

# Intracellular bacteria in Hodgkin's disease and sclerosing mediastinal B-cell lymphoma: sign of a bacterial aetiology?

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## Summary

**Background:** The aetiology of Hodgkin's disease is still unknown more than 160 years after its original description. In recent years a viral aetiology was the preferred hypothesis. Epidemiological, clinical, laboratory, and histological findings, however, point rather to a bacterial aetiology.

**Methods:** In the histological work-up of tissues from patients suffering from malignant lymphoma periodic acid-Schiff (PAS) stains are routinely done. In several bacterial infections intracellular PAS-positive material can be observed. We examined PAS-stained slides at magnifications of 1000× of six Hodgkin and twelve Non-Hodgkin patients.

**Results:** We found PAS-positive diastase resist-

ant intracellular rods and spheres in all Hodgkin patients and in all of the six patients suffering from sclerosing mediastinal B-cell lymphomas, but not in the other Non-Hodgkin lymphomas.

**Conclusions:** The diastase resistant PAS-positive structures are compatible with intracellular bacteria. After gastric MALT-lymphoma and gastric non-cardia adenocarcinoma it appears that Hodgkin's disease and sclerosing mediastinal B-cell lymphomas may also be human tumors related to bacteria.

**Key words:** Hodgkin's disease; sclerosing mediastinal B-cell lymphoma; aetiology; bacteria

## Introduction

During the last few years the discussion about the bacterial aetiologies of malignant tumors was revived. In *Xenopus* a mycobacterium was shown to be related to a lymphosarcoma [1]. In mice a *Helicobacter* species was found to be a likely candidate for the aetiology of hepatocellular tumors [2]. A connection of bacteria to human neoplasms was shown for gastric MALT-lymphoma [3–5] and for gastric non-cardia adenocarcinoma, where *Helicobacter pylori* has been accepted as a definite biological carcinogen by the WHO / IARC [6]. Some benign human tumors are also related to bacteria such as cutaneous bacillary angiomatosis (*Bartonella*, formerly *Rochalimaea quintana*) [7], or benign lymphomas (*Bartonella henselae*) [8]. *Agrobacterium tumefaciens* is known to induce malignant tumors in plants [9]. The relationship now being established between bacteria and certain plant, animal, and human malignant tumors gives rise to the following question: are there more tumors where bacteria might play an aetiological role? We propose that the pathogenesis of Hodgkin's disease is

similar to the one of crown gall tumors in plants [10], where a natural exchange of genetic material from *Agrobacterium tumefaciens* (oncogenic plasmids) to plant cells induces malignant tumors in dicotyledones [11]. The "crown gall" hypothesis for Hodgkin's disease would explain the clinical observations of a bacterial infection and the behaviour as a malignant tumor. In addition regression of Hodgkin's disease by antibiotics has been described [12].

Periodic acid-Schiff (PAS) staining is known to be an important clue to the histological identification of intracellular bacteria such as *Tropheryma whipplei* [13]. In the histological work-up of tissue of malignant lymphomas periodic acid-Schiff (PAS) stains are routinely done, but not examined at magnifications above 400× where bacteria could be detected. We screened the PAS-stained slides at a magnification of 1000× of twelve patients suffering from Non-Hodgkin's lymphoma and six patients suffering from Hodgkin's disease.

## Methods

PAS-stained slides of six Hodgkin and twelve Non-Hodgkin patients were screened for PAS-positive intra-

cellular structures at a magnification of 1000× (oil immersion); at least 2400 cells in each slide were examined.

## Results

PAS-positive diastase-resistant intracellular rods and spheres could be observed in Hodgkin's disease and sclerosing mediastinal B-cell lymphomas, but not in other non-Hodgkin lymphomas (table 1). Figure 1 shows the rods and the spheres. The rods are about 3 µm in length and 0.5 µm in width.

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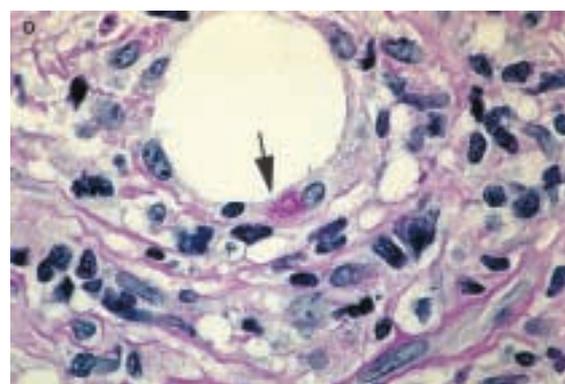
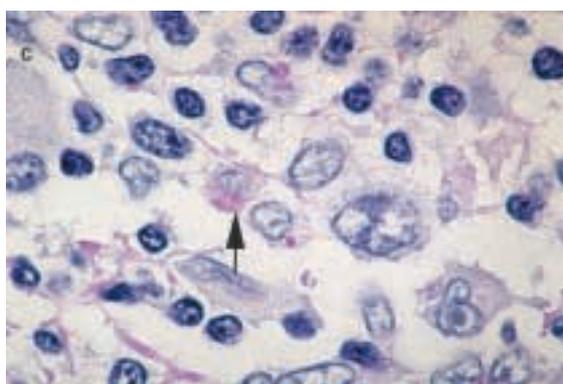
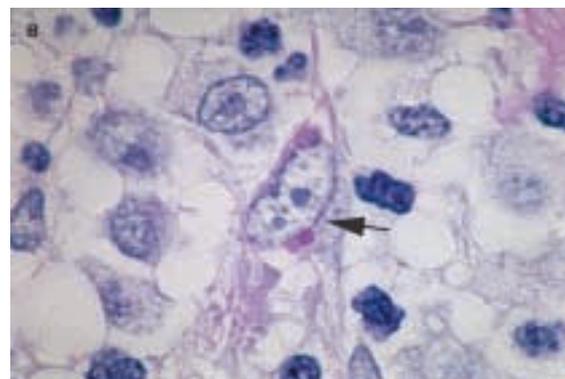
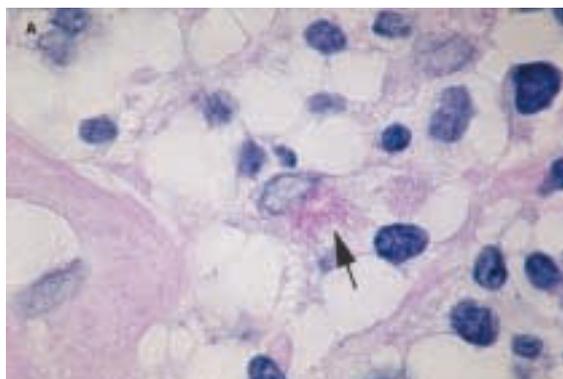
**Table 1**

Microscopic examination of PAS-stained slides at a magnification of × 1000; number of cells containing rods and spheres per about 2400 cells examined per slide.

Patient	diagnosis	rods	spheres
1-6	Non-Hodgkin lymphoma, large cell	0	0
7	Hodgkin's disease, nodular sclerosis	3	23
8	Hodgkin's disease, nodular sclerosis	13	33
9	Hodgkin's disease, nodular sclerosis	10	7
10	Hodgkin's disease, nodular sclerosis	4	8
11	Hodgkin's disease, nodular sclerosis	6	12
12	Hodgkin's disease, nodular sclerosis	14	33
13	Sclerosing mediastinal B-cell lymphoma	18	4
14	Sclerosing mediastinal B-cell lymphoma	6	4
15	Sclerosing mediastinal B-cell lymphoma	6	11
16	Sclerosing mediastinal B-cell lymphoma	9	8
17	Sclerosing mediastinal B-cell lymphoma	3	1
18	Sclerosing mediastinal B-cell lymphoma	5	7

**Figure 1**

Periodic acid-Schiff (PAS) stained sections of lymph nodes: (A), (B), (C): Hodgkin's disease (× 2000). (D): Sclerosing mediastinal B-cell lymphoma (× 1000). A: Intracellular rods (arrow). B: Intracellular spheres and rod (arrow). C: Adjacent cells with rods (arrow) and sphere. D: Intracellular rods (arrow).



## Discussion

In 1995 one of the authors put forward the hypothesis that Hodgkin's disease is a human counterpart of bacterially induced crown-gall tumors in plants [10], where *Agrobacterium tumefaciens* is the aetiological agent [9]. In Hodgkin's disease many features suggest a bacterial infection. Ever since the first description of Hodgkin's disease the search for aetiological agents including bacteria and fungi was intensive but unsuccessful. A viral aetiology was thought more probable when Epstein-Barr virus DNA was detected in Hodgkin's disease tissue [14]. Epidemiological, clinical, laboratory, histological and treatment features, however, point rather to a bacterial aetiology:

*Epidemiology:* Two main epidemiological patterns are found in Hodgkin's disease: 1. In developing countries a first peak is found in childhood, a low incidence in the third decade, and a second peak in older adults [15]. 2. In industrialized countries a low incidence is observed in children, a first peak in young adults and a second one in older adults. These epidemiological patterns may suggest a bacterial disease such as tuberculosis. Epidemiological studies by Vianna et al. [16] suggest an incubation period of years like for lepra.

*Presentation:* In over 90% of Hodgkin patients the disease manifests itself in lymph nodes draining the respiratory tract [17]. With this pattern of presentation an airborne infection is quite probable.

*Symptoms and laboratory findings:* The fluctuating fever, chills, and night-sweats as observed in Hodgkin patients are most typical of a chronic bacterial infection. The laboratory findings (neutrophilia, increased blood sedimentation rate and elevated C-reactive protein concentrations) point in the same direction.

*Histology:* In contrast to "true" neoplasms there is no coherent tumor cell population. A mixture of lymphocytes, macrophages, eosinophils, plasma cells, fibroblasts, and others is found in the tissue affected by Hodgkin's disease. Hodgkin / Reed-Sternberg cells which are believed to be the malignant cell population represent only about 0.1% to 1% [18]. The histological picture is rather compatible with a granuloma as seen in chronic bacterial infections than with "true" neoplasms.

*Treatment:* Hodgkin's disease is successfully treated by radiotherapy and chemotherapy. Is this success compatible with a bacterial aetiology? We think it is at least in early Hodgkin's disease. Prior to the use of antibiotics localised infections were successfully treated by radiotherapy [19]. Cytotoxic drugs used in the treatment of Hodgkin's disease show antibacterial activity [20]. The C-reactive protein (CRP) is typically elevated in bacterial infections and Hodgkin's disease. After the implementation of a successful antibiotic treatment of a bacterial infection the CRP serum-level decreases by about 50% within 24 hours [21]. The CRP serum-concentration during the first days of a successful chemotherapy of Hodgkin's disease shows a similar kinetic [22].

The dimensions of the intracellular rods we observed (about 3  $\mu\text{m}$  in length and 0.5  $\mu\text{m}$  in width) and the diastase-resistant PAS-positivity are compatible with several groups of bacteria such as the  $\alpha$ -2 subgroup of proteobacteria: *Bartonella* is related to benign human tumors including lymphomas. *Agrobacterium tumefaciens* belonging to the same subgroup of proteobacteria is known to induce malignant tumors in plants [9]. The PAS-positive spheres we interpreted as accumulation of bacterial residues in phagosomes as in *Tropheryma whipplei* infections. As in other bacteria-induced tumors (e.g. crown-gall disease, gastric MALT lymphomas) the rods are not found in the tumor cells but in macrophages like in *Tropheryma whipplei* infections. The rods observed here cannot be ceroid pigments because of their morphology and staining properties. PCR and electronmicroscopic investigations are under way.

In conclusion we propose that bacteria play an aetiological role in Hodgkin's disease and sclerosing mediastinal B-cell lymphoma which is a well defined disease entity [23].

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