Chronic orbital inflammatory disease: parasitisation of orbital leucocytes by mollicute-like organisms

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SUMMARY Chronic orbital inflammatory disease (COID) is usually considered non-infectious and idiopathic. Treatment is empirical, palliative, and may not prevent disease progression. COID occurs in isolation or in association with various systemic diseases. Exophthalmos may be an important presenting sign. Vasculitis, lymphoid infiltrates, and granulomas are common. Mollicute-like organisms (MLO) parasitising and destroying vitreous leucocytes are often found to cause human chronic uveitis when an appropriate search is made. Inoculation of these MLO into mouse eyelids produced chronic uveitis and exophthalmic orbital inflammatory disease. Mollicutes are cell wall deficient bacteria. Extracellular mollicutes cause human and animal diseases characterised by lymphoid infiltrates, immunosuppression, and autoantibody production. Intracellular morphologically similar bacteria are non-cultivable pathogens termed MLO. Identification is based on direct detection in diseased cells by transmission electron microscopy. MLO are cytopathogenic and detection is aided by the alterations they produce. MLO replace the cytoplasm, destroy the organelles, and alter the nucleus. This results in cell proliferation, destruction, and dysfunction. MLO parasitise lymphocytes, monocytes, and polymorphonuclear leucocytes. This report describes orbital leucocytes parasitised by MLO in three patients with isolated COID. Rifampicin treatment of MLO disease is discussed.

Chronic orbital inflammatory disease (COID) is usually considered to be non-infectious and idiopathic. It occurs in isolation or in association with various systemic diseases. Exophthalmos may be an important presenting sign. Vasculitis, lymphoid infiltrates, and granuloma formation are common pathological features. Mollicute-like organisms (MLO) parasitising and destroying vitreous leucocytes are often found to cause chronic uveitis in patients when an appropriate search is made. Inoculation of these MLO into mouse eyelids produced chronic uveitis and exophthalmic orbital inflammatory disease. MLO became disseminated to produce lethal chronic inflammatory disease in all organs. MLO-parasitised leucocytes were detected in all the diseased organs. The disease was progressive and similar in all the organs. The initial lesion was a vasculitis. This led to tissue lysis, lymphoid infiltrates, and granuloma formation.

Mollicutes are cell-wall-deficient bacteria. They can be overlooked or confused with viruses. Their ultrastructural appearance is characteristic. Extracellular mollicutes are fastidious, lipid-rich, and contain a variety of potent cytotoxic substances including nuclease. Extracellular mollicutes cause human and animal diseases characterised by lymphoid infiltrates, immunosuppression, and autoantibody production.

Some morphologically similar organisms are intracellular pathogens. Despite considerable efforts at cultivation by many investigators the consensus is that these mollicute forms are non-cultivable. Their taxonomy is uncertain and they are termed MLO. Identification of MLO is based on direct detection within parasitised cells by transmission electron microscopy. MLO are cytopathogenic and their detection is aided by the ultrastructural alterations they produce. They replace the cytoplasm and destroy the organelles and they also alter the nucleus, probably as a result of nuclear parasitisation and release of nuclease.
This results in cell proliferation, destruction, and dysfunction. A common and important feature of MLO disease is vascular involvement.

MLO parasite lymphocytes, monocytes, and polymorphonuclear leucocytes. They appear as undulating filaments of 0.005–0.01 μm diameter, and pleomorphic trilaminar membrane-bound 0.01–1.0 μm tubulospherical bodies. Some forms develop into spore-like 0.5–0.7 μm cocci by deposition of electron dense material within the trilaminar membrane. The cocci have distinctive ultrastructural features. The following report describes the results of a search for MLO parasitised leucocytes in the orbital tissues of patients with isolated COID.

Materials and methods

Three patients with COID served as sources of the study materials. None had a history of thyroid, bowel, or other systemic disease. On physical examination all had normal signs and no evidence of peripheral lymphadenopathy. The results of routine studies of urine, blood cells and chemistry, and thyroid function were within normal limits in all three, and the chest roentgenogram of each was normal. The COID of each patient was considered to be non-infectious. Microbiological studies were not conducted on the excised orbital tissues.

Patient 1 was a 17-year-old female with a two-year history of progressive left upper eyelid ptosis. Orbital computed axial tomograms were normal. Two separate attempts at surgical correction were initially successful, but there was gradual return of the ptosis. During a third corrective procedure abnormal orbital tissue was noted and excised.

Patient 2 was a 61-year-old female with a six-year history of progressive exophthalmos limited to the right orbit. Computed axial tomograms performed at the onset of the disease showed right orbital patchy non-confluent radiodensities and normal extraocular muscles. The left orbit was normal. Biopsy then showed chronic non-specific inflammation. Treatment at that time consisted of combination radiation and chemotherapy. This arrested the disease, but five years later progressive exophthalmos recurred. A repeated computed axial tomography displayed progression of the right orbital disease. The contralateral orbit was again normal. A biopsy of the diseased tissue was taken, and that material was studied in this report.

Patient 3 was a 65-year-old female with a five-month history of progressive exophthalmos of the right orbit. Computed axial tomograms showed patchy right orbital non-confluent radiodensities and normal extraocular muscles. The contralateral orbit was normal. An orbital biopsy was performed.

A portion of each patient’s specimen was fixed in 10% formalin, processed for routine paraffin-embedded histological sections, and stained with haematoxylin-eosin. Another portion of each patient’s specimen was finely minced, fixed in 150 mM cacodylate buffered 2% glutaraldehyde, post-

Fig. 1 Case 1. Chronic vasculitis involving arterioles, capillaries, and adventitia of small arteries.  
(Haematoxylin-eosin, ×110).
fixed in osmium tetroxide, and embedded in Araldite (R). All these materials were available for investigative purposes through an Institutional Review Board exempt protocol. The sections stained with haematoxylin-cosin were studied for COID diagnostic concurrence under a light microscope. Thick sections embedded in Araldite (R) and stained with toluidine blue were also studied for leucocyte-rich foci under a light microscope. Multiple ultrathin sections were prepared from such foci and stained with uranyl acetate-lead tetroxide, and embedded in Araldite (R). All these sections were available for leucocyte alterations and MLO parasitisation by a transmission electron microscope.

Results

HISTOLOGICAL STUDIES
The numbers of leucocytes varied on each patient's specimen (Figs. 1-3). Lymphocytes were the predominant leucocyte in each specimen, but a few scattered monocytes and polymorphonuclear leucocytes were also present. Vasculitis was detected in each specimen, but it was most evident in the specimen from patient 1 (Fig. 1). Each specimen also showed scattered minute lytic foci in the extra-vascular adipose tissue. The specimen from patient 2 showed an intense diffuse lymphoid infiltrate (Fig. 2). The specimen from patient 3 showed several non-necrotic, non-confluent, epithelioid granulomas interspersed between focally intense lymphoid infiltrates (Fig. 3). Ziehl-Nielsen, Gomori's metha-

amined silver, and periodic acid Schiff stains on this specimen were negative for acid fast bacilli and fungi.

TRANSMISSION ELECTRON MICROSCOPIC STUDIES
About 2–10% of all the leucocytes in each patient's specimen displayed intracytoplasmic undulating filaments, 0.005–0.01 μm diameter, and pleomorphic trilaminar membrane-bound 0.01–1.0 μm tubulospherical bodies (Figs. 4,5). Some of these bodies exhibited deposition of intramembranous electron dense material to form 0.5–0.7 μm cocci (Fig. 5). All these cocci had thick cell walls, a distinct periplasm, and complex internal tubulofilamentous substructures. Nuclei of leucocytes harbouring the abnormal bodies often had ragged, irregular contours, chromatin clumping with lysis, and prominent perinuclear electron lucency (Figs. 4,5). In the polymorphonuclear leucocytes these bodies were usually associated with cytoskeletal lysis and a paucity of normal granules (Fig. 5).

Discussion
This study shows the diagnostic value of examining leucocyte COID lesions by transmission electron microscopy. Clinically and pathologically each patient's disease was considered to be non-infectious and idiopathic. However, the leucocytes in each specimen contained intracytoplasmic bacterial-like bodies. Most of these lacked a cell wall. Some
of these forms elaborated distinctive spore-like cocci. The organisms were morphologically indistinguishable from the MLO that produced chronic orbital inflammatory disease in mice. They produced nuclear and cytoplasmic alterations similar to those caused by the other MLO. They parasitised a similar wide range of leucocyte cell lines. We believe that these bacteria were MLO and caused the COID in these patients.

Study of polymorphonuclear leucocytes facilitates MLO detection. As in other chronic inflammatory diseases, polymorphonuclear leucocytes are present in MLO diseases. Prominent cytoplasmic lysis occurs in these leucocytes as a result of MLO parasitisation. The smallest MLO particles, namely, the 0.005-0.01 μm diameter filaments, are readily detected in the lysed cytoplasm. Furthermore, cell wall material forming distinctive spore-like cocci is often deposited in these cells. The lipid rich polymorphonuclear leucocyte cytoplasm may enhance MLO growth and development.

Chronicity was a noteworthy clinical feature of the MLO orbital disease in all three patients. Progression to exophthalmos occurred in two of them. All had chronic inflammation with various features including vasculitis, tissue lysis, lymphoid infiltrates, and
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Fig. 5 Several spore-like cocci are apparent. Undulating pleomorphic tubulospherical bodies (arrows), some with internal structures, are also detectable. The cytoplasm shows patchy lysis and a paucity of normal granules. The nucleus displays several electron lucent chromatin foci of 'moth eaten' appearance, a ragged irregular contour, and a perinuclear electron lucent halo. (Uranyl acetate-lead citrate, ×20,900).

granuloma formation. In the mouse orbit MLO inoculation produced similar chronic inflammation. The mouse disease was progressive, leading to exophthalmos, and vasculitis of small vessels was the initial lesion. The mouse MLO orbital inflammatory process resulted in tissue lysis, lymphoid infiltrates, and granuloma formation. Disease chronicity and progression in the mouse was attributed to MLO disruption of the host inflammatory process.

MLO were found in all three patients studied. A search for MLO as a cause of COID in other healthy patients needs to be conducted to expand these observations. Moreover, the results of MLO experimental mouse disease suggest that MLO may be a cause of human idiopathic systemic inflammatory diseases in addition to orbital disease.

COID frequently occurs in association with chronic bowel, thyroid, and joint diseases. COID patients with such associated systemic diseases need to be investigated for MLO. The search should be conducted on both the orbital and systemic disease.

MLO diseases respond to long-term administration of appropriate antibiotics. Rifampicin was tried in the treatment of MLO experimental mouse disease and it significantly decreased the morbidity and mortality in comparison with untreated infected controls. Rifampicin was subsequently used to treat human MLO uveitis unresponsive to corticosteroids in uncontrolled studies. With prolonged rifampicin administration the ocular inflammation subsided, allowing reduction or discontinuation of corticosteroid. Rifampicin is a broad spectrum intra-
cellularly acting inhibitor of bacterial DNA-dependent RNA polymerase and is safe for prolonged administration.29 It seems to be indicated in the treatment of orbital disease where MLO are demonstrated. Empirical administration of rifampicin may be considered in COID patients where MLO cannot be excluded by transmission electron microscopy of tissue leucocytes and the disease is progressive.

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