

Lymphocyte Function in Hodgkin's Disease^{1, 2}

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Summary

The immunologic deficiency of Hodgkin's disease includes skin anergy, delay in homograft rejection, negative transformation response of blood lymphocytes, and susceptibility to infections, particularly those of fungal or viral etiology (1, 3, 5). Since thymus-derived lymphocytes normally mediate these functions, a disturbance of this cell system would account for the observed immunological deficiencies in Hodgkin's disease.

The majority, if not all of thymus-derived lymphocytes belong to the pool of recirculating, long-lived, small lymphocytes and an unimpeded circulation of these cells between lymph and blood is considered essential for a complete expression of their immunocompetence. Blockage and alteration of this circulation at the level of the lymph node is likely to occur in patients with Hodgkin's disease because of the characteristic infiltration of the lymph nodes by neoplastic cells.

The question arises, therefore, whether the immunological deficiencies of Hodgkin's disease are due to intrinsically incompetent lymphocytes or the result of a faulty circulation of intrinsically competent lymphocytes. To answer this question the thoracic duct lymphocytes as well as blood lymphocytes of a patient with Hodgkin's disease have been studied with regard to re-circulation and immunocompetence.

Case Report

J.A., a 44 year old male, presented in 1968 with fever, night sweats and cervical lymph adenopathy. Biopsy of a cervical lymph node revealed Hodgkin's disease, mixed cellularity type. Chest X-ray demonstrated mediastinal lymphadenopathy and abdominal lymphangiography revealed enlarged aortic lymph nodes suggestive of Hodgkin's disease involvement. The patient was classified as Hodgkin's disease, Stage III-B.

Of significance in the patient's past history was pulmoanry tuberculosis for which he was frequently hospitalized between 1948 and 1953. In 1956, he had a right upper lobectomy. There has been no reactivation of the tuberculous process.

At his admission in June 1968, the WBC was 4700/mm³ and the blood lymphocyte count 1800/mm³. The treatment consisted of nitrogen mustard (0.4 mg/kg) followed by radiotherapy approximately 4000 rads in 4 weeks to lymph nodes above the diaphragm, followed by 3600 rads in 6 weeks to lymph nodes below the diaphragm. The patient has remained in apparent remission to the present time (1971).

On 1-17-71, the patient was brought into hospital for the purpose of thoracic duct cannulation and lymphocyte study. He was asymptomatic and the physical examination was unremarkable except for a few pea-size lymph nodes in the left axilla. Blood chemistries were within normal limits. The WBC was 4200/mm². Skin tests for TBC, mumps, blastomycosis, histoplasmosis, and candida were negative. DNCB skin test was equivocal.

Methods

1. *Thoracic duct cannulation.* A thoracic duct side-fistula was established in the neck. The cannula was placed in the duct to allow intermittent diversion of the entire lymph flow to the outside for measurement and sampling, while permitting a normal thoracic duct circulation be-

1. This study was supported by Grant CI No. 25 of the American Cancer Society

2. Presented at the 4th International Congress of Lymphology, Tucson, Ariz., 1973.

tween sampling (4). This sampling was obtained by simple gravity drainage. The flow per minute was obtained from a 15 minute collection. The cell concentration was determined in a hemocytometer. The lymphocyte output/minute was taken as the product of the two previous determinations. Repeated lymph samples were processed for cytologic examination.

2. *Simulation response of lymphocytes*; Blood lymphocytes were separated for culture using the sedimentation method described by *Hersh and Irwin* (5). Thoracic duct (TD) lymphocytes were collected in sterile tubes with heparin. The lymphocytes were washed once in Hank's balanced salt solution (BSS) (Microbiological Associates). Both samples of lymphocytes were diluted to a concentration of $2-2.5 \times 10^6$ cells/ml in Eagle's Minimum Essential Medium (Microbiological Associates, Bethesda, Maryland).

The following cultures were prepared:

- a) Blood Lymphocytes only, b) TD lymphocytes only, and
- c) 3:1 mixture of TD and blood lymphocytes 15% by volume autologous serum was added to each culture

Following the method of *Schwarz* (7), pokeweed mitogen (PWM, Grand Island Biological, Grand Island, New York). 0.1 ml/ml of culture was used as the mitogen. Cultures were incubated for 72 hours at 37 °C. Three hours before harvest, tritiated thymidine (TTh, 10 Ci/mmole, Schwarz Bioresearch, Inc.) 10 µc/ml, was added to the cultures.

At harvest each culture was centrifuged at 1000 rpm 10 min (200 g) washed twice in Hanks BSS, and resuspended in 1 cc of Hanks BBS. Cell concentration was measured in a hemacytometer. 0.4 ml was pipetted off and centrifuged as before. The cell pellet was suspended in a drop of serum and smeared on clean slides, fixed in methyl alcohol, and stained with MacNeal's tetrachrome or Jenner-Giemsa.

0.6 ml of each suspension was prepared for liquid scintillation counting. The cell suspension was dissolved in 5.4 ml NCS Reagent (Nuclear Chicago), decolorized by the addition of 0.1 ml saturated solution of benzoyl peroxide in toluene and transferred to vials in 14.5 ml toluene: liquefluor for counting.

Transformation of lymphocytes was assayed by the appearance of blast cells on stained smears and TTH uptake per 10^6 lymphocytes measured in a Beckman liquid scintillation counter. 500 lymphocytes were counted on each slide and the percentage of lymphoblastoid cells was determined.

Results

TD Lymph measurements. During the period of observation, the thoracic duct circulation remained unimpaired, as demonstrated by measurements of normal thoracic duct pressure and radiological demonstration of a free flow of lymph into the venous system past the site of cannulation.

Repeated thoracic duct lymphocyte output measurements were obtained over a period of three weeks and were as follow: lymph flow rate, 0.3-0.9 ml/min (average, 0.7 ml/min); lymphocyte concentration, 1000-2400/mm³ (average, 1500/mm³); lymphocyte output: 300, 000-1, 300,000/min. (average, 700,000/min). Cytologic examination showed a Reed-Sternberg cell and malignant reticular cells (Fig. 1).

In vitro lymphocyte stimulation results. TTH responses in PWM stimulated and control cultures are shown in Fig. 2. Stimulation response of the blood lymphocytes alone to PWM was negligible. However, the TD lymphocytes demonstrated a seven fold increased uptake of TTH, while the TTH uptake in the mixture of TD and blood lymphocytes was maximal.

The cytological results are presented in Table 1. The percentage of lymphoblasts was not increased in the blood lymphocyte sample by addition of PWM. The TD lymphocyte cultures containing PWM showed a definite lymphoblastoid stimulation, while maximum stimulation was noted in the 3:1 mixture of TD and blood lymphocytes.

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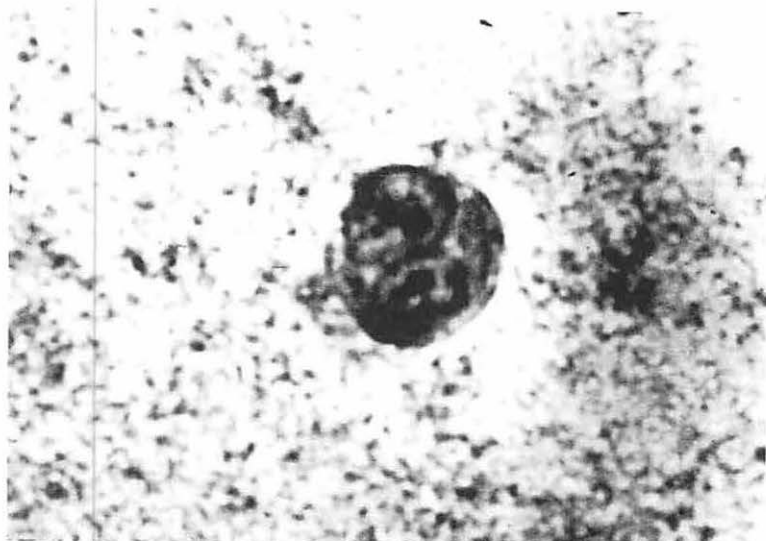


Fig. 1 Reed-Sternberg cell, binucleate with prominent nucleoli. Cytologic studies by Dr. Marta Garret.

Fig. 2 Counting times were sufficient to permit an error of 2 S.D. of $\pm 0.3\%$ for all four determinations. The radioactivity measurements are in counts per minute per 10^6 lymphocytes. P.W.M. = pokeweed mitogen.

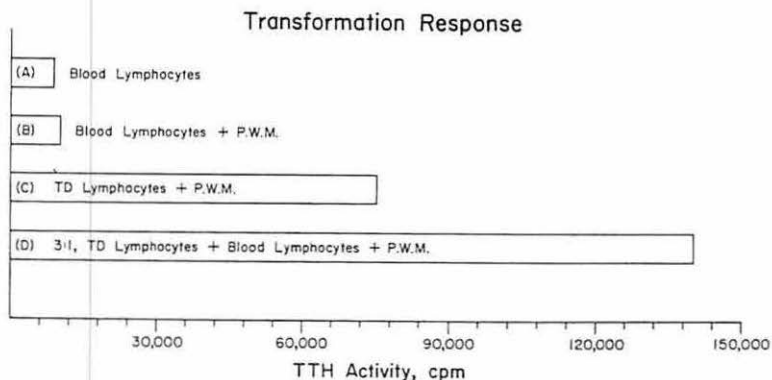


Table 1 Percentage Lymphoblastoid Transformation on Smears

	Without PWM (S.D.)	With PWM (S.D.)
Blood lymphocytes	2 (± 1)	7 (± 2)
T.D. lymphocytes	3 (± 1)	53 (± 6)
3 parts T.D. lymphocytes	-	71 (± 5)
1 part blood lymphocytes	-	-

Discussion

The above results are similar to those of Iverson (6) who showed in rats that the in vitro phytohemmagglutinin response of lymphocytes depended on an optimum ratio of circulating and non-circulating lymphocytes, and that when the recirculating lymphocytes were diverted from the blood by a TD drainage, the remaining blood lymphocytes failed to transform.

Our patient demonstrated a low TD lymphocyte output (2) and immune deficiency as shown by skin energy and failure of his blood lymphocytes to be transformed by the mitogen PWM. However, his TD lymphocytes demonstrated a maximum response.

These findings support our working hypothesis that the ID of Hodgkin's disease may be related at least in part to impaired recirculation of long-lived lymphocytes rather than an inherent defect in the lymphocyte.

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INTERNATIONAL SOCIETY OF LYMPHOLOGY FORTHCOMING CONVENTIONS

Lymphology meeting in Hiroshima

In connection with the 10th International Congress of Angiology in Tokyo, August 30 - September 3, 1976, a post-congress meeting of lymphologists will be organised by Dr. Y. Nisiimaru in Hiroshima.

For further details, please contact:

Yasuyosi Nisiimaru, M.D.

President of the Japanese Society of Angiology

2 Umeki-cho (TEMO)

KURE / Japan

VI International Congress of Lymphology

General Information

The Congress will be held at the Hotel Inter-Continental, Praha 1, from June 20-25, 1977

Main topics

1. Methods of investigation in lymphology
2. Lymphangiopathies
3. The lymphatic system and drugs
4. The lymphatic system and immunology
5. Pathophysiological aspects of the lymphatic system
6. Basic science

Three types of scientific papers are planned:

1. Review lectures

These lectures will cover recent progress in most perspective areas of lymphology with special attention to postgraduate education. Papers by invitation, reading time up to 45 minutes

2. Symposia

The Symposia will be organized by the scientific coordinators of the individual topics. Only a limited number of papers can be accepted. Reading time established individually up to 15 minutes

3. Communications

Papers pertaining to selected topics will be preferred. Reading time up to 10 minutes including projection of slides.