L-Tryptophan and the Eosinophilia-Myalgia Syndrome: Current Understanding of the Etiology and Pathogenesis

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The eosinophilia-myalgia syndrome (EMS) is a newly recognized illness that occurred in an epidemic form during the summer of 1989. The illness was characterized in the acute phase by myalgia and eosinophilia, followed in many patients by chronic cutaneous lesions, progressive neuropathy, and myopathy. EMS was associated with ingestion of L-trypto-phan, an essential amino acid marketed as a nutritional supplement but widely used as a therapeutic agent. Evidence of abnormal L-tryptophan metabolism has been described in patients with EMS, and most likely reflects increased activity of indoleamine 2,3-dioxygenase, the rate-limiting enzyme of tryptophan metabolism. A contaminant identified in EMS-associated L-tryptophan preparations has been isolated and characterized, but its biologic effects and role as the etiologic agent in EMS remain to be established. Pathologic observations and experimental studies indicate that eosinophils, mononuclear inflammatory cells, and fibroblasts are potential effector cells, and interleukin-5 and transforming growth factor-β are important mediators in the pathogenesis of the syndrome. Although few new cases of EMS occurred following the withdrawal of L-tryptophan, affected patients continue to manifest late sequelae of the disease, including dermal fibrotic conditions. This tragic outbreak of a newly recognized illness has focused interest on the role of chemical and environmental agents in the pathogenesis of various idiopathic illnesses characterized by tissue inflammation and fibrosis.
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In October 1989, state health authorities in New Mexico identified a cluster of patients with an unusual acute illness characterized by intense myalgia and eosinophilia. Each patient had been ingesting L-tryptophan-containing preparations (LTCP) for treatment of insomnia. As many additional cases were identified from other states, the Centers for Disease Control (CDC) proposed the term ''eosinophilia-myalgia syndrome'' (EMS) to designate the previously unknown syndrome, and established the following criteria for the diagnosis: (1) peripheral blood eosinophil count exceeding 1000/mm^3, (2) generalized myalgia severe enough to affect the patient's ability to pursue usual daily activities, and (3) the absence of other illnesses that could account for the eosinophilia [1]. A case-control study initiated by the state health department in Oregon showed that there was an unequivocal association between EMS and the consumption of LTCP [2]. Following the recall of LTCP by the Food and Drug Administration (FDA), the number of new cases of EMS reported to the CDC decreased dramatically. As of June 1991, 1543 cases of EMS had been registered, and it was suggested that the actual number may be considerably higher [3]. Nearly one-third of the patients required hospitalization, and 27 deaths directly attributable to EMS have been reported [4].

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Abbreviations: ANA, antinuclear antibodies; CDC, Centers for Disease Control; EBT, 1,1′-ethylidenebis[tryptophan]; EF, eosinophilic fasciitis; EMS, eosinophilia-myalgia syndrome; FDA, Food and Drug Administration GM-CSF, granulocyte/macrophage colony-stimulating factor; HPLC, high-performance liquid chromatography; IDO, indoleamine 2, 3-dioxygenase; IHES, idiopathic hypereosinophilic syndrome; LTCP, L-tryptophan-containing preparations; TGFβ, transforming growth factor-β; TOS, toxic oil syndrome

CLINICAL FEATURES

Most EMS patients first developed symptoms between July 1989 and February 1990. The dose of L-tryptophan ingested by these individuals varied from 10 mg to 15 g/d (median 1.5 g/d) [2]. The clinical presentation, laboratory findings, and pathologic features of EMS have been described in a number of recent publications [5–13]. The frequency of acute and chronic manifestations of the disease based on an analysis of several studies [5–13] is summarized in Table I.

In its early phase (0–2 months), EMS was characterized by diffuse myalgia, low-grade fever, swelling of the face and extremities, and a variety of skin rashes. A substantial proportion of patients with EMS developed a protracted course despite the discontinuation of LTCP use. Approximately one-half of the patients developed cutaneous induration resembling diffuse fasciitis or scleroderma [6,7,10,12]. These changes frequently affected the extremities, but spared the hands and feet (Fig 1). Histopathologic examination of full-thickness skin biopsy specimens showed marked thickening of the fascia and dermis due to accumulation of collagen and mucopolysaccharides, accompanied by inflammatory cell infiltration with mononuclear cells and eosinophils (Fig 2). Diffuse sensorimotor peripheral neuropathy and myopathy manifested by paresthesias, painful muscle cramps, or proximal weakness were frequent late sequelae [12,14,15]. Other chronic manifestations of EMS include pulmonary hypertension, cardiac arrhythmias and cardiomyopathy, hypothyroidism, alopecia, and cognitive abnormalities [6,10–13]. Most patients had striking peripheral blood eosinophilia in the initial phase of the illness. Bone marrow examination showed eosinophilic hyperplasia with normal precursor maturation. In contrast to other disorders associated with eosinophilia, IgE levels were not elevated in the serum of EMS patients [10].

Treatment with glucocorticoids resulted in improvement of the acute manifestations of EMS, and in rapid decrease of the peripheral blood

Table I. Prominent Clinical Manifestations of EMS[a]

Clinical Manifestation	Frequency (%)
Early (0–2 months)	
Myalgia	100
Skin rash	71
Edema	52
Fever	41
Arthralgia	35
Respiratory symptoms	32
Late (2 months to 1 year)	
Weight loss	50
Muscle weakness	44
Paresthesia	42
Scleroderma-like induration	42
Xerostomia	36
Alopecia	33

[a]Data compiled from [10–13].

eosinophil count. However, skin induration, myopathy, or neuropathy generally responded poorly to glucocorticoids, and the long-term outcome of patients treated with glucocorticoids was not statistically different from that of untreated patients [13–16]. Other treatments have been reported to be of benefit in EMS, but in the absence of controlled trials, these results are difficult to interpret. At the time of this writing, some 2 years after the EMS epidemic, the full clinical spectrum, natural history, optimal therapy, and long-term prognosis of this novel disease are unknown.

ETIOLOGY

Immediately following the recognition of the EMS epidemic and the realization of its magnitude, intense research efforts were undertaken to identify the cause of the disease. Although the specific etiologic mechanisms have not been fully elucidated to date, two hypotheses have been advanced: (1) EMS is triggered by a contaminant in LTCP that arises as a by-product of the manufacturing process or is introduced exogenously; and (2) EMS develops in predisposed individuals who have an inborn or acquired alteration in the enzymes involved in L-tryptophan metabolism. In the following section, we will critically review the data supporting these alternatives.

Although EMS appears to be a ''new'' disease, clinical and pathologic similarities between EMS and several previously described idiopathic syndromes associated with eosinophilia were recognized. Prominent among these are eosinophilic fasciitis (EF), idiopathic hypereosinophilic syndrome (IHES), and toxic oil syndrome (TOS). Each of these conditions

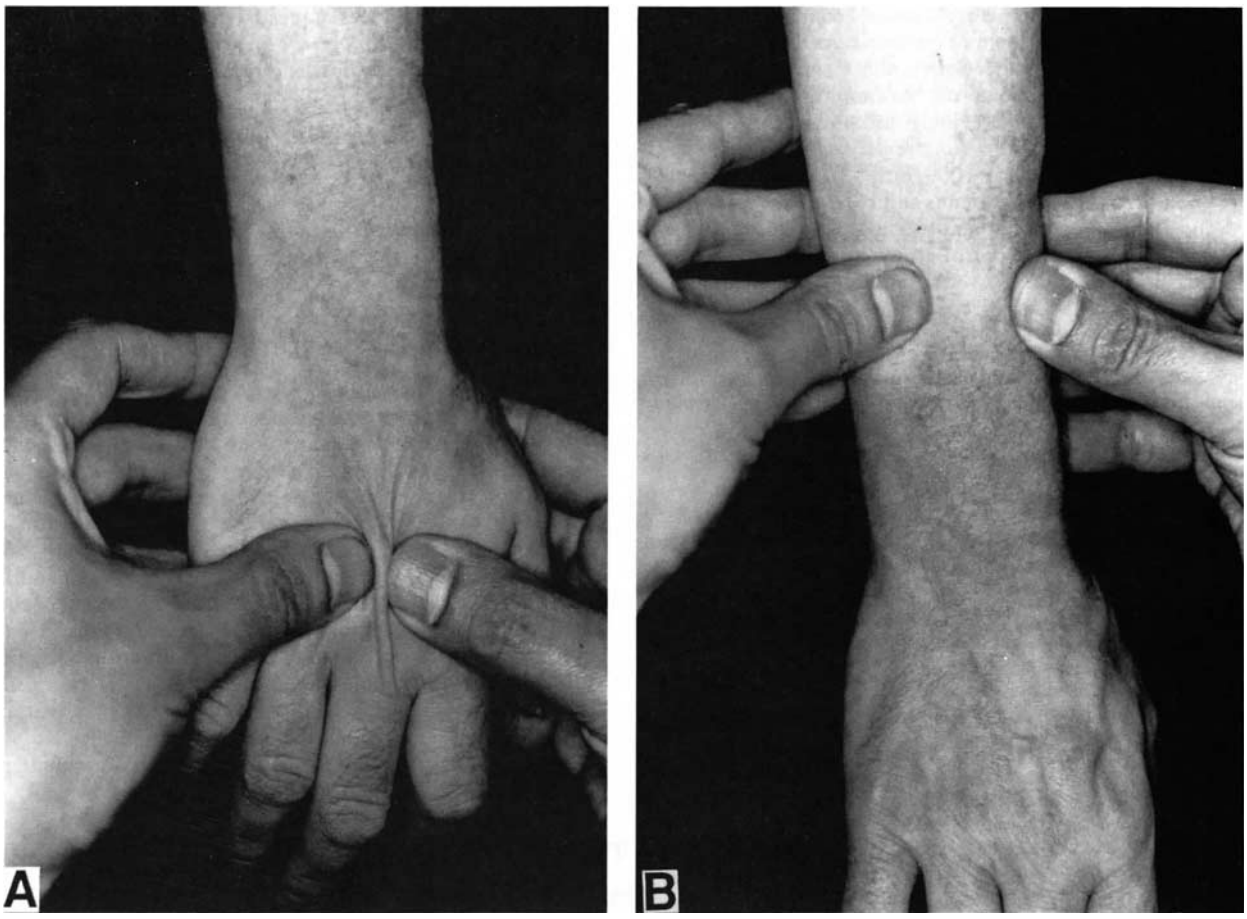

Figure 1. Induration of the skin in EMS. Note that the hands are unaffected *(A),* whereas the forearm is hidebound *(B).*

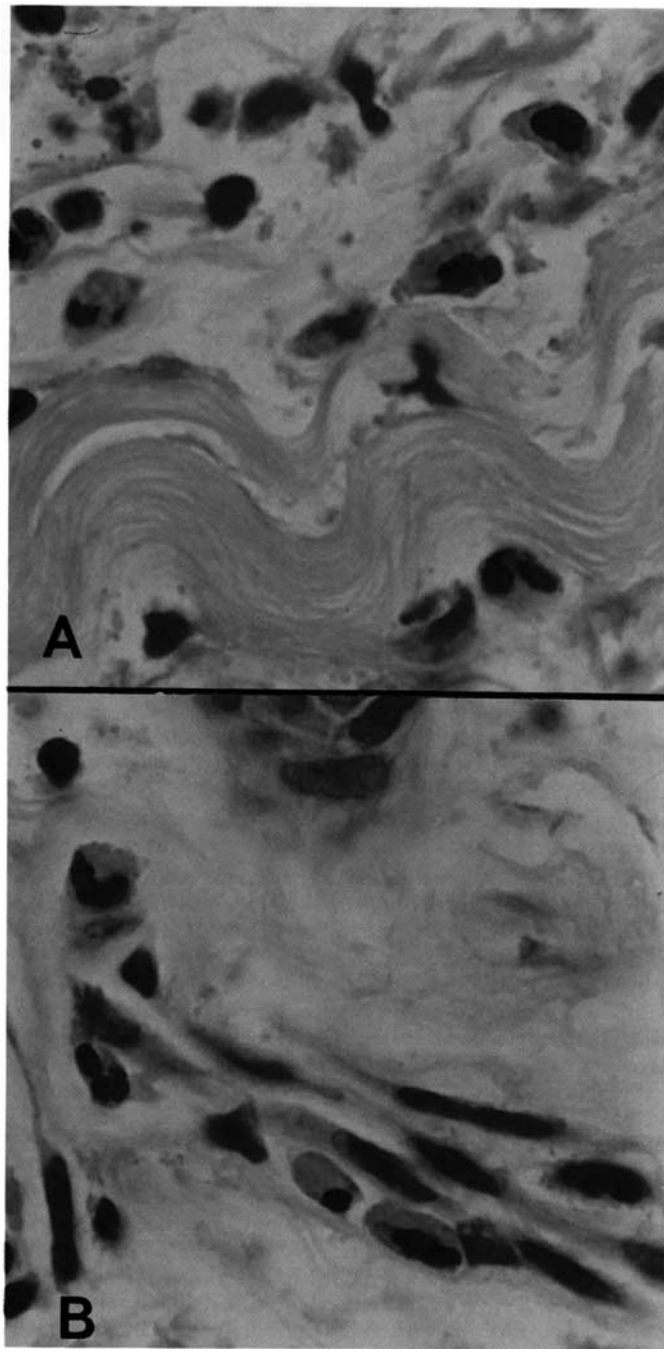

Figure 2. Histopathologic findings in EMS. *(A)* Abundant eosinophils in the deep dermis are in proximity to undulating collagen fibers. *(B)* Many eosinophils are juxtaposed to fibroblasts (hematoxylin and eosin, magnification × 100) (from [6]).

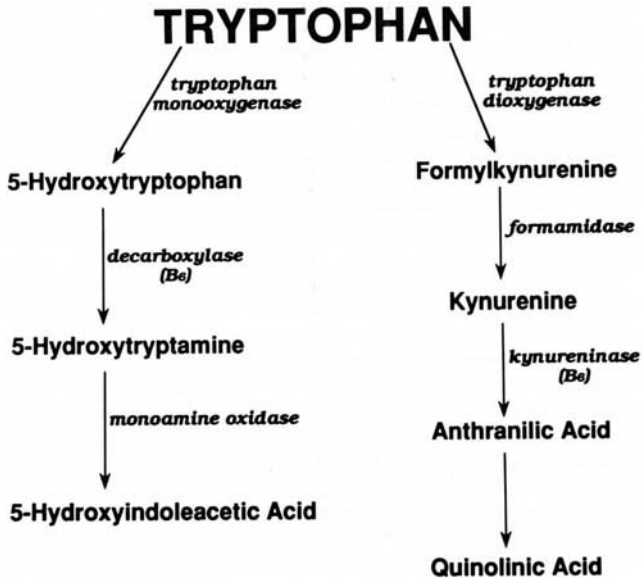

Figure 3. Metabolic pathways of L-tryptophan in humans. Important enzymes are indicated in *italics*. Vitamin B6 (pyridoxine) is required for some metabolic steps, as shown.

is characterized by eosinophilia, infiltration of various tissues by mature eosinophils, and some degree of visceral organ dysfunction. EF, a relatively uncommon idiopathic condition first described by Shulman in 1974, is characterized by diffuse fasciitis and peripheral blood eosinophilia. Although significant internal organ involvement is uncommon, the fascial inflammatory process may extend deep into the muscle [16]. It has been pointed out that many patients diagnosed as having EF before 1989 were retrospectively found to have ingested LTCP prior to becoming ill [17]. Some of these may have been pre-epidemic cases of L-tryptophan-associated EMS [18]. In fact, it has been suggested that EF and EMS are identical diseases. However, despite similarities between the two syndromes, a careful analysis shows that several clinical and laboratory features clearly distinguish EF from EMS, indicating that they are related but distinct clinical entities [19].

EMS bears a particularly striking resemblance to TOS. TOS was an explosive illness associated with eosinophilia and multisystem manifestations in the acute stages, and the development of late sequelae including chronic skin induration in many patients. The TOS epidemic, which swept Spain in 1981, affected over 20 000 people and was associated with significant mortality and morbidity [20]. The outbreak of the TOS epidemic was attributed to consumption of denatured rapeseed oil originally intended for industrial use. Similarities between EMS and TOS include the abrupt onset of symptoms and eosinophilia, the development of chronic neuromuscular manifestations in a subset of patients despite discontinuation of the etiologic agent (contaminant), a female predominance in the late stages of the disease, abnormalities in tryptophan metabolism, and evidence of striking eosinophil infiltration and degranulation in affected tissues [21]. Despite intensive investigation during the past decade, the responsible etiologic agent of TOS has not been identified to date and no animal models of this disease have been generated [22].

L-Tryptophan The virtually universal association between L-tryptophan ingestion and the development of EMS in the 1989 epidemic suggests the existence of a cause-effect relationship. L-tryptophan is an indole amino acid that must be obtained from dietary sources. Although it is the least abundant amino acid in most proteins, tryptophan is converted into a large number of biologically important metabolites [23]. The activity of the enzymes regulating tryptophan metabolism is influenced by several factors, including corticosteroids, alcohol, and anti-depressant medications, as well as by interferon [23]. Whereas most dietary L-tryptophan is utilized in protein synthesis, a small fraction is metabolized via two pathways (Fig 3). In peripheral tissues, greater than 90% of the L-tryptophan not required in protein synthesis undergoes oxidation to formylkynurenine. This step is catabolized by the enzymes tryptophan 2,3-dioxygenase in the liver and indoleamine 2,3-dioxy-genase (IDO) in extrahepatic tissues. Following a series of intermediate steps, quinolinic

acid, a potent endogenous neurotoxin, is generated from kynurenine in this pathway. Quinolinic acid has been the subject of great interest, because it has been implicated in the pathogenesis of several metabolic and neurologic conditions. Recent evidence suggests that quinolinic acid also plays a role in the encephalopathy associated with human immunodeficiency virus infection [24]. A rise in plasma L-tryptophan induces the activity of IDO, and therefore augments the biosynthesis of kynurenines. Importantly, extracellular fluid levels of quinolinic acid following L-tryptophan loading may equal or even exceed those that have been shown to be neurotoxic *in vitro* [25].

The minor metabolic pathway of L-tryptophan involves its hydroxylation to 5-hydroxytryptophan, and occurs primarily in the central nervous system. 5-hydroxytryptophan may undergo decar-boxylation to 5-hydroxytryptamine (serotonin), an important neurotransmitter and a potent vasoconstrictor. Although the serotonin pathway normally accounts for only 1–3% of L-tryptophan metabolism, large amounts of dietary L-tryptophan may be diverted to this metabolic pathway in patients with functioning carcinoid tumors.

Ingestion of 0.5–1.0 g of L-tryptophan causes behavioral alterations in humans, including drowsiness [26]. Because serotonin plays an important role in the mediation of sleep, it has been assumed that the somniferous effects of L-tryptophan result from increased availability of serotonin in the brain [27]. Despite inconclusive evidence for its effectiveness, L-tryptophan has been widely promoted for the treatment of a variety of problems including insomnia, pre-menstrual tension, obesity, and depression. In the United States, LTCP were not labeled for these medical indications but were sold as food supplements, and as such were not subject to regulatory control by the FDA. By 1989, more than 2% of households had at least one member who was consuming LTCP [28]. Prior to the outbreak of the EMS epidemic, some investigators suggested that excessive L-tryptophan ingestion may result in accumulation of toxic levels of quinolinic acid in the central nervous system [29]. It has also been pointed out that amino acids ingested as supplements were metabolized quite differently than amino acids ingested from dietary sources, and should be considered to be drugs rather than food supplements [30]. However, with the exception of a transient ''serotonin reaction'' characterized by paresthesia and myoclonus, seen in some individuals taking L-tryptophan in combination with anti-depressant medications containing monoamine oxidase inhibitors [31], few adverse effects of L-tryptophan have been reported.

The epidemic nature of EMS, and its apparent rarity in countries where the processing and distribution of LTCP is carefully regulated (such as Canada), suggest that a contaminant or by-product in the L-tryptophan preparations, rather than abnormalities of its metabolism, may have been responsible for the development of EMS. Furthermore, there is no evidence that L-tryptophan or related compounds participate in the activation or degranulation of eosinophils. It is of great interest, therefore, that a large body of clinical and experimental observations accumulated during the past three decades suggests that L-tryptophan – derived compounds or alterations in L-tryptophan metabolic pathways may have a role in the pathogenesis of certain features of EMS. Particularly intriguing in this regard is the evidence linking L-tryptophan and related compounds to abnormal fibrogenesis (Table II). For example, it has been shown that serotonin stimulates collagen synthesis *in vitro,* and causes fibrosis in experimental animals [32,33]. Patients with carcinoid syndrome occasionally develop cutaneous and retroperitoneal fibrosis [34]. The distribution of skin induration in these cases closely resembles that observed in EMS, *i.e.,* proximal fibrosis with sparing of the hands and feet.

Systemic sclerosis and scleroderma-like syndromes are associated with alterations in both pathways of normal tryptophan metabolism. For example, increased serum levels of tryptophan metabolites have been demonstrated in East African populations that subsist on a diet rich in bananas and have a high frequency of endocardial fibrosis [35]. In patients with systemic sclerosis, an exaggerated rise in serum levels of kynurenine and urinary excretion of quinolinic acid following tryptophan loading has been described [36]. Several studies that evaluated tryptophan metabolism in patients with EMS found that their plasma concentrations of kynurenine were higher than in healthy individuals [7,14,37]. Untreated EMS patients with the most active disease displayed the most significant elevations in kynurenine [7,14]. We examined cerebrospinal fluid levels of quinolinic acid in EMS patients and demonstrated a marked increase associated with neuropathy [14]. Although these observations implicate quinolinic acid in the development of EMS neuropathy, quinolinic acid causes an axon-sparing neurologic lesion, unlike that seen in EMS [38]. Whereas the specific biochemical abnormalities responsible for these alterations in tryptophan metabolism have not been identified, it is currently thought that increased activity of the rate-limiting enzyme IDO is responsible for the increase in kynurenine pathway intermediates in EMS [7]. IDO is readily induced by a variety of cytokines *in vitro,* and is activated in patients treated with such cytokines [39]. Although it has been proposed that EMS-associated LTCP directly trigger activation of IDO, recent studies examining the effect of L-tryptophan preparations on normal mononuclear cells *in vitro* indicated that EMS-associated LTCP did not induce increased IDO activity in these cells [40]. Alterations in tryptophan metabolism in EMS are unlikely to be primary phenomena and probably reflect activation of IDO by inflammatory mediators. Nevertheless, elevated levels of certain tryptophan metabolites may exert direct toxicity on tissues or may promote abnormal fibrogenesis. Those possibilities need to be carefully examined.

Table II. Association of Tryptophan and Related Biogenic Amines with Tissue Fibrosis

In vivo fibrosis
Methysergide
Bananas, plantains
Carcinoid syndrome
Eosinophilia-myalgia syndrome
Animal and *in vitro* models
Subcutaneous fibrosis
Synovial fibrosis
Stimulation of collagen synthesis
Idiopathic fibrosing syndromes
Eosinophilic fasciitis (Shulman's syndrome)
Systemic sclerosis

TOXICOLOGY OF L-TRYPTOPHAN

Persuasive arguments have been advanced to support the possibility that contaminated L-tryptophan preparations were responsible for the outbreak of EMS. At the time of the epidemic, bulk L-tryptophan was produced by several manufacturers, each located in Japan. Following importation to the United States, L-tryptophan was packaged in the form of capsules and tablets and sold as nutritional supplements [41]. Almost 200 brands of LTCP were in use in the United States in 1989, but the development of EMS was associated only with batches of L-tryptophan originating from a single manufacturer (Showa-Denko, Tokyo) [41]. This distribution pattern indicated a point-source contaminant. The possible etiologic role of a contaminant is suggested by the observation that EMS-associated LTCP cause secretion of cytokines, including granulocyte/

mono-cyte colony-stimulating factor (GM-CSF) from normal peripheral blood mononuclear cells *in vitro* [42]. No such effect was noted with non-EMS-associated tryptophan lots. Furthermore, Crofford and colleagues recently were able to induce a syndrome with features of EMS using implicated L-tryptophan [43]. These investigators found that administration of EMS-associated L-tryptophan to rats caused inflammation of the subcutaneous fascia and perimysium. The changes could only be induced in Lewis rats, a strain highly susceptible to a variety of inflammatory illnesses. The affected animals did not develop blood eosinophilia or histologic evidence of pulmonary or skin involvement. DeSchryver-Kecskemeti and colleagues found increased perivascular inflammatory infiltrates and mast cell de-granulation in small intestinal lamina propria of rats treated with EMS-associated L-tryptophan [44]. Animals treated with vehicle control or pure L-tryptophan did not show similar histopathologic abnormalities.

Mass production of L-tryptophan involves a bacterial fermentation process during which multiple secondary metabolites may be generated. Several purification steps, including adsorption with activated carbon and reverse-osmosis membrane filtration must be employed to remove cellular debris, by-products, and other impurities [28]. Analysis of the manufacturing conditions at Showa-Denko revealed that between December 1988 and June 1989 several changes in L-tryptophan production were instituted. These included the use of a new bacterial strain (strain V) *of Bacillus amyloli-quefaciens* that had been genetically manipulated to produce L-tryptophan with a high yield, bypass of the reverse-osmosis filtration step, and reduction in the amount of powdered activated carbon used for purification. A significant correlation between the reduction of powdered activated carbon and the development of EMS was noted.

Analysis of EMS-associated L-tryptophan lots by high-performance liquid chromatography (HPLC) indicated the presence of numerous peaks beside tryptophan, each corresponding to one or more chemical impurities [28]. A single absorbance peak with a retention time of 25 min was consistently found in EMS-associated L-tryptophan lots. This peak, labeled ''peak E'' by investigators at the Mayo Clinic, is identical to peak 97 demonstrated by the CDC [45]. The contaminant represented by ''peak E'' has been isolated and purified. Analysis by a variety of techniques indicated the structure 1,1′-eth-ylidenebis[tryptophan] (EBT) [46,47]. EBT appears to be a novel amino acid, composed of a tryptophan dimer with two L-tryptophan molecules joined together by an ethylidene bridge between the two indole ring nitrogens (Fig 4). As implicated lots of LTCP contained 99.5% pure L-tryptophan, the contaminant represented by ''peak E'' or its breakdown products was present in very small amounts, and must have had extremely high biologic activity in order to trigger EMS. Alternately, ''peak E'' may be a surrogate marker for other etiologic substance(s). Indeed, numerous additional peaks have been demonstrated by HPLC analysis of Showa-Denko-produced L-tryptophan. These include 1-methyl-1,2,3,4-tetrahydro-β carboline, which may be generated by hydrolysis of EBT, and various tryptophan breakdown products, as well as a baci-tracin-like contaminant [48].

The consumption of Showa-Denko's LTCP was not uniformly associated with the development of EMS. In one cohort study, approximately half of the patients ingesting more than 4 g/d of L-tryptophan originating from Showa-Denko became ill, whereas the attack rate was substantially lower in those ingesting less than this amount, suggesting a critical threshold dose of exposure necessary to trigger EMS [49]. In addition to exposure to the etiologic agent, individual predisposition may also play a role in determining the risk of developing EMS.

PATHOGENESIS

The Role of the Eosinophil The striking eosinophilia in the blood and in some tissues of patients with EMS implicates eosinophils as effector cells in the syndrome. The growth and maturation of eosinophils in the bone marrow, their migration to sites of inflammation, and their viability and function are regulated by three cytokines. Interleukin-3 (IL-3), IL-5, and

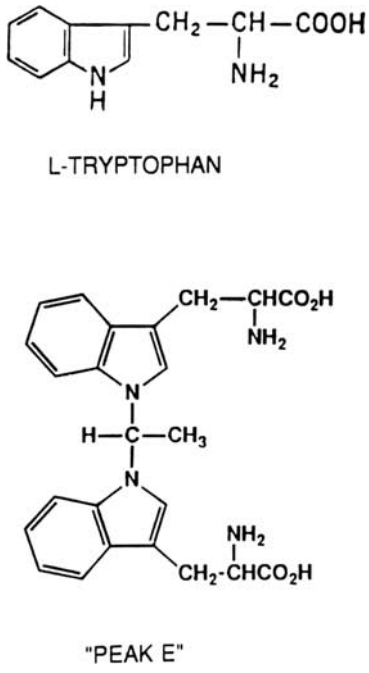

Figure 4. Structure of L-tryptophan and of the compound represented by ''peak E.'' This compound is l,l′-ethylidenebis[tryptophan] (EBT), a dimer formed by two L-tryptophan molecules joined by an ethylidene bridge between the two indole ring nitrogens.

GM-CSF are produced by activated T lymphocytes, mast cells, neutrophils, and eosinophils themselves [50,51]. These cytokines promote the development of eosinophil precursors, enhance the eosinophil's ability to respond to chemotactic factors, and stimulate the conversion of normal eosinophils to eosinophils with abnormally low sedimentation density (hypodense eosinophils) *in vivo* and *in vitro* [52]. Hypodense eosinophils are activated cells with increased expression of complement receptors, prolonged survival *in vitro,* and markedly enhanced release of leukotrienes and other inflammatory mediators [53]. The proportion of hypodense eosinophils in the blood is increased in hypereosinophilic disorders, indicating activation mediated by eosinophilopoitins. IL-5, in particular, has been implicated in the abnormal eosinophil proliferation and activation characteristic of IHES [54].

Cytokine-induced stimulation of eosinophils appears to be an important feature of EMS. Gradient sedimentation analysis of eosinophils isolated from patients with EMS revealed a fourfold increase in the proportion of hypodense cells, compared to eosinophils from normal donors [55]. Furthermore, EMS sera, but not normal human sera, markedly increased the *ex vivo* viability of normal eosinophils. This effect was attenuated by a neutralizing antibody to IL-5. These observations indicate that EMS sera have increased IL-5 activity and suggest that IL-5 may be responsible for the eosinophilia and elevated proportion of hypodense eosinophils primed for augmented biologic response to activating stimuli.

Activated eosinophils release toxic granule proteins, including major basic protein (MBP) and eosinophil-derived neurotoxin (EDN) at sites of inflammation. Determination of urine and serum levels of MBP and EDN showed marked elevation in patients with EMS, indicating ongoing eosinophil degranulation [5,8]. In addition, striking extracellular deposition of MBP was demonstrated by immunofluorescence in the lung, myocardium, muscle, and other affected tissues, suggesting that eosinophil-derived granule proteins may contribute to tissue injury in EMS [8]. Although eosinophil activation is clearly important in the pathogenesis of EMS, severe injury has been noted in tissues in the

absence of eosinophils or their degranulation products. These observations imply that infiltration and degranulation by eosinophils cannot be entirely responsible for the full spectrum of pathology in EMS [8].

Autoimmune Mechanisms Recent observations indicate that in EMS, both cellular and humoral autoimmune mechanisms may contribute to tissue injury (Table III). The cellular immune abnormalities in EMS include the presence of activated lymphocytes and mononuclear cells in muscle and fascia, alterations in peripheral blood mononuclear cell phenotypes, and increased expression of surface activation markers on circulating T lymphocytes. Examination of muscle biopsy specimens in EMS reveals an often striking inflammatory infiltrate, composed predominantly of mononuclear cells. In contrast to idiopathic inflammatory myositis, EMS is characterized by perimyositis, with infiltrating cells found almost exclusively at perivascular, perimysial, and fascial locations [8,14]. Quantitative immunocytochemical analysis demonstrated a predominance of activated T cells at these sites [56]. Macrophages account for 20–40% of infiltrating cells. Eosinophils are only rarely noted in muscle biopsy specimens, despite peripheral blood eosino-philia at the time of the biopsy. Analysis of peripheral blood mononuclear cells indicated alterations in CD4+ cell subpopulations reflecting activation of T cells, as well as a reduction in suppressor-inducer T cells with a concomitant increase in the helper-inducer population [53].

Abnormal humoral immunity is evidenced by the frequent occurrence of antinuclear antibodies (ANA) in EMS, but the extent to which autoantibodies contribute to the pathogenesis is unclear. By indirect immunofluorescence on Hep-2 cell substrate, circulating ANAs can be detected in up to 50% of patients [8,10,12]. Specific antibodies associated with autoimmune diseases, such as antibodies to the extractable nuclear antigens Sm and RNP, Ro, La, topoiso-merase-1, and kinetochore, have not been described in EMS. We recently evaluated autoantibodies in a large group of EMS patients by immunoprecipitation. Several polypeptides were recognized by EMS sera, including a 105-kD polypeptide that was immunoprecipi-tated by sera from 15% of the patients (Targoff I, Varga J, KaufFman L, Jimenez SA: unpublished observations). The antigens recognized by autoantibodies in EMS sera have not been characterized to date. In one EMS patient with chronic neuromuscular involvement, high titers of an autoantibody reacting with the nuclear membrane were detected by indirect immunofluorescence, and were identified as anti-lamin C by immunoblotting to purified lamin C. Autoantibodies with this specificity have been described previously only in patients with chronic hepatitis delta infection [57]. Anti-lamin C antibody titer decreased in this case following discontinuation of L-tryptophan ingestion and treatment with glucocorticoids [59].

Table III. Immunologic Abnormalities in EMS

Cellular
Inflammatory cell infiltration in fascia, muscle:
Monocytes, macrophages, lymphocytes
Activated mononuclear cells
In tissue
In blood
Preponderance of CD8+ T cells in affected tissues
Humoral
Circulating immune complexes
Antinuclear autoantibodies

Fibrosis Fibrosis of the dermis and fascia, perimysium and peri-neurium, and connective tissue surrounding blood vessels is the most dramatic histopathologic finding in late EMS. Massive accumulation of collagen, fibronectin, and other extracellular matrix components occurs at these sites (Fig 5). Increased expression of the corresponding genes has been demonstrated [6,58]. Fibroblasts derived from the involved skin of patients with untreated EMS show increased rates of collagen synthesis and transcription of the al(I)procollagen gene *in vitro* compared to age and sex-matched healthy controls [60]. These alterations appear to

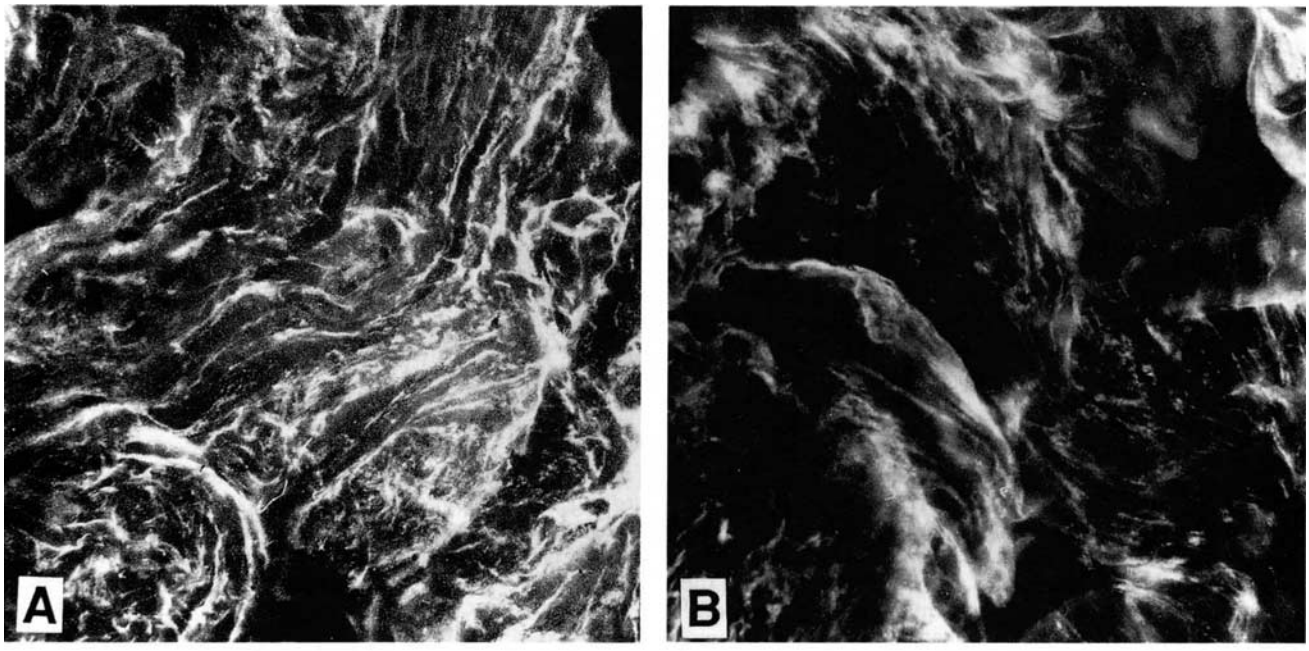

Figure 5. Excessive deposition of fibronectin *(A)* and type VI collagen *(B)* in the subcutaneous fascia, as demonstrated by indirect immunofluorescence of a biopsy specimen from affected skin in EMS. (From [58].)

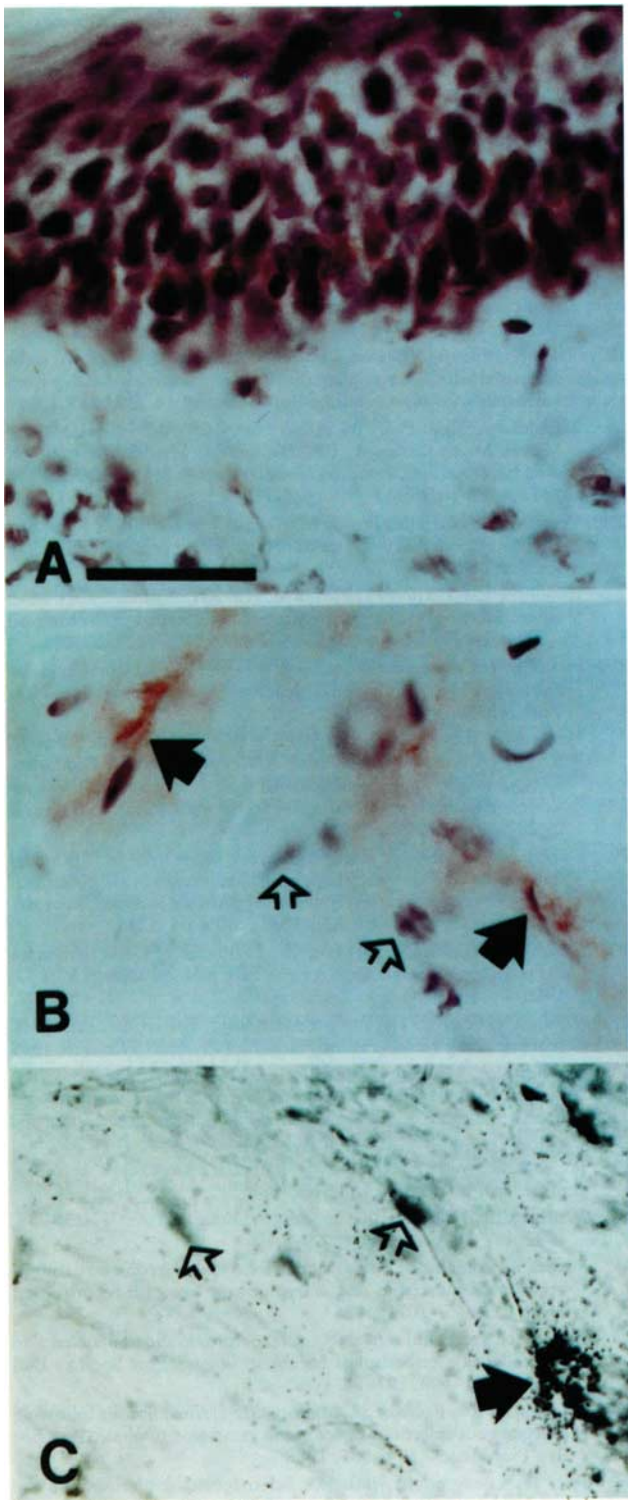

Figure 6. Expression of TGF-β in EMS. *A,B:* peroxidase – anti-peroxidase staining for TGF-β protein. Note positive immunoreaction in association with cells of lower epidermis *(A)* and within a subpopulation of fibroblastic cells in the fascia *(B, arrow),* whereas the majority of the cells are negative *(B, open arrows). C: in situ* hybridization with a cDNA for TGF-β_1. The autoradiographic grains represent radiolabeled cDNA/mRNA hybrids. The TGF-β_1, gene is expressed in the fascia by a subpopulation of fibroblastic cells *(arrows).* Most of the cells, however, are negative *(open arrows),* in a pattern resembling that noted for TGF-β_1, protein (hematoxylin counterstain). *Bar,* 50 μm (from [58]).

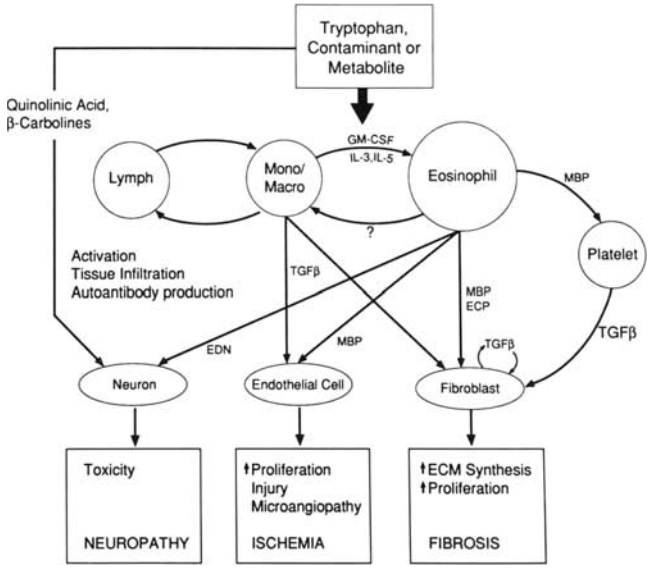

Figure 7. Overview of the pathogenesis of EMS, emphasizing the role of effector cells and regulatory cytokines (see text for discussion). MBP, major basic protein; PDGF, platelet-derived growth factor; ECP, eosinophil cat-ionic protein; EDN, eosinophil-derived neurotoxin; ECM, extracellular matrix.

persist during several serial passages. Numerous fibroblasts displaying elevated procollagen mRN A levels were found in the dermis and fascia by *in situ* hybridization of biopsy specimens from involved skin in patients with EMS [6]. These studies also demonstrated an absence of topographic association between eosinophils and biosynthetically active fibroblasts, suggesting that direct cell-cell interactions may not be required for the activation of fibroblast connective tissue production in EMS [58]. In addition to increased synthesis of extracellular matrix components, EMS fibroblasts *in situ* also display elevated expression of the gene for transforming growth factor β_1, (TGF-β1) (Fig 6) [58], TGF-β_1, plays a critical role in the physiologic regulation of connective tissue metabolism, and appears to participate in the pathogenesis of several experimentally induced and spontaneously occurring fibrotic conditions [56–58,60–62]. Although direct confirmation is lacking, the evidence from immunohistologic and *in situ* hybridization studies suggests that TGF-β_1 may mediate the development of EMS-associated fibrosis via autocrine and paracrine mechanisms.

Additional cytokines and cells may also contribute to fibrogenesis in EMS. Of particular interest in this regard is the potential role of eosinophils. The participation of eosinophils in the development of fibrosis is supported by several recent observations. Crude eosinophil extracts have been shown to induce fibroblast proliferation and glucosamine synthesis *in vitro* [63], and eosinophil-derived granule proteins induce secretion of platelet α granule components, including fibrogenic cytokines such as TGF-β [64]. Furthermore, under certain circumstances eosinphils synthesize and secrete transforming growth factor α, which is a potent stimulus for fibroblast proliferation [65]. Therefore eosinophils are capable of elaborating potentially fibrogenic cytokines and other, as yet unidentified, mediators that may contribute to connective tissue activation. An important role for eosinophils in the pathogenesis of pathologic fibrosis is also indicated by the recent demonstration of eosinophil activation in systemic sclerosis, a prototypic human fibrotic disease [66]. In this study, serum levels of eosinophil degranulation products were found to be markedly increased in patients with systemic sclerosis. In addition, degranulation was demonstrated by immuno-cytochemistry in the affected skin of these patients.

A hypothetical scheme combining these pathogenetic mechanisms is shown in Fig 7. The initial triggering agent of EMS, such as a contaminant

in LTCP, activates inflammatory cells including lymphocytes, monocytes, and perhaps other cells. These cells are induced to secrete cytokines that cause activation of eosinophils (IL-5, GM-CSF) and fibroblasts (TGF-β). Activated eosinophils may release cytokines, toxic granule contents, and other inflammatory substances, resulting in amplification of the immune response and tissue injury. Activated fibroblasts produce increased amounts of collagen and other extracellular matrix components. These cells may also undergo autoinduction via TGF-β, thus perpetuating the fibrotic response.

CONCLUSIONS

Although the clinical and pathologic features of EMS are well characterized, the etiology and pathogenesis of this recently described disease remain poorly understood. The presence of trace impurities in some LTCP was associated with the development of EMS, and preliminary results from *in vitro* studies and from a rodent model of the disease support the possibility of a contaminant as the etiologic factor. A compound highly associated with EMS has been identified and characterized, but its role as an etiologic agent in the syndrome has not been established. At the same time, abnormalities in the metabolism of tryptophan have been demonstrated in some EMS patients. These abnormalities could play a role in the pathogenesis of EMS or may simply reflect inflammation. It may be that the development of EMS is triggered by an interaction of these two factors, *i.e.,* the introduction of a contaminant of LTCP and abnormal metabolism of L-tryptophan in predisposed hosts. Activation and degranulation of eosinophils, possibly mediated by cytokines, appear to be important early events. In addition, cellular immune mechanisms appear to be responsible for many of the clinical and pathologic manifestations of EMS. Toxic tryptophan metabolites, such as the neurotoxin quinolinic acid are generated in excess, and may also contribute to tissue injury. The recognition that consumption of a widely used essential amino acid sold as a food supplement, and not subject to regulatory control, may result in the development of a serious multisystem disease with chronic autoimmune features has focused interest in the potential role of chemical and environmental agents in triggering idiopathic eosinophilic and fibrotic disorders. Careful study of the EMS epidemic will undoubtedly enhance our understanding of these incurable illnesses, and will be of great value in efforts to develop new strategies for their prevention and treatment. Furthermore, the tragic epidemic also emphasizes that amino acids and other ''nutritional supplements'' must be regulated when they are used as drugs.

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