Eosinophilic fasciitis associated with Mycoplasma arginini Infection

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Eosinophilic fasciitis (EF) with generalized sclerodermiform skin lesions developed over a 19-month period in a previously healthy 23-year-old man. Although we confirmed EF by skin histology and laboratory tests, the recurrent fevers and the clinical observation of sclerotic prepuce with urethritis indicated further bacteriological analysis by conventional microbiological and DNA-based tests. Urethra cultures were positive for an arginine-hydrolyzing mycoplasma and Ureaplasma urealyticum. The patient also had serum IgM antibodies to Mycoplasma pneumoniae using enzyme-linked immunosorbent assay (ELISA)-based qualitative detection. Mycoplasma arginini was isolated from two independent venous blood serum samples and was identified by conventional microbiological tests and sequencing of the 16S rRNA and rpoB genes (GenBank sequence accession numbers HM179555 and HM179556, respectively). M. arginini genomic DNA also was detected by species-specific PCR in the skin lesion biopsy sample. Treatment with corticosteroids and long-term courses of selected antibiotics led to remission of skin symptoms and normalization of laboratory values. This report provides the first evidence of EF associated with mycoplasma infection and the second report of human infection with M. arginini and therefore suggests that this mycoplasma infection might have contributed to the pathogenesis of the disease.

CASE REPORT

A 23-year-old male, a former bodybuilder, sought medical treatment for progressive generalized skin tightening. His previous medical history was unremarkable. The patient had terminated sport exercises 19 months earlier due to a sudden, progressive skin induration, which led to restricted movement of wrists and ankles within several months, and cutaneous symptoms gradually extended to the extremities and to the upper body. At the beginning of his illness, he quit body building and stopped anabolic steroid consumption. Within 1 year, he lost 23 kg, he became febrile in the afternoons, and his skin disease slowly progressed. Serological tests for HIV, hepatitis C virus (HCV), and Lyme borreliosis were negative. His crista biopsy specimen revealed eosinophilia. The patient was treated with a very low dose of prednisone (10 mg/day orally [p.o.] for the next 6 months without dermatological improvement. Any attempt to decrease the prednisone dosage resulted in return of arthralgia and fever.

Upon initial examination, the patient suffered from generalized skin tightening and tautness on his whole trunk and on all the extremities except the fingers and toes. He also exhibited multiple cutaneous venous furrowing on the arms (Fig. 1). The shiny, brownish, firm skin with some porcelain-whitish patches was tightly bound to the underlying tissues, resulting in a restricted movement of the chest, wrists, elbows, ankles, and knees. The genital region was also involved; the indurated, sclerotic, depigmented prepuce led to phimosis and showed lichen sclerosus-like symptoms with an inflamed urethral orifice.

Initial laboratory tests showed peripheral blood eosinophilia (12.3 × 10^9/liter; normal, 0.0 × 10^9 to 7.0 × 10^9/liter), high serum IgG concentration (50 g/liter; normal, 7 to 16 g/liter), elevated serum IgG1 concentration (29.5 g/liter; normal, 5.2 to 12.7 g/liter), increased IgE concentration (408 U/ml; normal, 0 to 100 U/ml), elevated C-reactive protein (CRP) (25.6 mg/liter; normal, 0.0 to 5.0 mg/liter), and elevated erythrocyte sedimentation rate (ESR) (61 mm/h; normal, 1 to 20 mm/h). Skin histology from the right upper arm revealed a mildly atrophic epidermis, with a fibrotic reticular dermis and homogenized, hyalinized connective tissue in the lower dermis. There was no clear border between the lower dermis and the subcutis, where almost all the subcutaneous fat was replaced by horizontally running, homogenized collagen fibers intermingled with chronic inflammation with lymphocytes, macrophages, a few eosinophils, and several plasma cells up to the fascial layer. Thus, the laboratory data, skin histology, and the clinical symptoms were in agreement with the diagnosis of eosinophilic fasciitis (EF).

Cardiac echocardiogram, electrocardiogram, and chest, skull, and barium swallow X-ray values were within normal limits. The bone scintigraphy showed increased tracer activity in the wrists, ankles, shoulders, elbows, and knees.

An arginine-hydrolyzing mycoplasma and Ureaplasma urealyticum were cultured (Mycoplasma Duo kit; Bio-Rad, France) from the urethra. The initial isolate of the mycoplasma was not identified or saved. Qualitative enzyme immunoassays (EIAs) (Ani Labsystems Ltd. Oy, Vantaa, Finland) for detection of serum IgA, IgG, and IgM antibodies to Borrelia burgdorferi and Mycoplasma pneumoniae were performed and showed that the patient had serum IgM antibodies to M. pneumoniae. A quantitative assay to determine the titer of these IgM antibodies was not done. Isolation of M. pneumoniae from the patient was not attempted. Since results from high-resolution computed tomography (CT)
scanning of the lungs and pulmonary function tests were within normal limits, the presence of a possible extrapulmonary manifestation of mycoplasma infection (37) or the presence of IgM antibodies due to past exposure to *M. pneumoniae* (13, 28, 38) could perhaps explain this positive result in the IgM EIA.

DNA extracted (MiniPrep Express Matrix kit; MP Biomedicals, Irvine, CA) from a deparaffinized skin biopsy specimen taken from the patient before the initiation of antibiotic therapy was PCR positive for the presence of mycoplasma. The skin DNA was tested by SYBR green real-time PCR with mycoplasma-specific universal primers as described previously (20, 35). On the basis of these findings, two independent venous blood serum specimens were collected from the patient at recurrent episodes of fever and were cultured (PPLO broth; BD Biosciences, Franklin Lakes, NJ) for the presence of mycoplasma. Both venous blood serum-inoculated cultures were positive for a pure growth of arginine-*M. arginini* (Fig. 2) based on the sequencing data, as well as the presence of DNA of *M. arginini* cultured from the skin biopsy specimen reamplified with the species-specific primers for the *rpoB* gene (rpoB_argininiF, 5′-TTTGAC GGGTTGTGATAACGT-3′, and rpoB_argininiR, 5′-CAGCT AATCCTAGTGTAATTCGAG-3′), and the PCR products were directly sequenced using the same primers. Therefore, the presence of DNA of *M. arginini* in the patient’s skin biopsy specimen was confirmed, and the sequences of *M. arginini* cultured from the venous blood serum and PCR amplified from the skin sample were identical. Species-specific PCR detection of *M. pneumoniae* in the DNA extracted from the skin biopsy specimen was attempted using published primers (7, 18), but the result was negative. The *in vitro* antimicrobial susceptibility testing (17) was done, and the MICs were as follows: doxycycline, 0.25 mg/ml; erythromycin, 0.5 mg/ml; enrofloxacin, 1.0 mg/ml; lincomycin, 2.0 mg/ml; chlorotetacycline, 4.0 mg/ml.

Based on the classical clinical picture and the confirmed diagnosis of EF, we initiated high doses of methylprednisone (1.5 mg/kg of body weight/day, p.o.). Because microbiological and PCR data indicated the presence of mycoplasma infection in urethra, skin lesions, and blood, when multiple 1/2- to 2-cm large unilateral and then bilateral skin ulcers developed above the ankles and progressed further on the right skin (Fig. 3), intravenous (i.v.) erythromycin was added to the treatment regimen. For long-term therapy, methylprednisone treatment was gradually decreased (1.5 to 1.0 to 0.5 to 0.3 mg/kg/day, p.o.) in combination with a full dosage of i.v. clarithromycin or p.o. erythromycin, clarithromycin, or doxycycline. After 5 months of the combination treatment, the patient felt significantly better for the first time since the onset of his illness and his ulcers healed. Within the following 2 years, the patient’s skin became soft, his movement was free and comfortable, and the phimosis was resolved without the otherwise refused circumcision. In the first year of that treatment, he received 1-week courses of doxycycline (100 mg, twice a day [BID], p.o.) in every second month and took methylprednisolone continuously (4 mg every second day, p.o.). Within the second year, the patient received doxycycline (100 mg, BID, p.o., for a week) in every third month and took 2.0 mg methylprednisone on every second day.

EF is a scleroderma-like disease of unknown origin and is considered to be an autoimmune or recently also an autoinflammatory disease (26), supported by the fact that not only steroids but also immunosuppressive drugs, or biologics such as tumor necrosis factor alpha (TNF-α) inhibitors, seem to be an effective treatment (19, 22). Some of the autoimmune phenomena associated with EF are characteristic for the disease, like high serum IgG concentrations, while others such as hemolytic anemia; thrombocytopenia; aplastic anemia; arthralgia or arthritis; the presence of antinuclear, antihyroglobulin, and anti-thyroid peroxidase antibodies; livedo reticularis; and IgA nephropathy are rare (1, 3, 8, 24). Most of these autoimmune phenomena also have been associated with mycoplasma infections in humans (15, 37). While the etiology of EF has remained obscure, the following remarks should be noted: (i) the presence of granzyme B-positive CD8+ T lymphocytes observed in the EF skin lesions suggests a cytotoxic immune response to infectious or environmental agents (34); (ii) levotryptophan (LT) consumption, presumably containing biological or chemical impurities, has been associated with EF or eosinophilia-myalgia syndrome (EMS) (2, 8–12). Here we report a young EF patient with mycoplasma bacteremia (*M. arginini*) and possibly concurrent infection, or previously infected–recently recovered status, with another mycoplasma species (*M. pneumoniae*), who was successfully treated with high-dose corticosteroids in combination with long-term high doses of antibiotics.

The presence of a positive result for IgM antibodies to *M. pneumoniae* and explanation of their association with a possible concurrent *M. pneumoniae* infection of the respiratory tract in the patient are difficult due to the absence of clinical symptoms and...
FIG 2 Dendrograms showing phylogenetic relationships based on nucleotide sequence data for the 16S rRNA (A) and rpoB (B) genes for *M. arginini* isolated from the patient and closely related species of the family *Mycoplasmataceae*. The trees were constructed by the minimum evolution method in the MEGA 4 package. The bootstrap values presented at corresponding branches were evaluated from 1,000 replications. GenBank accession numbers are indicated for each strain used in creating the dendrograms.
the presence of normal chest X-ray and CT scan of the lungs and pulmonary function tests in the patient. The EIA (Ani Labsystems) used for detection of serum IgM antibodies to *M. pneumoniae* is based on the surface-exposed P1-enriched protein (31), which is the main virulence factor of *M. pneumoniae*, and the assay has been shown to have good performance in terms of sensitivity and specificity early in the course of infection (6). The possibility of cross-reactivity of anti-*Mycoplasma arginini* IgM antibodies with *M. pneumoniae* P1 protein in this test has never been investigated due to the absence of clinically documented importance of *M. arginini* in humans. Because IgM antibodies to *M. pneumoniae* may persist for several months in the circulation (13, 28, 38), the patient may have had the positive result in the EIA due to past exposure to *M. pneumoniae*.

We speculate that mycoplasma infection may have triggered EF in our patient. Non-EF skin manifestations during *M. pneumoniae* infection have been reported (16, 27, 30, 34, 37). The following known triggering factors were identified in our patient: (i) LT-rich food additive consumption derived partially from uncontrolled sources and (ii) strenuous exercises. We also wish to highlight some additional suspicious observations for this patient that may have contributed to EF: (i) regular use of p.o. and intramuscular (i.m.) anabolic steroids derived partially from uncontrolled animal sources, (ii) mycoplasma bacteremia, (iii) the presence of mycoplasma and ureaplasma urethral infection, and (iv) the presence of mycoplasma DNA in the diseased skin.

The fact that we have detected the presence of *M. arginini* in the blood and also in the skin lesion of the patient needs to be elucidated. This organism is normally associated with animals (5, 21), and the source of this *M. arginini* infection in the patient is unclear. The patient had regularly used nutrition supplements rich in proteins and amino acids (i.e., LT and arginine) for the past 10 years, and he reported the combinational use of anabolic steroid injections and tablets (testosterone enanthate, nandrolone phe- nypropionate, clenbuterol, and methandrostestanolone) for the past 3 years. All the products were from local distributors, and the majority of these nutritional supplements were derived from animal materials of uncontrolled sources. Although speculative, it is possible that the patient acquired the infection through the consumption of his dietary supplements and/or from hormone injections derived from uncontrolled animal materials. However, we were not able to identify this source retrospectively.

While most mycoplasma species are host specific, there are reports of the presence of mycoplasma in hosts that are not perceived as their normal habitat. Thus, in addition to *M. arginini*, rare human infections with *Mycoplasma bovis* (23), *Mycoplasma phocicercale* (4), and presumably animal hemotropic mycoplasma species (14, 29, 32, 33) have been reported. In some cases, the “jump” of animal mycoplasma to humans may have a pathological impact, particularly under predisposing conditions such as immunodeficiency (25, 39). Mycoplasma infections have been associated with autoimmune disorders (15, 37), but their role in disease pathogenesis is unclear.

Our case is the second report of documented *M. arginini* infection in humans. The first human case was a fatal *M. arginini* septicemia with pneumonia reported in a 64-year-old slaughterhouse worker with advanced non-Hodgkin’s lymphoma (39). Our EF patient may represent a case of induced autoimmunity, where the consumption of LT and anabolic steroids from uncontrolled sources could contribute to disease development. We think that the presence of these factors in the medical history might indicate a search for potential infectious triggers, including the presence of mycoplasma infections. Our data also suggest that complementary antibiotic therapy may be necessary in some patients with EF.

**Nucleotide sequence accession numbers.** The sequences were deposited at GenBank under accession numbers HM179555 and HM179556.

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**REFERENCES**


**FIG 3** Development of skin ulceration during high-dose steroid therapy.