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THE EOSINOPHILIA-MYALGIA SYNDROME AND TRYPTOPHAN

Edward A. Belongia,[1] Arthur N. Mayeno,[2] and Michael T. Osterholm[1]

[1]Acute Disease Epidemiology Section, Minnesota Department of Health, Minneapolis, Minnesota 55440; [2]Departments of Immunology and Medicine, Mayo Clinic and Foundation, Rochester, Minnesota 55905

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INTRODUCTION

During the summer and fall of 1989, an epidemic of a new, multisystem illness occurred in the United States. The disease was characterized by severe

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muscle pain and profound eosinophilia. It was initially recognized in October when physicians in New Mexico treated three women with similar clinical findings. They suspected an association with tryptophan consumption after observing that all three had consumed the food supplement prior to onset of illness (35). This finding was publicized by the local news media, and additional cases were reported by other New Mexico physicians. Shortly thereafter, cases were also recognized in other regions of North America and Europe. The major clinical features formed the basis for the name of the new disease: eosinophilia-myalgia syndrome, or EMS.

In early November, case-control studies were initiated in Minnesota and New Mexico to determine if there was an epidemiologic association between tryptophan use and EMS, or if tryptophan use was a surrogate for another unidentified risk factor. Both investigations demonstrated a significant association between antecedent tryptophan consumption and EMS (9, 23). A national surveillance program was initiated by the Centers for Disease Control (CDC). The case definition was developed based on review of the clinical findings of the initial cases. It included (*a*) eosinophil count greater than 1,000/mm^3, (*b*) generalized debilitating myalgia, and (*c*) no evidence of infection or neoplasm that would explain the clinical findings.

On November 11, 1989, the US Food and Drug Administration issued a warning that advised consumers to discontinue use of tryptophan food supplements. The agency subsequently requested a nationwide recall of all over-the-counter food supplements that contained at least 100 milligrams of tryptophan in a daily dose. Although over 1500 EMS cases were identified, the epidemic was essentially halted by the removal of tryptophan from the consumer market.

THERAPEUTIC USE OF TRYPTOPHAN

Medical research in the 1970s and early 1980s suggested that tryptophan might be useful for the treatment of depression (17, 32, 84). Since then a number of investigations have examined its efficacy for a variety of other conditions, including insomnia, chronic pain, schizophrenia, premenstrual syndrome, affective disorders, and behavioral disorders (6, 12, 16, 20, 25, 31, 33, 49, 52–55, 64). The emphasis on treatment of psychiatric and behavioral disorders stemmed in part from the observation that brain serotonin content could be altered by changes in plasma tryptophan levels (24).

During the 1980s, reports in the popular press encouraged consumers to use tryptophan for therapeutic purposes. The product was widely available without a prescription, and it was promoted as an over-the-counter remedy for a variety of problems (51). A 1990 survey of tryptophan use in the Minneapolis-St. Paul area found that 4% of households had at least one person who had

used tryptophan between 1980 and 1989 (3). The prevalence of use increased markedly between 1985 and 1989 and was highest in women (Figure 1). Although many consumers purchased tryptophan for therapeutic use, it was marketed as a food supplement. The manufacturers made no claims regarding therapeutic efficacy, and the product was not regulated or approved by the US Food and Drug Administration (5a).

BIOCHEMISTRY AND METABOLISM OF TRYPTOPHAN

Tryptophan is an essential amino acid. It is catabolized in mammals along two main pathways, resulting in the formation of kynurenine and serotonin (Figure 2). Most ingested tryptophan is degraded via the kynurenine pathway and provides precursors for the biosynthesis of niacin (nicotinic acid) and nicotinamide adenine dinucleotide. In this pathway, tryptophan is first oxidized to *N*-formylkynurenine by tryptophan 2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase (IDO). This first enzymatic step is the rate-limiting step in the degradation of tryptophan. TDO (also called tryptophan pyrrolase) is localized to the liver while IDO is distributed throughout various tissues. Induction of IDO or TDO increases tryptophan catabolism and the formation of kynurenine and its metabolites. TDO activity is partially regulated by the hypothalamic-pituitary-adrenal axis. The enzyme is induced by glucocorticoids and adrenocorticotropic hormones; it is down-regulated by growth hormone. TDO activity is also increased by tryptophan loading. IDO activity is induced by gamma interferon (IFN-γ), and administration of this cytokine leads to increased levels of tryptophan metabolites in vitro and in vivo (8).

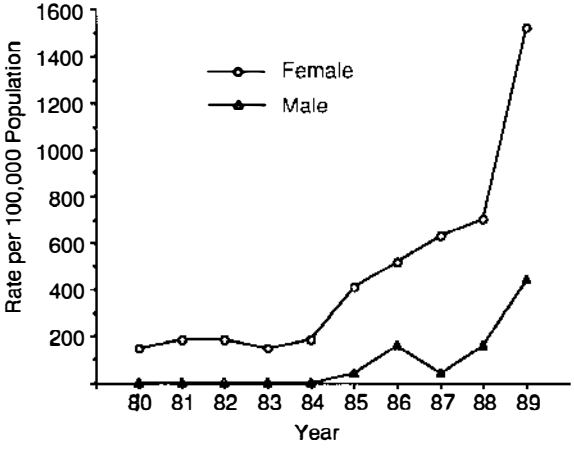

Figure 1 Prevalence of tryptophan use among male and female household members in a survey of 2012 randomly selected households in metropolitan Minneapolis-St. Paul (from Reference 3).

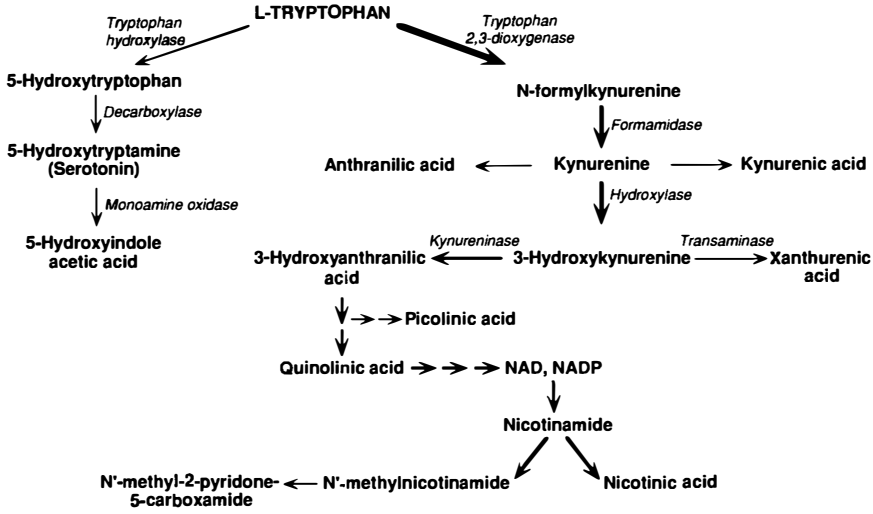

Figure 2 Major metabolic pathways of tryptophan degradation in humans.

Administration of interleukin 2 also induces IDO (7). Unlike TDO, IDO is not induced by either glucocorticoids or tryptophan loading.

Kynurenine is catabolized through several routes. The major pathway involves hydroxylation to 3-hydroxykynurenine, followed by degradation to 3-hydroxyanthranilic acid. Transaminases convert small portions of kynurenine and 3-hydroxykynurenine to kynurenic acid and xanthurenic acid, respectively.

A small portion of ingested tryptophan is converted to serotonin, a neurotransmitter. This metabolic pathway is found primarily in the central nervous system. Serotonin is degraded by monoamine oxidase and is excreted as 5-hydroxyindoleacetic acid. In the carcinoid syndrome, excessive amounts of serotonin and 5-hydroxyindoleacetic acid are produced by this route. A small proportion of ingested tryptophan is also metabolized by bacteria in the large intestine to indole, skatole, and other indole derivatives. Indole is converted to indican, which is excreted in the urine along with other indole compounds such as tryptamine, indole pyruvic acid, and indole acetic acid.

EOSINOPHILIA-MYALGIA SYNDROME

National Surveillance Data

By mid-1990, a total of 1531 EMS cases had been reported to the Centers for Disease Control, including 27 deaths (74). Eighty-four percent of patients

were female, 97% were non-Hispanic white, and 86% were over 34 years old (median age, 49 years). Surveillance data demonstrated a dramatic increase in the incidence of EMS during the summer and fall of 1989 (Figure 3).

The prevalence of EMS was higher in the western United States than in other parts of the country, possibly because of a higher rate of tryptophan consumption in those states (74). High prevalence rates were also found in states that carried out investigations of EMS, including Minnesota, South Carolina, New Mexico, and Oregon. The high prevalence in these states may be partly attributed to more active surveillance and case identification.

The true prevalence of EMS is underestimated by surveillance reports. Persons with mild disease were excluded by the surveillance case definition even if the clinical diagnosis was consistent with EMS. In addition, surveillance data were compiled from reports submitted by physicians, and it is likely that a number of cases were diagnosed but not reported to state or federal health agencies.

Epidemiologic Studies

After initial case-control studies implicated tryptophan consumption as a major risk factor for EMS, additional investigations sought to determine the basis for this association. Two hypotheses were initially advanced to explain the association. According to one hypothesis, tryptophan itself triggered EMS in susceptible individuals, possibly owing to abnormalities of tryptophan

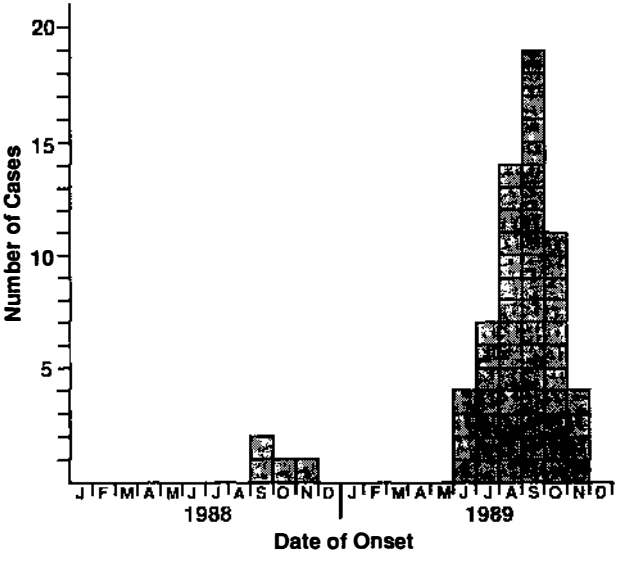

Figure 3 Cases of eosinophilia-myalgia syndrome in Minnesota by date of symptom onset (from Reference 3).

metabolism (15). According to the other hypothesis, EMS was triggered by a contaminant that was present in some lots of manufactured tryptophan. The latter hypothesis was consistent with the sudden appearance of the outbreak after tryptophan had been marketed for several years with no apparent ill effects. Epidemiologic investigations subsequently demonstrated that EMS was not triggered by tryptophan per se, but rather by exposure to a contaminant in tryptophan manufactured by one company.

Evidence for a tryptophan contaminant was provided by two case-control studies. In Minnesota, EMS patients and two control groups were evaluated to determine the manufacturer of their tryptophan and to assess potential risk factors (3). One control group consisted of self-referred, asymptomatic tryptophan users; the other group included asymptomatic tryptophan users who were randomly selected during a telephone survey. Retail lot numbers were obtained and traced back to determine the tryptophan source, and lots of bulk tryptophan were analyzed using high performance liquid chromatography.

Analysis of the tryptophan source for case patients and controls demonstrated a strong association between EMS and consumption of tryptophan manufactured by Showa Denko, K.K. (Tokyo, Japan). Twenty-nine (97%) of 30 case-patients consumed tryptophan (during the month before onset) that was manufactured by this company, compared to 21 (60%) of 35 in the combined control groups (odds ratio 19.3; 95% confidence interval 2.5 to 844.9). The tryptophan consumed by the 29 case-patients was manufactured by Showa Denko between October 1988 and June 1989. To assess the role of manufacturing changes during this time period, additional analyses were carried out utilizing the 29 case-patients and 21 controls who consumed tryptophan that was manufactured by Showa Denko. Product lots that were used prior to illness onset were considered "case lots", whereas lots that were not consumed by any patients were considered "control lots."

The company utilized a fermentation process to manufacture tryptophan. A strain of *Bacillus amyloliquefaciens* was used to synthesize tryptophan from precursors. Following fermentation, the tryptophan was extracted from the broth and purified using a series of filtration, crystallization, and separation processes. In December 1988, the company introduced a new strain of *B. amyloliquefaciens* (strain V) that had been genetically modified to increase the synthesis of intermediates in the tryptophan biosynthetic pathway. In 1989 the company also processed some fermentation batches with a reduced amount of powdered activated carbon (10 kg) in one of the purification steps. According to the company, these changes did not significantly alter the purity of the tryptophan produced, which was maintained at 99.6% or greater.

In the comparison of "case lots" and "control lots," both a reduction in the amount of powdered activated carbon and use of *B. amyloliquefaciens* (strain V) were significant manufacturing changes according to univariate analysis.

The independent contribution of each manufacturing change could not be assessed because there was a high correlation between them. Studies carried out by the company suggested that the biochemical and physiologic characteristics of *B. amyloliquefaciens* (strain V) did not differ from those of earlier strains. At this time it is not known whether the production of contaminant varied with different bacterial strains or if the efficiency of removal varied with changes in the separation and purification processes. Possibly both factors contributed to the presence of the etiologic agent.

The association between EMS and consumption of Showa Denko tryptophan was also found in an investigation of EMS patients and asymptomatic tryptophan users in Oregon (67). Ninety-eight percent of case patients had consumed tryptophan manufactured by Showa Denko, compared to 44% of controls. Eighty-five percent of the case lots were manufactured from January through May, 1989. An analysis of manufacturing conditions for case lots and control lots was not reported.

Chemical analyses of bulk tryptophan lots provided additional support for the epidemiologic findings (3). High performance liquid chromatography demonstrated a unique pattern, or "fingerprint," for tryptophan manufactured by different companies. The chromatographic pattern consisted of multiple peaks, with each peak representing a trace chemical constituent other than tryptophan. The chromatogram for Showa Denko tryptophan was distinctive and included 5 "signature" peaks that were present in all tryptophan manufactured by this company. Comparison of individual peaks in case and control lots demonstrated one peak ("peak E") that was significantly associated with case lots. This peak was present in 9 (75%) of 12 case lots and 3 (27%) of 11 control lots (odds ratio, 8.0; 95 percent confidence interval, 0.9 to 76.6; $P = 0.022$). The presence of peak E was also associated with the manufacturing changes described earlier. The chemical structure of peak E was subsequently determined to be 1,1'-ethylidenebis[tryptophan] (EBT) (Figure 4) (50, 68). The chemical is hydrolyzed under acidic conditions, and its biologic activity is uncertain. However, preliminary results from animal studies suggest that EBT may cause abnormalities of the fascia and microvasculature (46).

In addition to EBT, two other contaminants have been reported to be associated with case lots of tryptophan manufactured by Showa Denko (77). Both were found by using high performance liquid chromatography. One of the peaks, labeled UV-5 (also called FL-7), eluted before tryptophan and may be a low molecular weight aromatic compound. The mean concentration of UV-5 was lower than that of EBT. The other peak (UV-28) eluted much later than EBT and was present in even lower concentrations. The chemical structure of these peaks had not been reported as of January 1992.

There is no convincing evidence that any EMS cases were caused by

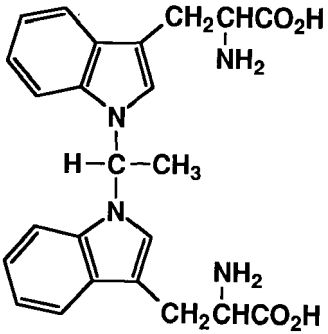

Figure 4 Structure of 1,1'-ethylidenebis[tryptophan], a tryptophan contaminant that has been associated with EMS in epidemiologic studies.

consumption of tryptophan manufactured by companies other than Showa Denko. One patient in the Minnesota series consumed tryptophan that was traced back to another manufacturer. This person had reportedly consumed a lifetime total of 20 tablets from one bottle during a two-month period. The tablets from this bottle yielded a chromatogram that was characteristic of tryptophan manufactured by Showa Denko, including the presence of EBT. These data suggest that her tryptophan was manufactured by Showa Denko rather than by the company identified by the product traceback. The explanation for the discrepant results from the product traceback is unclear. In the Oregon study, one EMS patient reportedly took tryptophan manufactured by another company, but this patient had also used another unknown brand of tryptophan before onset of her symptoms. Thus, exposure to tryptophan manufactured by Showa Denko could not be ruled out.

Data from a cohort of tryptophan users in a South Carolina psychiatric practice provides an estimate of the EMS attack rate for persons exposed to the etiologic agent (37). In this group, 157 persons consumed a single brand of tryptophan that was manufactured by Showa Denko. The product traceback indicated that only three manufacturer lots (all from Showa Denko) were represented in the tablets. The EMS attack rate was 29% in this group. An additional 23% were classified as "possible cases" because they had some clinical findings of EMS with suggestive symptoms, but without incapacitating myalgia. Thus, the pooled attack rate may have been as high as 52% among persons exposed to the etiologic agent.

RISK FACTORS

Few risk factors for EMS have been identified other than consumption of implicated tryptophan lots. The amount of tryptophan consumed has been

identified as a risk factor in three investigations. In Minnesota, EMS patients consumed a median of 40.5 grams of tryptophan per month, compared to 6.0 grams for random controls and 15.0 grams for self-referred controls ($P < .001$) (3). In Oregon, EMS patients consumed an average of 1.3 grams of tryptophan per day, while the average in the two control groups was less than half that amount (67). In the South Carolina cohort, the risk of EMS was 3.5 times higher for persons taking more than 4 grams of implicated tryptophan per day than for those using 0.5 to 1.5 grams daily (37). This demonstrates a dose-response relationship between the amount of implicated tryptophan ingested and the risk of EMS.

There are several possible explanations for the association between tryptophan dose and risk of EMS. First, persons who consume larger doses of tryptophan may be exposed to greater quantities of the etiologic agent. Alternatively, persons consuming large doses (i.e. many tablets) may have a greater probability of encountering a contaminated tablet that causes illness. Evidence for a dose-response relationship in the South Carolina cohort suggests that the former hypothesis is correct; tryptophan dose is probably a surrogate for dose of ingested contaminant.

Age was also found to be a risk factor for EMS in two investigations. In the Minnesota study, the median age of case patients (45 years) was significantly older than the median age of randomly selected tryptophan users (39 years) after controlling for dose. In the South Carolina cohort, the risk of EMS was increased in older patients and was independent of dose (37). In Oregon, no significant difference in age was found between EMS patients and controls who used tryptophan (67). It is plausible that increasing age could increase the risk of EMS owing to physiologic changes in renal or hepatic function that alter the metabolism or delay the clearance of a toxic substance. Age-related changes in gastrointestinal absorption could also play a role.

There is no evidence that other host factors significantly alter the risk of developing EMS. A variety of factors were examined in both the Minnesota and South Carolina studies, including preexisting illnesses, asthma, smoking, alcohol consumption, and use of specific food supplements or prescription medications (e.g. nonsteroidal antiinflammatory drugs, tricyclic antidepressants, benzodiazepines, pyridoxine). None of these factors was significant when EMS patients were compared with controls who consumed tryptophan manufactured by Showa Denko. However, it is possible that other unknown genetic or environmental factors increase the risk of EMS after exposure to the etiologic agent.

Investigators have speculated that persons with impaired hypothalamic-pituitary-adrenal (HPA) function may be at increased risk of developing EMS when exposed to an inflammatory trigger (65). In the normal host, the presence of inflammatory mediators will signal the HPA axis to restrain the intensity of the inflammatory response through the release of glucocorticoids.

In Lewis rats, arthritis can be induced by exposure to streptococcal cell wall antigen, and susceptibility is increased if the HPA response to an inflammatory stimulus is impaired (70). However, at present no evidence indicates that EMS patients have impairment of the HPA axis.

CLINICAL AND PATHOLOGIC FEATURES

EMS is a syndrome with multiple clinical presentations and variable severity. Early clinical reports indicated that most patients developed profound eosinophilia and severe myalgias; these features provided the basis for the CDC case definition. In addition to myalgia, the most commonly reported early symptoms included arthralgias, weakness or fatigue, dyspnea or cough, rash, headache, peripheral edema, fever, and paresthesia (3, 10, 58, 74).

According to the CDC surveillance case definition for eosinophilia, all patients had at least 1,000 cells/mm.^{3} In different groups of EMS patients, the median eosinophil count has been reported to be 4,000 to 6,000 cells/mm^3 (3, 10, 67). The majority of patients also had an elevated leukocyte count with abnormally high levels of serum aldolase, a marker for muscle injury. Serum creatine phosphokinase, another indicator of muscle injury, was normal in most patients. Approximately one half of patients had abnormal liver function tests. The erythrocyte sedimentation rate, rheumatoid factor, and levels of IgE, complement, cryoglobulin, and thyroid stimulating hormone were normal in most patients tested (10, 29, 38, 58, 74).

Pathologic studies have demonstrated a perivascular, lymphocytic infiltrate in the dermis, fascia, and skeletal muscle, with variable numbers of eosinophils (34, 69). The perivascular infiltrate was accompanied by thickening of the capillary and arteriolar endothelium in dermal, fascial, and muscle vessels. The frequent occurrence of microangiopathy in biopsy specimens suggests that ischemia may contribute to tissue injury (69). Cytotoxic eosinophil degranulation products can be found in affected tissue, and these may play an important role in pathogenesis (35, 47).

The cutaneous and subcutaneous induration in EMS patients may be attributed to excessive accumulation of connective tissue in the affected fascia and lower dermis (79). Immunohistochemical studies have demonstrated increased deposition of transforming growth factor-β_1 (TGF-β_1), type VI collagen, and fibronectin in the extracellular matrix of the affected fascia (57). Fibroblasts in affected fascia demonstrate increased expression of several genes by in situ hybridization, including type I procollagen, type VI collagen, and TGF-β_1 (79). Since TGF-β_1 has been shown to play a prominent role in the regulation of fibroblast connective tissue production (61), this cytokine may play a role in the pathogenesis of EMS.

Most patients with EMS reported paresthesias, and in some patients peripheral neuropathy was the most prominent clinical feature. In some patients, persistent paresthesias have been accompanied by axonal and demyelinating abnormalities revealed by electrophysiologic testing (78). Perineural inflammation and type II fiber atrophy with denervation features have been observed, but muscle fiber necrosis was uncommon (80). The severe myalgias may be related to inflammation of nerves in the fascia or muscle, peripheral nerve injury caused by eosinophil-derived neurotoxin, or ischemia of nerves caused by occlusive microangiopathy (47).

Respiratory symptoms have been reported frequently by EMS patients, but the proportion with significant pulmonary disease is unknown. In the South Carolina cohort, 22% of hospitalized patients with EMS had radiographic evidence of pulmonary infiltrates (37). Lung biopsies performed in a small number of patients have demonstrated a vasculitis and perivasculitis with a chronic interstitial pneumonitis (73, 76). Disturbances of cardiac rhythm and conduction have also been documented. Examination of autopsy specimens has demonstrated neural lesions throughout the conduction system, similar to the neuropathology seen in skeletal muscle (36). Inflammatory lesions of the small coronary arteries were also present. The prevalence of cardiac abnormalities among all patients with EMS is unknown, although life-threatening rhythm disturbances appear to be rare.

The clinical and histopathologic findings of EMS overlap those of eosinophilic fasciitis (22). The latter is a scleroderma-like syndrome characterized by tender swelling and induration of the subcutaneous tissue, primarily in the arms and legs. Eosinophilia and hypergammaglobulinemia are characteristic findings, along with inflammatory infiltrates in the fascia and dermis. Eosinophilic fasciitis is distinguished from systemic sclerosis (scleroderma) by the relative absence of visceral involvement, digital ulcerations, and Raynaud's phenomenon (44), although the two diseases share many common features and may be variants of the same pathologic process.

The relationship between EMS, eosinophilic fasciitis, and systemic sclerosis was investigated in a retrospective review of patients diagnosed with eosinophilic fasciitis between 1977 and 1989 (48). Eight (24%) of 34 patients with onset from 1986 to 1989 had taken tryptophan before onset of symptoms. However, none of 25 patients with onset from 1977 to 1984 had consumed tryptophan ($P < .001$). In addition, none of 11 patients with systemic sclerosis had used tryptophan before the onset of illness. Review of biopsy material demonstrated no histopathologic differences between tryptophan-associated eosinophilic fasciitis and idiopathic eosinophilic fasciitis.

These results indicate that the histopathologic features of EMS and eosinophilic fasciitis are identical and that some cases of eosinophilic fasciitis were caused by tryptophan consumption. However, eosinophilic fasciitis may

also be triggered by factors other than tryptophan consumption. Few, if any, eosinophilic fasciitis cases with onset before 1986 can be attributed to tryptophan use. This finding could be explained by manufacturing changes that caused sporadic contamination of tryptophan manufactured from 1985 to 1988, with an increase in the quantity or concentration of this contaminant in 1989.

Abnormalities of tryptophan metabolism have been reported in patients with EMS, the toxic oil syndrome, and other scleroderma-like conditions, leading to speculation that one or more metabolites may play a role in the pathogenesis of these diseases (5, 59, 65, 71). In patients with EMS, both kynurenine and quinolinic acid levels are elevated compared to those of controls (66), and quinolinic acid is a potential neurotoxin (26). However, the same abnormalities of tryptophan metabolism are found in unrelated conditions that involve chronic immune system activation, including HIV infection (27). These abnormalities appear to be mediated by IFN-γ, which induces IDO, the major rate-limiting enzyme in the kynurenine pathway. Administration of IFN-γ to cancer patients causes increased serum levels of kynurenine and quinolinic acid and decreased levels of tryptophan. However, it does not produce scleroderma-like changes or eosinophilia. Overall, studies of tryptophan metabolism suggest that the observed abnormalities are secondary to immune system activation; there is no evidence that they contribute to the specific pathologic changes seen in EMS.

NATURAL HISTORY AND TREATMENT

Only limited information is available regarding the clinical progression of EMS and the response to treatment. In Washington, 45 patients were followed up with serial telephone interviews and review of physical exam findings for up to 15 months after illness onset (19). The initial symptoms were myalgia and fatigue in most patients, followed by pulmonary symptoms (cough or dyspnea) within several weeks. Pruritic rash, peripheral edema, and/or paresthesias also developed in over 75% of patients, typically during the first two months after onset of myalgia. Some patients had relapses of myalgia after nearly complete symptom resolution. Neurologic symptoms generally consisted of diffuse paresthesias, but 15% of patients also described neurocognitive symptoms such as memory loss and difficulty concentrating. Scleroderma-like skin changes developed in 42%, but this finding was not observed until later in the disease course (median, 80 days after onset). The changes included dry, leathery, thickened skin, usually accompanied by changes in pigmentation. After 6 months, there was a steady decrease in the number of patients reporting severe myalgia, pulmonary symptoms, rash, and edema. One year after onset, myalgias had resolved in 42% of patients and had improved by an average of 72% in the remainder.

In New York, a follow-up questionnaire was completed by 91 patients (11); the median interval from symptom onset was 16 months (range, 11 to 40 months). At follow-up, 64% of patients reported persistent EMS symptoms that were "moderate" or "extreme." The most commonly reported persistent symptoms included fatigue (64%), muscle weakness (60%), muscle cramps (57%), myalgia (55%), and arthralgia (48%). Only 10% of patients reported complete resolution of symptoms, although the majority had experienced some reduction of severity.

Both of the previous investigations relied primarily on subjective reports of symptoms and severity to assess the progression of EMS. Objective measurements, such as serial muscle biopsies or laboratory results, were not available. The findings suggest that EMS symptoms improve gradually in most patients, but complete recovery is uncommon during the first one to two years after onset. Additional studies are needed to determine if tissue histopathology correlates with symptoms over time.

The response to therapy has been disappointing. Multiple therapeutic interventions have been suggested, but no clearly effective treatment has been identified (14). Because of the nature of the outbreak, it has not been possible to assess treatment regimens in a randomized, prospective study. Glucocorticoid treatment (usually prednisone) has been reported to cause symptomatic improvement in some patients, with a reduction of the eosinophil count (42). However, some patients have not responded to high doses of prednisone, and others have had an exacerbation of symptoms when the dose was tapered (29, 48). There is no evidence that prednisone therapy alters the natural history of the disease or the risk of neuropathy (19). Other treatments that have been utilized include nonsteroidal antiinflammatory drugs, cyclosporin A, cyclophosphamide, hydroxychloroquine, D-penicillamine, methotrexate, octreotide (a somatostatin analogue), and plasmapheresis (13, 14, 29, 38, 48, 72). Many of these therapies have been tried in patients with severe illness, and insufficient information is available to assess efficacy.

EMS AND THE TOXIC OIL SYNDROME

The clinical and pathologic findings of EMS bear a striking resemblance to those of the toxic oil syndrome (TOS). The latter outbreak occurred in Spain during 1981. Nearly 20,000 persons were affected, including 315 who died (41). Unlike EMS, respiratory symptoms (cough or dyspnea) were prominent and severe in TOS during the first week of illness. Bilateral pulmonary infiltrates due to noncardiogenic pulmonary edema were present in over 90% of patients with chest radiographs (2). The respiratory symptoms usually resolved and the chest radiographs returned to normal, although some patients developed pulmonary hypertension (30). Other early symptoms included fever, malaise, headache, nausea, splenomegaly, diffuse adenopathy, and

pruritic rash (41, 75). In some patients, the disease progressed to an intermediate and chronic phase that more closely resembled EMS. The intermediate phase (2 to 8 weeks after onset) was characterized by eosinophilia and leukocytosis. Patients who progressed to the late phase developed muscle cramps and severe myalgias, peripheral edema, scleroderma-like skin changes, and polyneuropathy. The histopathology of skin, nerve, and skeletal muscle is remarkably similar in EMS and TOS (34, 63).

Epidemiologic investigations of TOS implicated consumption of denatured industrial rapeseed oil that was illegally sold by itinerant salesmen (60). Chemical analyses of implicated oil samples and "control" oil samples demonstrated that free aniline and fatty acid anilides were significantly associated with case-related samples (4, 40). However, efforts to evaluate the biologic activity of these substances have been limited by the absence of an animal model.

The strong similarities between EMS and TOS suggest that they may share the same final common pathway that leads to neuromuscular damage. However, they may not be triggered by the same etiologic agent. The vehicle of transmission was clearly different (oil versus manufactured tryptophan), and no contaminants common to both vehicles have been reported. In addition, the type and level of exposure to the etiologic agent may have been different for TOS compared to EMS. In the TOS epidemic, inhalation of oil vapor (during or immediately after cooking) could have been an alternate source of exposure and might account for the more severe pulmonary pathology.

A population-based follow-up evaluation of patients with TOS demonstrated that the majority reported an improvement of symptoms over 8 years (1). Overall, 49% had complete resolution. Of those with residual symptoms, only 7% had significant functional impairment. If the natural history of EMS is similar, severe long-term disability may be uncommon, and complete recovery may be expected in approximately half of affected patients.

PROGRESS TOWARD UNDERSTANDING ETIOLOGY AND PATHOGENESIS

Research to elucidate the etiology and pathogenesis of EMS is ongoing. Development of an appropriate animal model has been a priority. Ideally, such a model would be based on an inexpensive, readily-available species that develops eosinophilia and typical pathologic changes after exposure to implicated tryptophan. Investigators have also attempted to develop an in vitro system to study the mechanism of disease at the cellular level.

Animal Models

Lewis rats have been proposed as an animal model for EMS (18). This species is known to be susceptible to several inflammatory diseases in response to

inflammatory stimuli. To assess the utility of the model for EMS, Lewis rats were given either implicated tryptophan or pharmaceutical grade tryptophan at a dose of 1,600 mg/kg per day. Eosinophil counts were obtained weekly and histopathologic changes were assessed after 38 days. Muscle biopsy specimens demonstrated perimysial inflammation in 7 of 9 animals receiving implicated tryptophan, compared to 0 of 10 receiving USP grade tryptophan ($P < .001$, Fisher exact test). A significant increase in fascial thickening was also observed in rats receiving implicated tryptophan. However, leukocyte counts and eosinophil counts remained normal in both groups. Gastrointestinal changes were also noted with an increased number of degranulating inflammatory cells in the lamina propria of the rats that received case-implicated tryptophan (21).

Subsequent studies have demonstrated that Lewis rats treated with EBT or case-associated tryptophan developed significant fascial thickening compared to rats that received pure (nonimplicated) tryptophan or methyl cellulose vehicle (46). Although the animals did not develop eosinophilia, this is the first evidence that EBT, a contaminant that is epidemiologically associated with EMS, may cause pathologic changes in an animal model. These findings have not yet been confirmed by other investigators, and no other animal model for EMS has been proposed.

In Vitro Studies

In vitro investigations have attempted to clarify the mechanism of immune activation. One study has reported that EBT induces interleukin 5 (IL-5) production from human T cells in a dose-dependent manner (83). In addition, data from a small number of EMS patients suggests that IL-5 is elevated in the serum (56). Levels of granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin 3 were normal. IL-5 can increase eosinophil production, degranulation, and in vitro survival (28, 39). It can also convert eosinophils to a hypodense phenotype with enhanced antibody mediated cytotoxicity (62). Thus, the immunologic effects of IL-5 may mediate some of the end-organ damage in EMS. Additional investigations are needed to confirm this hypothesis.

Peripheral blood mononuclear cells were used by Gleich and coworkers to evaluate an in vitro bioassay for EMS. The cells were exposed to case- and control-lots of tryptophan and incubated for 24 h. GM-CSF and eosinophil viability enhancing activity were measured in the supernatant (manuscript in preparation). Case-associated tryptophan did not stimulate release of GM-CSF from mononuclear cells, and the supernatant did not enhance eosinophil viability. Endotoxin was found in random lots of tryptophan and was associated with release of GM-CSF; endotoxin was not associated with case lots.

Possible Pathogenetic Mechanisms

The sequence of events leading to the pathologic changes of EMS is undoubtedly complex. However, a preliminary framework for possible mechanisms can be advanced based on current knowledge. One scenario involves a direct effect of the etiologic agent on mononuclear cells, leading to production of IL-5 (83). This cytokine could then activate tissue eosinophils and convert them to a hypodense phenotype. Effector functions would be augmented with release of cytotoxic cationic molecules, including major basic protein, eosinophil-derived neurotoxin, and eosinophil peroxidase. In addition, eosinophils may release cytokines such as interleukin 3, GM-CSF, and leukotriene C4 (43, 82). A cascade of interacting cytokines, including those produced by eosinophils themselves, could then lead to recruitment of additional inflammatory cells and increased collagen synthesis by fibroblasts. Eosinophils might also interact collaboratively with lymphocytes and other cells through expression of CD4 and the major histocompatibility complex HLA-DR (81). Associated microvascular changes such as vasculitis and endothelial cell swelling could contribute to ischemia and peripheral neuropathy.

The predominance of inflammatory changes in fascia suggests that mediators produced by mesenchymal cells (fibroblasts and endothelial cells) may also play a role in the pathogenesis. For example, fibroblasts have been shown to augment IL-5-dependent eosinophil survival and stimulate conversion to the hypodense phenotype (62). Fibroblasts can also produce interleukin 8, which recruits neutrophils and lymphocytes when injected in vivo (45). Thus, one can speculate that the etiologic agent interacts with these cells to stimulate release of inflammatory mediators and increase collagen synthesis.

This framework is consistent with epidemiologic evidence for a dose-response relationship between tryptophan consumption (a surrogate for exposure to the etiologic agent) and risk of EMS. It is also consistent with recent reports that EBT may stimulate IL-5 production in a dose-dependent manner and that IL-5 is elevated in the serum of some patients with EMS. In this model, the degree of eosinophil activation would be correlated with the amount of etiologic agent consumed. Clinical signs and symptoms of EMS would presumably develop in persons who were exposed above a critical threshold level; the latter could vary depending on individual susceptibility. However, it is unclear why the inflammatory process can become self-sustaining in individuals after exposure to the etiologic agent is stopped (i.e. tryptophan use discontinued).

Another general hypothesis involves incorporation of the etiologic agent into metabolic or biosynthetic pathways that utilize tryptophan. EBT and tryptophan have obvious structural similarities. If EBT is the etiologic agent, it might function as a tryptophan analogue with adverse immunologic effects.

For example, if EBT is recognized by the transfer RNA that is specific for tryptophan, it might be incorporated into a nascent protein molecule, stimulating an autoimmune response. Alternatively, EBT might be incorporated into either the serotonin or kynurenine pathway of tryptophan degradation, leading to production of one or more toxic metabolites. However, the hypothesis that EBT acts as a tryptophan analogue does not explain the eosinophilia or the predilection for involvement of the fascia.

Additional work is needed to determine if EMS is triggered by exposure to EBT or the chemicals represented by peaks UV-5 and UV-28. It would be useful to determine if any of these substances can stimulate release of cytokines from different cell lines, particularly fibroblasts and endothelial cells. In addition, the reported IL-5-producing effect of EBT on mononuclear cells requires confirmation by other investigators. The hypothesis that EBT functions as a tryptophan analogue can be further evaluated by studies to determine if EBT (*a*) is recognized by transfer RNA and incorporated into protein, or (*b*) interacts with enzymes or intermediates in the tryptophan metabolic pathways.

CONCLUSION

EMS is a chronic inflammatory disease that occurred in epidemic proportions during 1989 and was associated with tryptophan consumption. It preferentially affected fascia with variable involvement of other tissues; typical findings included striking eosinophilia with severe myalgias. Pathologic changes included perimyositis, microangiopathy, and increased collagen deposition in fascia. Response to treatment has been poor, and over 50% of patients remain symptomatic after one year of follow-up. EMS is clinically and pathologically similar to eosinophilic fasciitis and the toxic oil syndrome, and some cases of the former have been attributed to tryptophan consumption.

Epidemiologic studies indicate that EMS is triggered by one or more contaminants in tryptophan that was manufactured by one company. The chemical that has been most strongly implicated is 1,1'-ethylidenebis[tryptophan] (EBT), a molecule that is structurally similar to tryptophan. Results from animal studies suggest that EBT may cause pathologic changes in fascia that resemble EMS. The mechanism of immune activation is unknown, but preliminary investigations suggest that EBT can stimulate production of IL-5, a potent eosinophil-activating cytokine. Two other contaminants (peaks UV-5 and UV-28) have also been reported to be associated with case lots of tryptophan, but their structures and biologic activity have not been described. Epidemiologic investigations have shown that the presence of EBT was associated with changes in the tryptophan manufacturing process. However, the specific manufacturing source of the contaminant has not been deter-

mined. Consumption of high tryptophan doses and increased age have been identified as possible risk factors in persons who consumed implicated tryptophan; tryptophan dose may be a surrogate for the amount of etiologic agent that is ingested.

Ongoing research in animals and in vitro systems is underway to elucidate the pathogenesis of the syndrome and identify the etiologic agent. Additional research is needed to determine how this substance was synthesized during the manufacturing process and to develop effective monitoring procedures to assure that it does not occur in other products. Success in these endeavors will greatly increase our understanding of eosinophilic and sclerosing diseases and may lead to more effective therapies.

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