

### Résumé

L'apparition d'adénites après la vaccination BCG ne fait que traduire l'invasion souhaitée de l'organisme par la voie lymphatique. Seules les adénites suppurées constituent un incident vrai de la vaccination. Cet incident ne doit se produire que chez 1% ou au maximum 1,5% des vaccinés. Tout accroissement, même faible, de ce pourcentage, (comme les auteurs en ont observé en 1957 et 1963) traduit un incident de préparation ou d'inoculation du vaccin.

### Summary

The occurrence of adenitis following the BCG vaccination is only a sign of expected invasion of the organism by the germ through the lymphatic way. Only, the suppurating adenitis is an undesirable complication of vaccination, which does not occur in more than 1% or 1,5% at most of the vaccinated people. Any increase, even slight, of this percentage (as it was observed by the authors in 1957 and 1963) may indicate errors in preparation or inoculation of the vaccine.

## THE MORPHOLOGICAL AND PROLIFERATIVE ASPECT OF LYMPH NODE OF HODGKIN'S DISEASE GROWN IN TISSUE CULTURE \*

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### Introduction

In the present report is described the morphological and proliferative aspects of involved lymph nodes in Hodgkin's disease grown in tissue culture. Some of the factors influencing the mode of growth, the rate of multiplication, the size of the nucleus, and the shape and number of nucleoli were investigated.

We do not think that at the present time we have come any closer to solving the problem of Hodgkin's disease than at the time of starting this project. We have, however, learned few things about the behavior of lymph nodes in vitro. We have reviewed the literature concerned with growth and differentiation of different cell types from lymph nodes in this disease and benefited from the observations of many workers on tissue culture which contributed greatly to our knowledge, in terms of general. (1-38, 40-45, 47, 49, 59, 61, 62, 64-81, 83-92, 94, 96, 97, 104, 106-109) and specific observations. (29, 39, 46, 48, 50-55, 57, 58, 59, 61, 63, 82, 93, 95, 98-103, 106, 110-114, 121)

### Materials and Methods

Lymph Nodes: Primary cell cultures were grown from lymph nodes of three patients suffering from Hodgkin's disease. These were obtained at the time of operation.

The first specimen was obtained on February 16, 1962 from a 10-year-old negro child suffering from a generalized lympho

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pathy and enlargement of the spleen and fever, of six months duration. The biopsy specimen from the servical region showed a lymph node of 15x15x10 mm in good condition. The cut surface disclosed a soft and gelatinous consistency, on which the small foci of yellow spots were striking. The histological evidence indicated Hodgkin's disease of sarcomatous type with numerous Strenberg cells.

The second specimen was removed on March 23, 1963, from a 42-year-old male. The biopsy specimen from the retroperitoneal mass showed a lymph node 20x20x10 mm in good condition. The cut part revealed a smooth surface of gelatinous consistency. The patient's history showed 5 years of complaint, involvement of stomach, liver, adenoid glands and retroperitoneal spaces. The histological evidence indicated Hodgkin's disease with an advanced sclerosing structure and rarity of Reed-Sternberg cells.

The third specimen came from a 60-year-old white male on May 6, 1963. The biopsy specimen was obtained from the inguinal lymph node. The histological evidence indicated a sarcomatous Hodgkin's disease with many Reed-Sternberg cells. This was a large lymph node, 20x20x30 mm obtained without sterile precautions. We cut off the surface of this lymph node under aseptic conditions and used the middle portion of it for tissue culture.

The roller tube technique was used for growing the lymph tissue in vitro, with reconstituted collagen or glass as substrate for the growing cells.

The following nutrient media were used to grow the cell from lymph nodes in vitro:

Medium I: Balance Salt Solution 2 Parts, AN-50\* 3 parts, human cord serum 5 parts, cells grown on reconstituted collagen (medium 2350 on collagen).

Medium II: Balance Salt Solution 1 part, AN-153 1 part, human cord serum 8 parts, cells grown on reconstituted collagen (medium 1180 on collagen).

Medium III: Balance Salt Solution 1 Part, AN-153 1 part, calf serum 8 parts, cells grown on reconstituted collagen (medium 1180 on collagen).

Medium IV: Balance Salt Solution 2 parts, o. 5% LAH\*\* 5 parts, calf

\* "AN" refers to special chemically defined media of very high value being evaluated by Dr. Chiun T. Ling et al.

\*\* LAH - lactalbumin hydrolysate.

serum 3 parts, cells grown on glass (medium 2530 on glass).  
Medium V: Waymouth's defined medium 20%, human cord serum 80%, cells grown on reconstituted collagen with and without antibiotics\* (medium 0280 on collagen).

Medium VI: Waymouth's medium 20%, calf serum 80%, cells grown on reconstituted collagen with and without antibiotics\* (medium 0280 on collagen).

Medium VII: Waymouth's medium 20%, serum 80%, cells grown on glass (medium 0280 on glass).

The nutrient media were replaced at 2 or 3 day intervals until the outgrowth around the explanted fragments was sufficient enough to start daughter subcultures and coverslip cultures. Many culture were stained (hematoxylin, PAS, Lielchowsky, and for lipids). Many of the cultures were sectioned to determine the character of the cell growth on collagen.

#### Observations

The growth of Hodgkin's lymph nodes in vitro seemed to depend upon the age of the person and the stage of the Hodgkin's disease at the time the node was obtained. The morphological and the proliferative aspects of Hodgkin's lymph node was characterized by four cytologically distinct phases:

- A— Simple emigrating phase
- B— Simple proliferative phase
- C— Complex proliferative phase
- D— Degenerative phase

#### A— Simple Emigrating Phase

At this phase, lasting from one to three days, all primary explants showed outgrowth of cells, except the explants grown in a medium with calf serum, without antibiotics. The medium containing calf serum and antibiotics produced some outgrowth during the second week of culturing. At this time, the colonies were small and contained rounded cells. Many of the small rounded or irregular polygonal cells resembled lymphocytes, other more expanded forms suggested macrophages, however, with less elaborate processes than the classical forms. Later on, many scattered cells were observed, many of which contained large vesicles. This observation suggests that there is a predominant activity of macrophages at the early stage of tissue

\* Penicillin (100 units) and Streptomycin (50 micrograms per ml of media).

outgrowth. On the second day. (the first case) and the third day (the second and third case) the cells described above as lymphocytes became pycnotic and exhibited phenomenon characteristic of dying cells. Although there are some investigators who emphasize that the lymphocytes are totipotent cells (111-112) however, the majority of them have no evidence confirming this conception.

#### B— Simple Proliferative Phase

At this phase, besides the lymphocytes and some large granular satellite cells with one or more nuclei suggesting macrophages, there were many spindle cells arranged in a radiating fashion. The latter cells were uniform in size and shape with relatively small amount of cytoplasm and a condensed centrally located nucleus containing one nucleolus. Also at this phase, the fibroblasts started to emigrate and after a few weeks the entire culture, on occasions, consisted of fibroblasts with only a few rounded cells. The fibroblasts were densely packed next to each other suggesting a tissular arrangement in which the cell and nuclear axes were parallel to each other. With appropriate subculturing, (for period of ten months and fifteen continuous passages) these fibroblasts were kept in tissue culture almost indefinitely.

#### C— Complex Proliferative Phase

In this phase, the fibroblasts lost their orientation and tissular arrangement by forming a loose network in which the cells were not connected to each other any longer. With each progressing day, the fibrospindle cells increased considerably and the round cell population decreased. The small spindle cell together with the large spindle cell made up the majority of the population at this time. The mitotic activity exhibited by the fibroblast cultures was of interest in this phase. During mitosis, the cells drew in their processes acquiring a spheroid shape, thus giving rise to two new spheroid daughter cells which subsequently returned to the spindle form. After two or three weeks *in vitro*, the original explants still showed large masses of rounded cells proliferating on the surface of fibro-spindle cells. It is of interest to note the similarity of this observation to the growth of MB III tumorous lymphoblasts and Yoshida rat sarcoma, observed by Dr. Gey. These were lymphoblastic tumors whose cells apparently required some unknown factor contributed by the stroma. The origin of the fibroblast in the lymph node cultures has been

questioned since they were described (2, 6, 9, 23). Some observers (39, 47, 114) considered them to be derived from the stroma of the lymph node. We are inclined to believe, at the present time, that many of these spindle cells with their rotated nuclei were reticular cells. These were not arranged in any specific order, but appeared as a different layer of cells. We think that these cells were of multipotent capacity and gave rise to other types of cells.

The first case exhibited features of an unhealthy culture with some areas of focal necrosis. The question arose as to whether some of these features were part of the Hodgkin's picture. In general, all of our cultures showed the following characteristics during this phase:

- 1— There was a predominance of fibro-spindle cells differing greatly in size and shape, some exhibiting pleomorphic features. These cells were at first closely associated with each other but this connection was soon lost. They enlarged and underwent further morphological changes. There were many of these cells in the first case and some in the second and third ones with two or more nuclei which could be confused with those of Reed-Sternberg cells. Isolated phagocytes with ingested lymphocytic bodies distributed throughout the explants. According to Dr. Gey, the occurrence of multinucleated cells is common in continuous tissue culture for many cell types. These cells seemed to be the result of fusion of spindle cells by disappearance of cellular border and shrinking the nucleus and nucleoli. The diameter of the nucleus in these cells was 2-4 times smaller than that of the normal cell. The cytoplasm surrounding the nucleus of these cells was denser at the periphery. The shape of these cells varied greatly.
- 2— In this phase, the growth was rapid for the first and third case while it was very slow for the second case (sclerotic form).
- 3— For the first and third case, a considerable amount of mucoid material was produced in all cultures. The deposition of this material was observed even in the smallest cell foci.
- 4— The presence of some large, irregular, vacuolated and sometimes vesicular and cystic cells with multilobulated nuclei in these cultures could be interpreted as a feature of Hodgkin's disease. These, therefore, could be called Reed-Sternberg cells.
- 5— Within the cell and on the cell surface there was very pronounced fine granulation.
- 6— There were many large, irregular, vacuolated, vesicular and cystic cells, often with huge nuclei. Some cells definitely had

signet cell character.

- 7— In all of our cases, some of the original explants produced focal collagen lysis within 3 to 10 days. This confirmed previous observations in this laboratory that histocytic cells of non-malignant origin have the potential to lyse reconstituted collagen. In the cultures derived from Hodgkin's lymph nodes (particularly the first and second cases) we observed the production of holes in the reconstituted collagen. Some of these lysed areas in the due course of time were bridged over by active fibroblasts and macrophages.
- 8— Rosette formation by lymphocytes was observed.
- 9— The intranuclear and intranucleolar vesicles were present (only in the first case).
- 10— There was dense outgrowth of abnormal cells on a background of apparently normal fibro-spindle cells.

In some cultures, reticular cells became predominant and we felt that selective growth had occurred. The normal and abnormal reticular cells varied greatly in size and shape and had rather large nuclei. The most important pathologic alteration of these cells was their huge, indented nuclei containing two or more large, irregular, elongated or angular and bizarre nucleoli. As a whole, the nucleoli were vacuolated and occupied the most part of the nuclei, and in some cells the ratio of nucleoli to the nucleus was about 1:5. In this condition, the increase in the number of nucleoli was well outstanding in the nuclei of the majority of cells. In most cells the nucleoli count was between 2 to 15. Some of the reticular cells had a large, regular nucleus while in others the nucleus was irregular with folded nuclear membrane. These were considered to be an intermediate stage of evolution between the reticular cell and the classical Reed-Sternberg cell. Occasionally, a cellular network observed which appeared sinusoidal in character. The cells making up this network were morphologically distinct and resembled those lining the blood vessels.

#### D— Degenerative Phase

This phase was not similar or constant in all of our cultures and somewhat overlapped the third phase. It seemed to be related to the histological appearance of lymph node's lesion and may even have some correlation with the patient's age. In our first case, the ten-year-old child, degenerative phase began after eight months of proliferation *in vitro* while in our second case, a 42-year-old male, it was observed after two months. In our third case, this phase still

was not impressive.

In this phase, the growth either stopped or slowed down and the cultures behaved differently as compared to the first three phases. The polymorphism of the cells was absent. Most of the cells were small elongated fibrospindle cells with an indistinct border. They were very granular, the shrunken nuclei could be hardly distinguished from the surrounding material. The cell outline progressively disappeared, the number of living and active cells were small. The explants and colonies tended to detach themselves from the tube wall. Degenerated cells, cell ghosts, debris, and granular material of cell origin could be found floating throughout the medium. Noteworthy at this time was the presence of some multinucleated cells which could be confused with the multinucleated cells seen previously. These cells originated from large broad cells in which the nucleus tended to vegetate and subsequently formed either small or large round protrusions. These were eventually detached from the main nuclear mass by breaking at the narrow base and forming a polynuclear cell. This possibly could be caused by aging of the cell. Another outstanding feature during this phase was the presence of intranuclear vacuoles and vesicles, especially in the first case. It would be interesting to study this in more detail.

#### Summary

The present report describes the morphological and proliferative aspects of lymph nodes in Hodgkin's disease grown in tissue culture.

The development of Hodgkin's lymph-nodes grown *in vitro* seems to depend upon the age of the person and the stage of the Hodgkin's disease at the time the lymph-node is obtained.

The morphological and the proliferative aspects of Hodgkin's lymph-node is characterized by four cytologically distinct phases:

- A- Simple emigrating phase.
- B- Simple proliferative phase.
- C- Complex proliferative phase.
- D- Degenerative phase.

The reticular cells, their evolution, and abnormalities, Sternberg cells and multinuclear giant cells have been studied and discussed.

### Résumé

Le présent rapport décrit les aspects morphologiques et prolifératifs des nodules lymphatiques de la maladie de Hodgkin, cultivées *in vitro*.

Le développement des nodules lymphatiques de la maladie de Hodgkin cultivées *in vitro* dépend de l'âge de la personne et de l'état de la maladie de Hodgkin au moment du prélèvement des nodules lymphatiques.

L'aspect morphologique et prolifératif des nodules lymphatiques de Hodgkin est caractérisé par quatre phases cytologiques distinctes

- a. - Phase d'émigration simple.
- b. - Phase proliférative simple.
- c. - Phase proliférative complexe
- d. - Phase dégénérative.

Des cellules réticulaires, leur évolution et anomalies et les cellules de Sternberg ainsi que les cellules multinucléaires géantes ont été étudiées et discutées.

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