline phosphatase[3] and hemosiderin[4]. Toluidin blue, Nile blue, polysacharides and luxol fast blue[5] were employed. Serum analysis revealed low-normal levels of ferrum-content (68 γ%) and ferrum-binding capacity (292 γ%). Bone marrow aspiration and bone marrow biopsy showed the presence of myelofibrosis and aplasia. No signs of hemochromatosis were found in liver and bone marrow biopsy.

Electron microscopy was carried out on unstained and double stained (uranyl acetate and lead citrate) sections at different magnifications from 2,000 to 50,000 with the Jem T7 and E-200 Philips electron microscopes[6–10].

Routine observations of peripheral blood smears (stained with MGG) revealed the presence of basophilic cytoplasmic inclusion bodies in about 20% of the lymphoid cells that comprised 85% of the peripheral leukocytes

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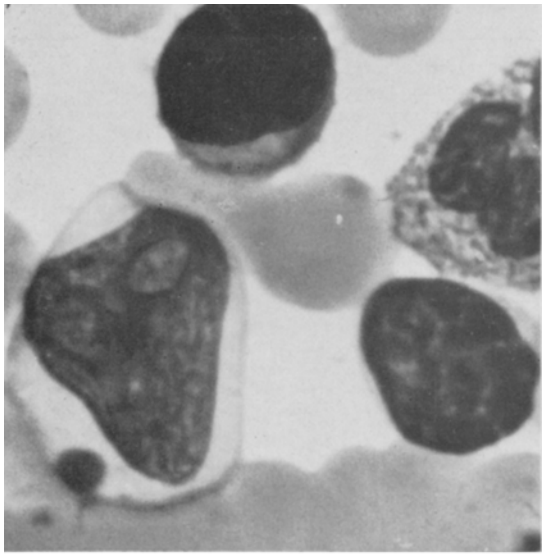

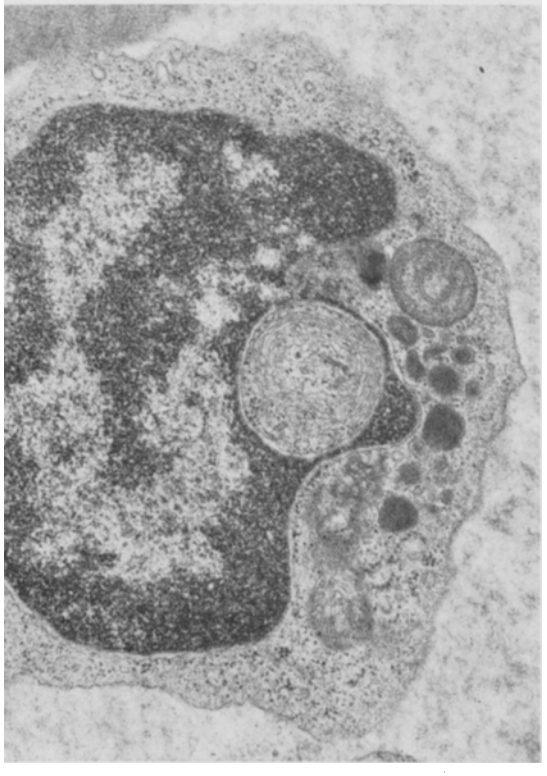

Fig. 1. Peripheral blood smear stained with MGG. The large lymphoid cell contains peripheral inclusion bodies (stained blue). ×5,000.

Fig. 2. Peripheral lymphoid cell. Note the irregularities of the cell membrane and the nuclear periphery. Intranuclear body surrounded by the nuclear membrane is present. ×12,000.

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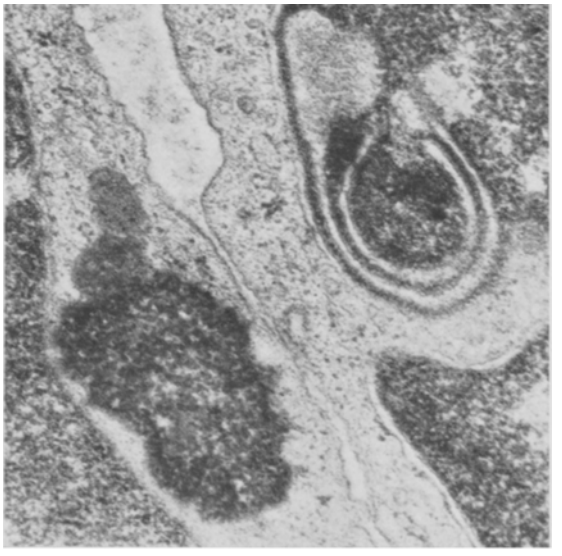
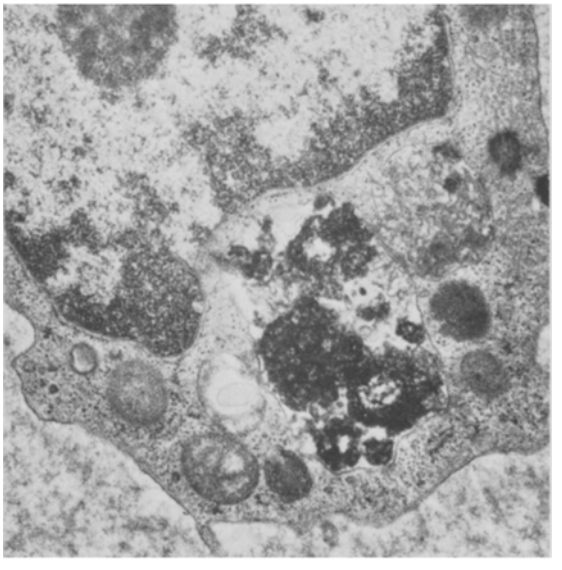
Fig. 3. Part of 2 adjacent peripheral lymphoid cells. Note the presence of the nuclear pockets in the upper cell and the granular cytoplasmic inclusion body in the lower cell. ×45,000.

Fig. 4. A large cytoplasmic phagosome surrounded by unit membrane. ×22,000.





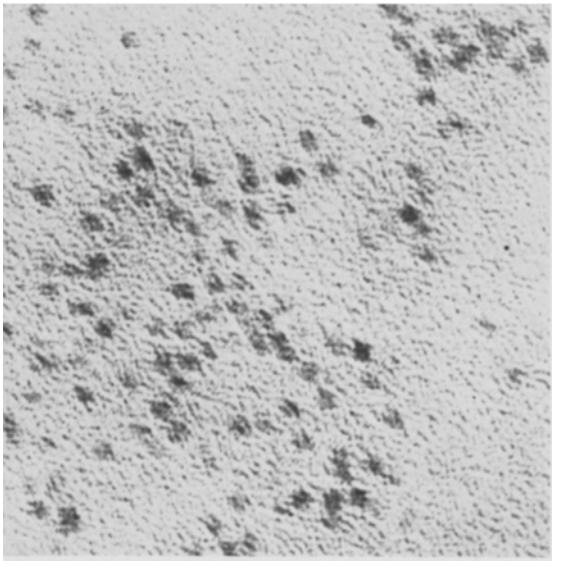


Fig. 5. Structures characteristic of ferritin observed in unstained sections of inclusion bodies of the lymphoid cell. ×400,000.

(Figure 1) These particles ranged from 0.5 to 1.5 μm in diameter and several inclusions were found in some cells. They reacted positively with methylene blue (blue), toluidine blue (blue), Nile blue sulphate (blue), and Prussian blue. Negative reactions were obtained with other dyes. The neutrophile granulocytes exhibited a very marked positive alkaline phosphatase reaction.

Ultrastructural observations revealed the presence of medium and large lymphoid cells with undulated cytoplasmic membrane and irregular nuclear surface. Nuclear pockets and nuclear inclusions were also observed (Figures 2 and 3). The cytoplasm contained mitochondria and the rough endoplasmic reticulum was poorly developed. Few lysosomes could be seen in the cytoplasm.

Phagocytic vacuoles containing electron-dense material of different consistency were regularly found in the lymphoid cells. A unit membrane surrounding the phagocytic vacuoles was always present. The material included in the vacuoles had an electron-dense appearance in unstained as well as in stained sections. High voltage electron microscopy of the unstained material revealed characteristic ferritin granules (Figures 4 and 5).

The supposition that the cytoplasmic inclusions described here are composed of serum-binding protein, most probably hemosiderin, as suggested from cytochemical evidence by KOSZEWSKI[1], was borne out by the electron micrographs presented in this article. KOSZEWSKI[1] and KOSZEWSKI et al.[11] in their previous studies have demonstrated the appearance of similar inclusions in man and animals treated with saccharated iron oxide compounds. In the case described here, iron therapy was omitted but a few blood transfusions were administered. Furthermore, KOSZEWSKI et al.[11] described the phagocytic activity of non-malignant lymphocytes. The present study showed that the same phenomenon could be brought about by the malignant lymphoid cells. Another hypothesis related to the presence of hemosiderin in lymphoid cells should be considered. It is still not known whether the lymphocytes serve as cells of origin of the blood corpuscules[11,12]. In patients with a malignant lymphoid disease treated with various antileukemia drugs, there is a possibility of the appearance of erythroid cells in the peripheral blood that are otherwise morphologically similar to the peripheral lymphocytes. It is suggested that further studies, as well as retrospective observations on this phenomenon, should be performed in order to clarify the underlaying mechanism.

Zusammenfassung. In den lymphoiden Zellen eines an chronischer lymphatischer Leukämie und Lymphosarcoma leidenden Patienten wurden cytoplasmische, Haemosiderin enthaltende und von Einzelmembranen umgebene Einschlusskörper gefunden. Es ist ungewiss, ob diese Erscheinung erhöhter phagocytischer Aktivität oder dem Auftreten anomaler peripherer erythroider Zellen im peripheren Blut zuzuschreiben ist.

B. PRESENTEY, I. HOD and A. J. ROSIN

District Laboratory, Health Insurance Institution Kupat Holim, Rehovot 76-100 (Israel); The Hebrew University of Jerusalem, Rehovot Campus, and Harzfield Chronic Diseases Hospital, Gedera (Israel), 30 October 1974.

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Suppression of Adjuvant Disease by Bacillus CALMETTE-GUÉRIN (BCG)

Modifications of the host immune mechanism produced by BCG are thought to be important in the response of patients with various malignancies after treatment with that agent[1-3]. We describe here the suppressive effect of BCG in a non-malignant experimental model, adjuvant disease in the rat[4].

This is a polysystemic syndrome of incompletely defined pathogenesis probably involving immune responses to one or several antigens[5-9]. It was induced in inbred male adult Wistar-Furth rats as described[10] by intradermal injection of adjuvant mixture into the left hind paw. The arthritis, which appears beginning at about day 10, is a major feature of the syndrome, and was scored on a four point scale (0–3) based on the degree of involvement in each of the limbs (exclusive of the adjuvant-injected hind paw)[10]. Rats were divided into 8 groups of 10, as shown in the Table. BCG was given according to 1 of 3 schedules to the appropriate groups. Each injection consisted of 25 mg BCG (BCG-S frais, Institute Pasteur, Paris, France) in 1.0 ml sterile 0.9% w/v NaCl in water given i.p.

Significant suppression of the disease by all BCG treatment schedules was observed, as shown in the Figure. In Figure A, it is shown that pretreatment with BCG significantly suppressed the arthritis relative to that of the adjuvant-injected controls ($p < 0.02$ on day 22), and also delayed its onset. BCG pretreatment followed by twice weekly BCG for 50 days completely prevented the disease until day 88. At that time, 3 of 10 rats developed mild, transient disease lasting only a few days. BCG therapy given after the adjuvant injection (Figure B), either before appearance of the arthritis or during the acute phase, significantly lessened the disease relative to that of the adjuvant-injected control group ($p < 0.02$ on day 14 and 19 respectively). It should be noted, however, that the arthritis in the post-adjuvant, BCG-treated groups progressed following the cessation of therapy to equal that of the non-treated adjuvant-injected control rats. ^{51}Cr-labelled thoracic duct (TD)

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