

Crohn’s Disease - A Mycobacterial Infection

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Introduction.

Mycobacterium species exist in two different forms, the cell-walled bacillary form, and the cell wall deficient mycobacterial form (CWDM). The latter form is associated with Crohn’s disease (CD) and has a simple structure consisting of a cytoplasm, an inner membrane, and an external biofilm matrix. CWDM have no cell wall [1].

CWDM are extremely difficult to culture successfully and there has been little interest for that reason. The exception is in relation to Mycobacterium avium ssp paratuberculosis,(MAP) the causative organism of Johne’s disease in ruminants, and a suspected cause of Crohn’s disease. However numerous efforts to cultivate the CWDM from CD tissues and blood have been unsuccessful until now [2].

The tissue study arose out of a technical training exercise. Wellington Public Hospital records were searched from 2015- 2019 for resections from Crohn’s disease patients (18). Controls of non-diseased colon were randomly selected . The tissues were stained with H&E, and several variants of the Ziehl-Neelsen stain. A particular variant of the ZN surprisingly demonstrated the present of acid-alcohol-fast spheroplastic CWDM forms in the ileal tissue of all Crohn’s disease patients, and in none of the controls (CWDM). Separately, cultures of blood from patients diagnosed with Crohn’s disease also carried viable CWDM in some numbers. Ethical approval was given by the local authority [3].

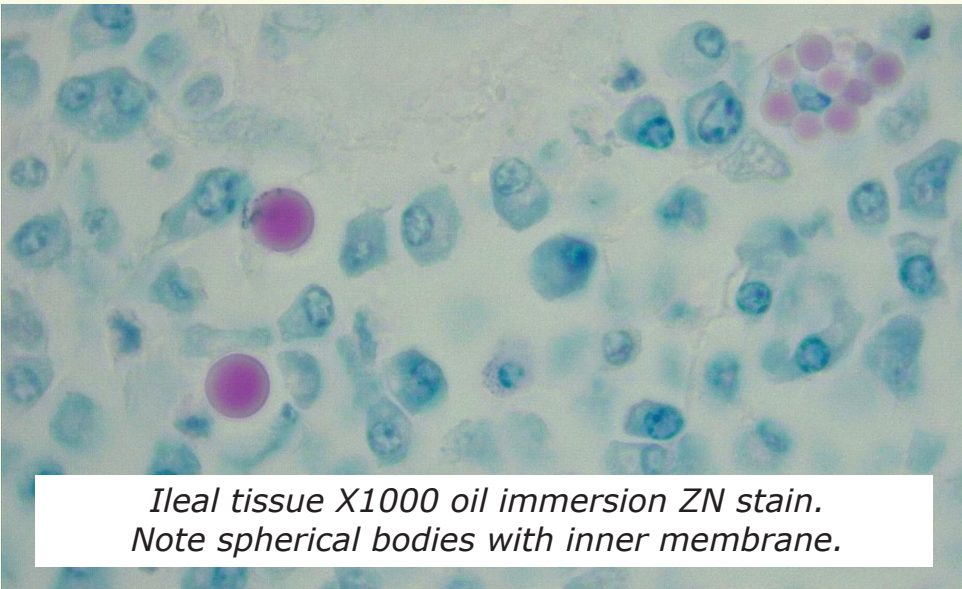
Methods.

Using the Ziehl Neelsen stain we were able to demonstrate the presence of CWDM in ileal tissue from patients with CD, and not in controls, thus fulfilling Koch’s first postulate.

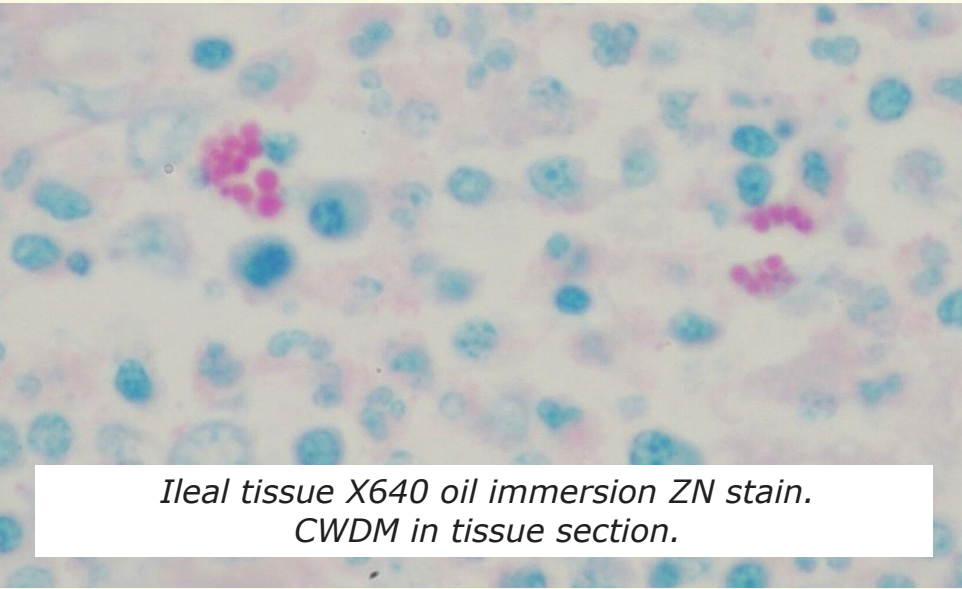
Using recently developed proprietary fluid medium we cultivated CWDM from tissues and blood samples from patients diagnosed with CD.

Results.

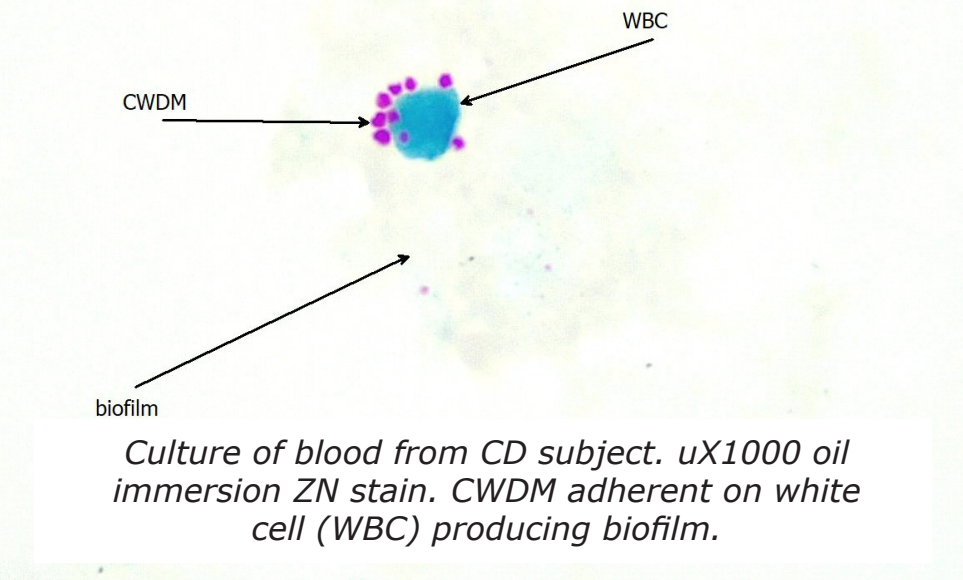
Ileal tissue
CD subjects 18/18 vs. Controls 0/15



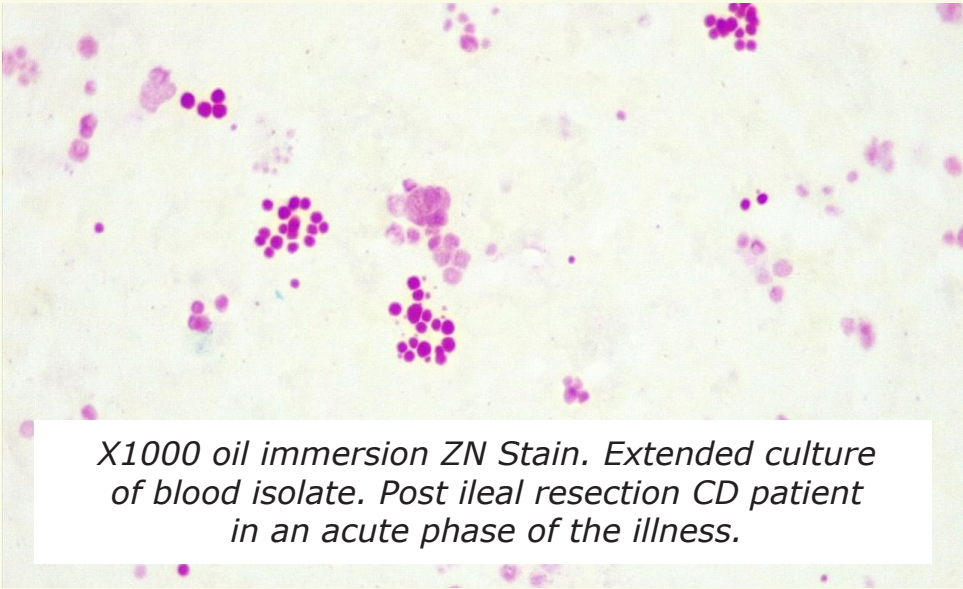
*Ileal tissue X1000 oil immersion ZN stain.
Note spherical bodies with inner membrane.*



*Ileal tissue X640 oil immersion ZN stain.
CWDM in tissue section.*



Culture of blood from CD subject. uX1000 oil immersion ZN stain. CWDM adherent on white cell (WBC) producing biofilm.



X1000 oil immersion ZN Stain. Extended culture of blood isolate. Post ileal resection CD patient in an acute phase of the illness.

Discussion.

The CWDM seen and isolated from CD patient samples in our studies are presently uncharacterised, but have the phenotypic characteristics of mycobacteria. They present as aerobic Mycobacterium species with defined nutrient requirements including mycobactin J. Whole genome sequencing is proceeding. Preliminary findings include the production of biofilm and mycolic acids. High numbers of CWDM are seen in blood cultures when patients are in an acute phase of the illness. CWDM cannot be detected in cultures or tissues by the ZN stain when the conventional acid-alcohol staining method is used, but are able to be seen in culture using an acid-fast ZN stain [4]. Decolourisation of tissue using 30% hydrochloric acid in isopropyl alcohol is recommended to stain the CWDM in tissue samples as well as preserving tissue architecture.

Global rates of inflammatory bowel disease are climbing rapidly [5]. The development of simple new methods for detecting CWDM associated with CD are urgently needed to meet this challenge.

[1] R. J. Chiodini, H. J. Van Kruiningen, W. R. Thayer, and J. A. Coutu, "Spheroplastic phase of mycobacteria isolated from patients with Crohn’s disease.," *Journal of Clinical Microbiology*, vol. 24, no. 3, Art. no. 3, 1986. [2] S. A. Naser, S. R. Sagrainsingh, A. S. Naser, and S. Thanigachalam, "Mycobacterium avium subspecies paratuberculosis causes Crohn’s disease in some inflammatory bowel disease patients," *World J Gastroenterol*, vol. 20, no. 23, pp. 7403–7415, Jun. 2014, doi: 10.3748/wjg.v20.i23.7403. [3] J. M. Aitken et al., "A Mycobacterium species for Crohn’s disease?," *Pathology*, pp. S0031-3025(21)00234–8, Jun. 2021, doi: 10.1016/j.pathol.2021.03.003. [4] J. M. Aitken, J. E. Aitken, and G. Agrawal, "Mycobacterium avium ssp. paratuberculosis and Crohn’s Disease—Diagnostic Microbiological Investigations Can Inform New Therapeutic Approaches," *Antibiotics*, vol. 13, no. 2, Art. no. 2, Feb. 2024, doi: 10.3390/antibiotics13020158. [5] L. Hracs et al., "Global evolution of inflammatory bowel disease across epidemiologic stages," *Nature*, pp. 1–9, Apr. 2025, doi: 10.1038/s41586-025-08940-0.