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Research Article

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Pathological and Immunological Effects of Ingesting L-Tryptophan and 1,1′-Ethylidenebis(L-Tryptophan) in Lewis Rats

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Abstract

The eosinophilia-myalgia syndrome (EMS) has been associated with ingestion of L-tryptophan (L-TRP) produced by a single manufacturer. Epidemiological data implicated 1,1′-ethylidenebis(L-tryptophan) (EBT) (peak 97 or peak E) as a possible etiologic agent. We showed previously that Lewis rats treated with the L-TRP implicated in EMS develop fasciitis and perimyositis similar to those seen in human EMS. We now report the pathology associated with the treatment of Lewis rats with synthetic EBT and/or L-TRP. All animals treated for 6 wk with case-associated L-TRP or EBT developed significant myofascial thickening, compared with animals in the vehicle control and control L-TRP groups. However, even those animals receiving the control L-TRP showed a mild but significant increase in the thickness of the myofascia, compared with vehicle-treated control animals. All animals except vehicle controls also exhibited significant pancreatic pathology, including fibrosis and acinar changes. Only animals treated with case-associated L-TRP for 6 wk showed evidence of immune activation with increased frequency of CD8, Ia, and IL-2 receptor–positive cells in the peripheral blood. Animals receiving L-TRP or EBT for < 6 wk did not show significant differences in myofascial thickness, although these animals did show pancreatic acinar changes. Although these results demonstrate for the first time the pathological effects of EBT, they do not rule out the possibility that other impurities in the EMS–case-associated L-TRP may also contribute to some of the features of EMS. (*J. Clin. Invest.* 1993. 91:804–811.) Key words: eosinophilia-myalgia syndrome • immune activation • fibrosis • pancreatitis • supplement

Introduction

Eosinophilia-myalgia syndrome (EMS)[1] is a recently described disease (1, 2) that occurred in epidemic fashion in the United States in the summer and fall of 1989 and has been associated with the use of L-tryptophan (L-TRP) produced by a particular manufacturer (3). To date more than 1,500 cases have met the Centers for Disease Control case surveillance definition of the disease (4, 5). The true incidence of the disorder is thought to be much higher because there appears to be considerable heterogeneity in the clinical presentation of the disease. Although HPLC analyses of batches of L-TRP from the implicated manufacturer reveal a number of discrete peaks that are not present in chromatograms from HPLC analyses of nonimplicated L-TRP, the impurity most strongly associated with EMS by epidemiological data is 1,1′-ethylidenebis(L-tryptophan) (EBT), previously called peak E or peak 97 (6–8).

We showed previously that Lewis (LEW/N) rats treated with L-TRP that was implicated in EMS develop fasciitis and perimyositis similar to those seen in human EMS, whereas LEW/N rats treated with L-TRP obtained from a nonimplicated manufacturer do not (9). Because EBT has now been synthesized in quantities sufficient for animal testing, we sought direct evidence that EBT, either alone or in combination with nonimplicated L-TRP, could cause changes similar to those of case-associated L-TRP in Lewis rats. We now report the pathology associated with acute and chronic treatment of female LEW/N rats with EBT and/or L-TRP. All animals treated with EBT or case-associated L-TRP for 6 wk developed significant increases in the myofascia, compared with the vehicle-treated control animals or those receiving control L-TRP. However, even those animals receiving the control L-TRP showed a mild but significant increase in the thickness of the myofascia, compared with the vehicle-treated control animals. All animals receiving EBT or any form of L-TRP also exhibited significant pancreatic pathology, including fibrosis and acinar changes. When the groups were analyzed for immunological differences, however, only those receiving long-term treatment with case-associated L-TRP showed differences in their peripheral blood mononuclear cell phenotypes (more macrophages, CD8, Ia, and IL-2 receptor–positive cells). Thus it appears that EBT can induce in Lewis rats some, but not all, of the pathology associated with chronic administration of case-associated L-TRP. Furthermore, the data from the short-term experiments indicate that the cumulative dose or duration of exposure may play a role in the development of the pathology.

Methods

Animals. 6-wk-old female LEW/N rats (specific pathogen free; ~ 100 g on arrival) were obtained from Harlan-Sprague-Dawley (Indianapolis, IN) and were housed and fed as previously described (9). Food consumption and body weight were measured weekly. Rats were randomly assigned to five groups of 16 animals each for the chronic admin-

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1. *Abbreviations used in this paper:* EBT, 1,1′-ethylidenebis(L-tryptophan); EMS, eosinophilia-myalgia syndrome; LEW/N, Lewis rats; L-TRP, L-tryptophan.

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istration study, and were treated by gavage 6 d/wk for 6 wk with: (*a*) case-associated L-TRP; (*b*) control L-TRP; (*c*) synthetic EBT; (*d*) EBT plus control L-TRP; or (*e*) methylcellulose, the vehicle control. For the acute study 10 rats/group per wk were randomly assigned to one of four treatment groups: (*a*) case-associated L-TRP; (*b*) control L-TRP; (*c*) synthetic EBT; or (*d*) methylcellulose, the vehicle control. These animals were treated 6 d per wk by gavage for 1 or 2 wk.

L-tryptophan samples. Two samples of L-TRP were used for this investigation. One sample, "case-associated L-TRP," was obtained directly from Showa Denko K. K. (Tokyo, Japan, lot 67116202). Chemical analysis showed that this tryptophan contained 140 μg EBT/g case-associated tryptophan. The other tryptophan sample, "control L-TRP," was a United States Pharmacopeia grade L-TRP from Tanabe U.S.A., Inc. (lot 9Y026; Chemical Sales Division, San Diego, CA), which has not been associated with cases of EMS. EBT was nondetectable in the control L-TRP.

Synthetic EBT. Synthetic EBT was prepared and analyzed as previously described (8). The purity of the test material was > 95% as determined by HPLC. The identity of this material was confirmed by mass spectrometric analysis.

Treatments. Suspensions of L-TRP or EBT were prepared in 0.5% aqueous methylcellulose (M-0387; Sigma Chemical Co., St. Louis, MO). L-TRP was administered at 2,000 mg/kg body wt per d, and EBT at 40 mg/kg body wt per d. Dosages of L-TRP and EBT were calculated on the basis of caloric intake, and represented a human dose of ~ 5–6 g L-TRP/d or 125–150 mg EBT/d. All treatments were administered in a total vol of 0.8–0.9% of body wt, 6 d/wk for the specified time period. At the termination of the particular study, all animals were randomly assigned coded numbers, so that pathological, immunological, and biochemical investigations were performed in a blinded manner. The animals were decapitated; blood was collected for cell counts, differentials, and serum assays; and the animals were completely autopsied.

Pathology. Portions of certain tissues (spleen, bone marrow, brain) were removed for further immunologic or biochemical analysis. Sections of skeletal muscle (gastrocnemius), heart, and liver were snap-frozen in isopentane cooled to −160°C with liquid N_2 and were stored at −80°C until use. The lungs were inflated with 10% formalin. The remainder of the tissues/organs, including an intact lower limb, were fixed overnight in 10% buffered formalin. After fixation, the tissues were sectioned, embedded in paraffin, and processed for routine hematoxylin and eosin staining. Tissues analyzed histopathologically included the lungs, liver, pancreas, kidney, heart, spleen, uterus, skin, and skeletal muscle. In the case of skeletal muscle, representative 5-μm cross-sections from both proximal (quadriceps) and distal (gastrocnemius) muscles were examined for pathological involvement (inflammation or myofiber necrosis, degeneration, or regeneration). The thickness of the fascia was measured directly on the stained cross-sections of gastrocnemius muscle. In a few cases, where the tissue was embedded in such a manner that the sections were tangential in orientation rather than in cross-section, the myofascial thickness was not measured.

Hematology. Total white blood cell counts with differentials were obtained at the beginning of the study for all animals, and thereafter were measured weekly for the animals in the acute study, and at weeks 3 and 6 for animals in the chronic study.

Immunological parameters. IgG monoclonal mouse anti-rat antibodies, W3/25 (CD4), OX8 (CD8), OX4 (MHC class II or Ia), OX41 (monocyte/macrophages), OX19 (CD5), OX1 (CD45) (Accurate Chemical & Scientific Corp., Westbury, NY), and ART18 (high affinity IL-2 receptor [IL2R]) (Boehringer Mannheim Biochemicals, Indianapolis, IN) were purchased as purified immunoglobulins. Optimal concentrations were determined in preliminary titration experiments.

All immunofluorescence staining was performed in 96-well, nonsterile, round-bottom microplates. Dilutions and washes were done with 0.1 M PBS, pH 7.0, supplemented with 2% heat-inactivated FBS and 0.05% sodium azide. 10 μl of heparin-treated rat tail vein blood was added to 100 μl of appropriately diluted monoclonal antibody or to PBS alone. The blood was suspended by gentle vortex mixing, and incubated at 4°C for 20 min. A cushion of FBS was layered under each sample and the plate was centrifuged at 500 *g* for 5 min. The pellets were resuspended in 100 μl of PBS, and 20 μl of a 1:10 dilution of phycoerythrin-conjugated donkey anti-mouse IgG was added to all wells. The plates were incubated at 4°C for 20 min and washed twice as described above. The samples were resuspended in 100 μl of solution (Immuno-lyse; Coulter Immunology, Hialeah, FL) for 2 min and fixed for 3 min with 25 μl of fixative (Coulter Immunology). The samples were washed two times with PBS and resuspended in 100 μl of PBS. Spleen cells were isolated and analyzed in a similar manner.

Fluorescence analyses were performed using a flow cytometer (Epics CS; Coulter Electronics, Hialeah, FL). The lymphocyte and monocyte populations were distinguished by gating of a forward versus 90° light scatter histogram. 5,000 cells were analyzed for each monoclonal antibody or control.

Statistical analysis. All data were collated and analyzed by using SAS (10) on a computer (370; IBM Corp., Danbury, CT). Continuous data (body and adrenal gland weights, immunological parameters, and myofascial thickness) were analyzed statistically by Wilcoxon rank sums (PROC NPAR1WAY); discontinuous data were analyzed by chi-square frequency distributions or by Fisher's exact test (PROC FREQ). A result was considered statistically significant if $P < 0.05$.

Results

Clinical observations. As in our previous study, no consistent clinical differences were noted among the rat groups in either

*Table I. Thickness of Gastrocnemius Fascia**

Week	Experimental group				
	Vehicle control	Control L-TRP	Case-associated L-TRP	EBT	EBT + control L-TRP
1‡	192.0±20.5	138.5±13.5	131.0±26.0	178.0±26.0	—
	(8)	(9)	(9)	(9)	ND
2‡	195.0±22.0	168.5±21.0	209.0±23.5	215.0±31.5	—
	(9)	(10)	(10)	(9)	ND
6	86.5±5.5	112.5±5.0§	202.5±19.0§‖	159.5±8.5§‖	124.5±11.5§¶
	(14)	(12)	(12)	(15)	(11)

* Mean diameter in μm±SEM, number of animals given in parentheses. ‡ No significant differences among the groups for weeks 1 and 2. Week 6: § Significantly different ($P \leq$) from the vehicle control: case-associated L-TRP (0.001), EBT (0.00001), EBT + control L-TRP (0.0076), and control L-TRP (0.0034). ‖ Significantly different from the control L-TRP group: case-associated L-TRP (0.0013), and EBT (0.004). ¶ EBT significantly different from the EBT + control L-TRP (0.0143). ND, not done.

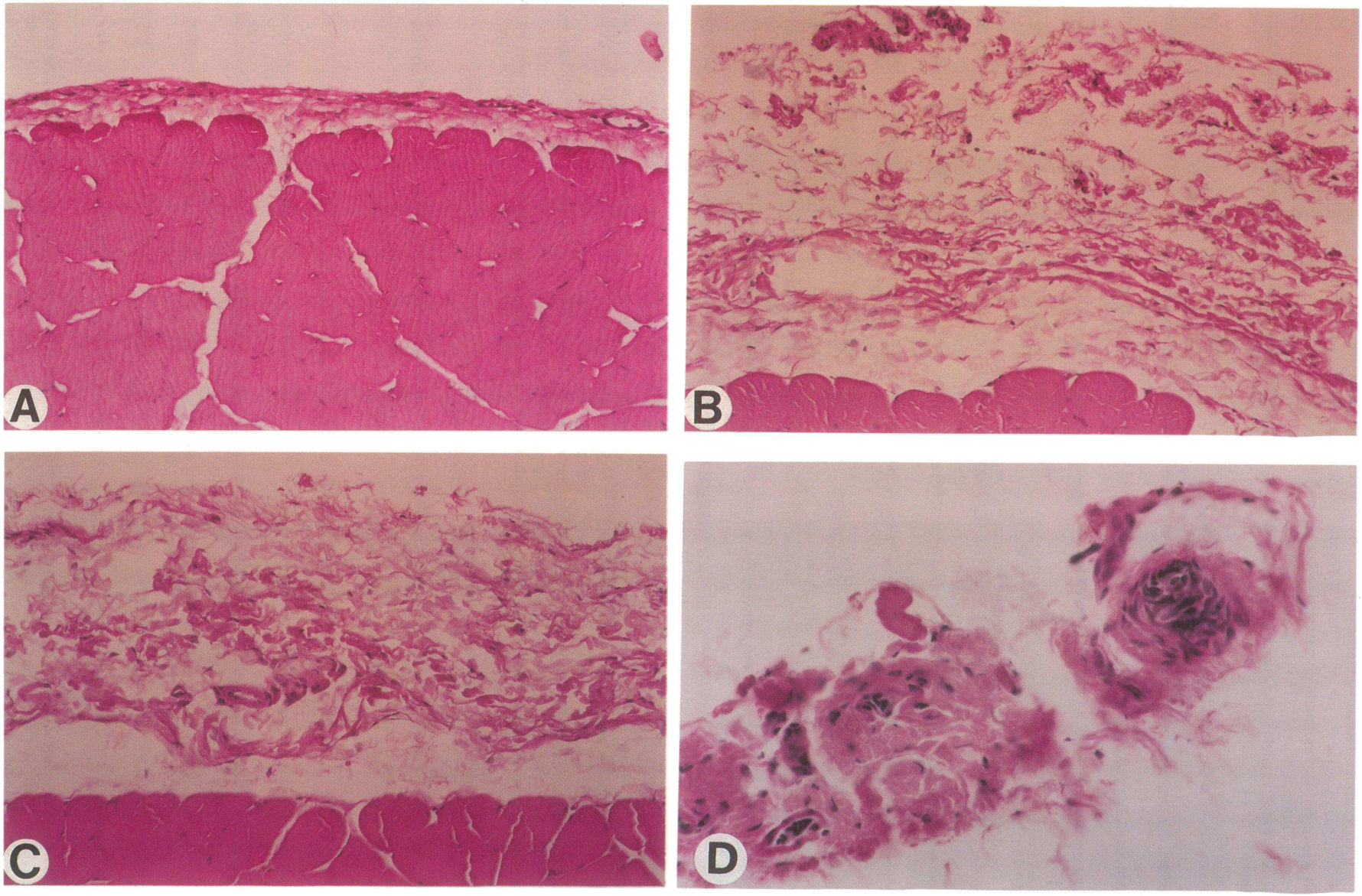

Figure 1. Myofascial thickness of gastrocnemius muscle in LEW/N rats treated for 6 wk with: (*A*) vehicle control, (*B*) case-associated L-tryptophan, and (*C*) synthetic EBT (hematoxylin and eosin, ×200). (*D*) Myofascial blood vessels in an animal receiving case-associated L-TRP showing perivascular fibrosis and swollen endothelial cells. The lumen of the upper right vessel is nearly obliterated by swollen, redundant endothelial cells (hematoxylin and eosin, ×400).

the acute or chronic studies. In addition, there were no significant differences in body weight gain or adrenal weight at autopsy in any of these groups (data not shown). During the 6-wk study, 10 animals (one vehicle control, three control L-TRP, three case-associated L-TRP, and three EBT plus control L-TRP) died as the result of gavage-related trauma, which was verified at autopsy. No animal died during the short-term study. Blood from the rats at the termination of both studies was negative for antibodies to mycoplasma, rat coronavirus, and sialodacryoadenitis virus. Analyses of sera in the animals treated for 6 wk revealed elevated kynurenine levels in rats receiving either control or case-associated L-TRP (6.0 or 6.57 μM, respectively, versus 3.19 μM for the vehicle control animals, 2.59 μM for EBT, or 2.91 μM for the EBT plus control L-TRP groups). These results are consistent with chronic ingestion of high doses of tryptophan, and are in agreement with our previous study (9).

Muscle and fascial pathology. In contrast to our previous experiment (9), we did not observe fascial or perimysial chronic inflammatory infiltrates in any of the animals from either the short-term or the longer studies. Rare focal endomysial infiltrates were noted, but there were no significant differences among any of the groups. However, all animals treated for 6 wk with L-TRP or EBT developed significant ($P < 0.05$) increases in the myofascia compared with the myofascia of the vehicle control animals (Table I and Fig. 1). The degree of fascial thickening was significantly greater in the rats treated with either EBT or case-associated L-TRP than in rats treated with either the vehicle control group or control L-TRP. Focal microangiopathy (reactive endothelial cells with intimal changes) and focal perineural fibrosis were noted in some animals treated chronically with case-associated L-TRP or EBT (Fig. 1 *D*). Animals receiving L-TRP or EBT for less than 6 wk did not show significant differences in myofascial thickness.

Other pathology. In addition to myofascial thickening, all animals in the 6-wk study except vehicle controls exhibited significant pancreatic pathology, including periductal and perivascular fibrosis and acinar changes (reactive and hyperplastic acinar epithelium, with ductal ectasia) (Figs. 2 and 3). Similar reactive epithelial changes were noted in the short-term study, but these changes were milder and were not always accompanied by significant fibrosis, as was the usual case in the chronic study. At week 1, all groups showed mild acinar changes, which were often accompanied by scattered parenchymal and perivascular mononuclear cells in the pancreas. Significant differences were noted at week 1 between the EBT group and either the case-associated or the control L-TRP group. By week 2, both L-TRP groups showed significantly increased pancreatic pathology, compared with the vehicle control or EBT group. A comparison of week-1 and week-2 results showed that the frequency of pancreatic pathology increased with increased treatment duration or cumulative dose in both L-TRP groups, but this was statistically significant only in the case-associated L-TRP group ($P < 0.031$).

Hydrometra (distention of the uterus by serous fluid) was significantly more common in the 6-wk experiment in those animals receiving EBT (44%) than in those receiving either case-associated L-TRP (7%) or control L-TRP (8%). The significance of this finding is unclear because hydrometra was found in all week-1 animals (range 10–60%) and in all week-2 groups except the vehicle control (range 20–30%), and there were no significant differences between the groups.

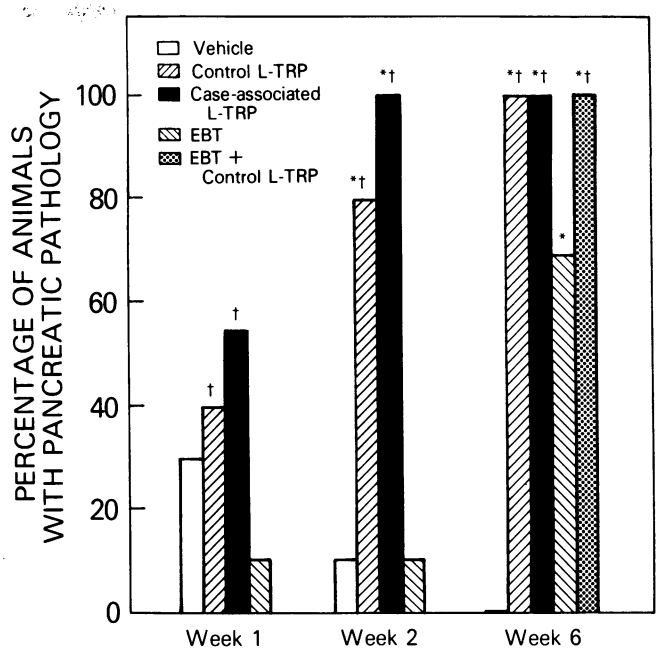

Figure 2. Percentage of animals with pancreatic pathology: mean percentage ±SEM. Significant differences in parentheses ($P \leq$): Week 1: (10 animals per group); †Significantly different from the EBT group: case-associated L-TRP (0.015), control L-TRP (0.025).Week 2: (10 animals per group): *Significantly different from the vehicle control: case-associated L-TRP (0.0001), control L-TRP (0.002). †Significantly different from the EBT group: case-associated L-TRP (0.0001), control L-TRP (0.002).Week 6: (16 animals per group for the vehicle control and EBT groups, 14 animals for the case-associated L-TRP group, and 12 animals per group for the control L-TRP and EBT plus control L-TRP groups): *All groups significantly different from the vehicle control, $P \leq 0.0001$. †Significantly different from the EBT group: case-associated L-TRP (0.022), control L-TRP (0.033), EBT + control L-TRP (0.033).

Although both gross and microscopic examination of the lungs from the animals of the 6-wk study showed evidence of pneumonia, including focal chronic and granulomatous interstitial inflammation and perivascular mononuclear infiltrates, there were no significant differences in the frequency of pneumonias among any of the groups. These changes are believed to be associated primarily with chronic gavage-related trauma and reflux, as these pulmonary findings were not noted in the animals treated for only 1 or 2 wk.

There was no evidence of significant differences in cardiac pathology in any of the groups from either study. Sections of skin and other organs/tissues revealed no specific differences among any of the animal groups. There were no differences in hematological parameters among vehicle control animals and treatment groups in either the acute or chronic studies.

Immunological parameters. Immunofluorescent staining of peripheral blood mononuclear cells in the 6-wk treatment study (Fig. 4) revealed that animals receiving case-associated L-TRP had elevated frequencies of OX8 (CD8), OX4 (Ia), ART18 (high affinity IL2R), and OX41 (monocyte/macrophages) positive cells, compared with animals receiving the vehicle control, control L-TRP, or EBT. These differences are all significant, except for the OX8 and ART18 levels in the comparison of the control L-TRP group with the case-associated L-TRP group. It was noted that animals receiving case-associated L-TRP had values for IL2R and OX41 that were

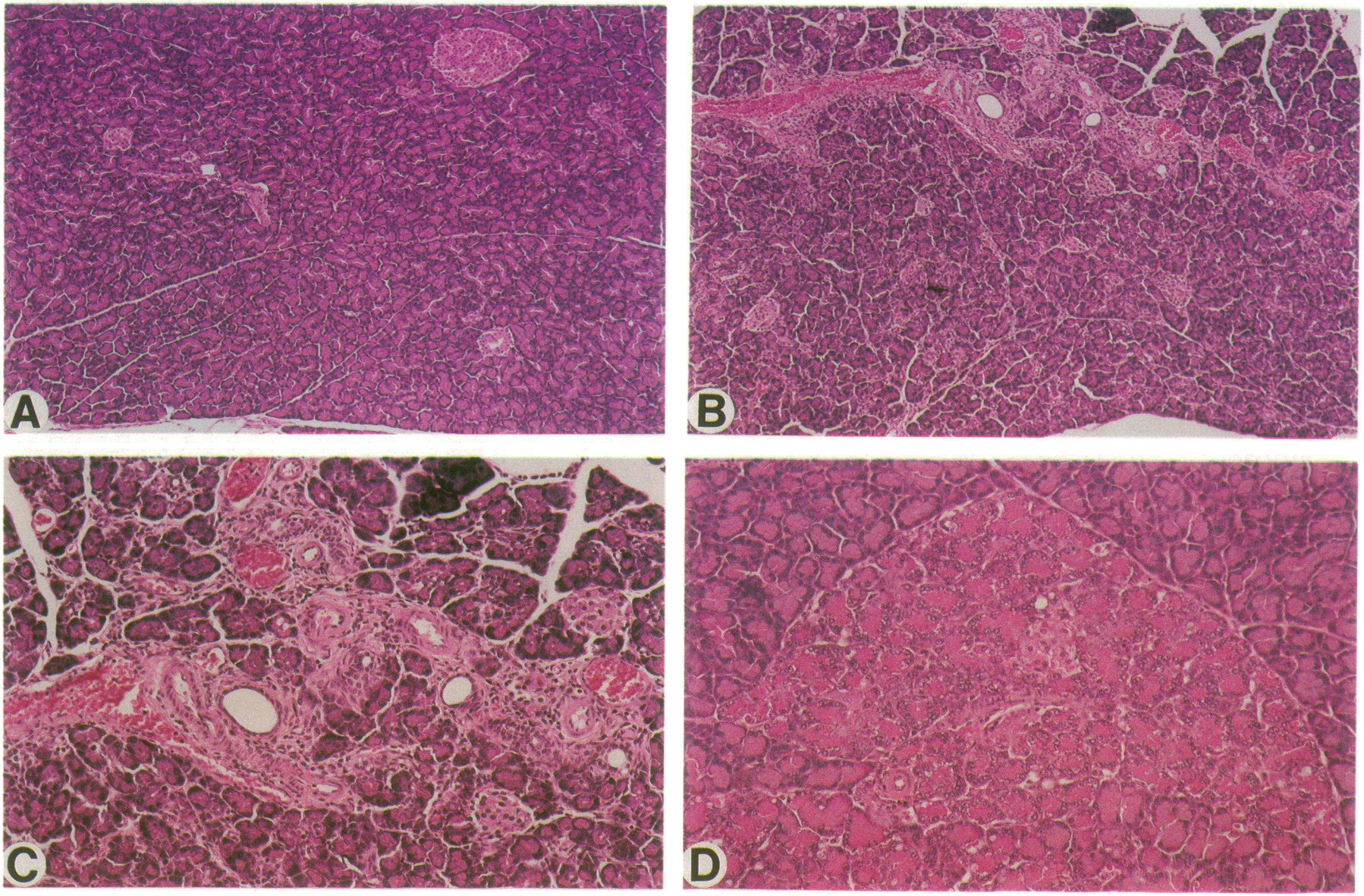

Figure 3. (*A*) Normal pancreas in vehicle control animal (×100). (*B*) Pancreas from an animal receiving case-associated L-TRP for 6 wk, showing periductal and perivascular fibrosis and ductal ectasia (×100). (*C*) Higher power magnification of *B* also showing focal periductal and perivascular mononuclear cells (×200). (*D*) Focal acinar hyperplasia in rat treated for 6 wk with EBT (×200). Changes similar to *B, C,* and *D* were seen in most animals receiving any L-TRP or EBT.

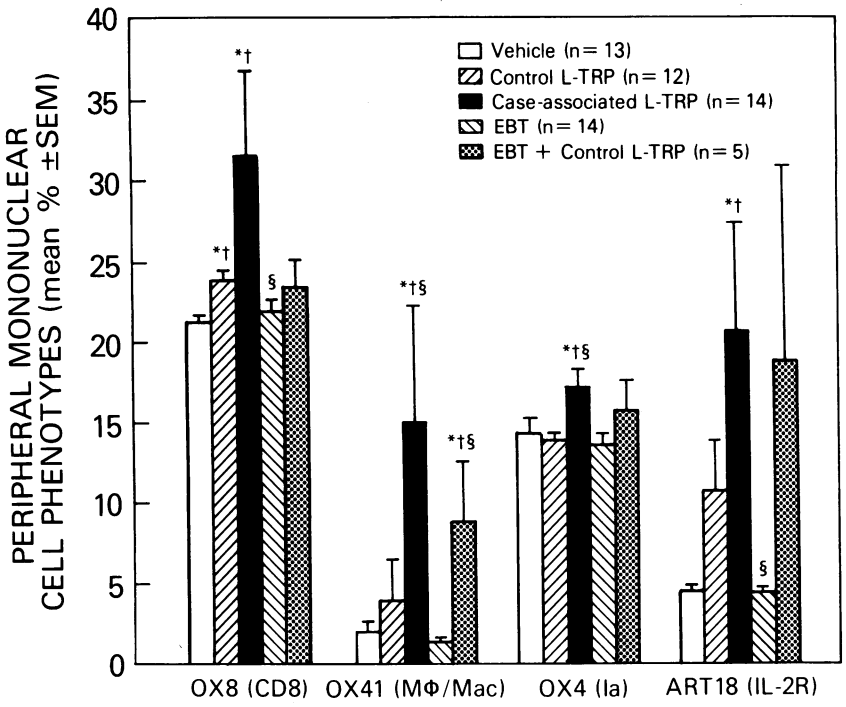

Figure 4. Percentage of phenotypic and activational markers on peripheral blood mononuclear cells in animals treated for 6 wk (mean ± SEM). Significant differences in parentheses ($P \leq$): *Significantly different from the vehicle control: case-associated L-TRP: OX1 (0.0422), OX19 (0.0317), W3/25 (0.0102), OX8 (0.007), OX4 (0.0155), OX41 (0.0155), and ART18 (0.0362); EBT: OX1 (0.0441); control L-TRP: W3/25 (0.002), and OX8 (0.001); EBT + control L-TRP: OX41 (0.0137), and OX19 (0.018). †Significantly different from the EBT group: case-associated L-TRP: OX8 (0.0171), OX4 (0.0043), OX41 (0.0019), and ART18 (0.0236); EBT + control L-TRP: OX41 (0.0035). §Significantly different from the control L-TRP group: case-associated L-TRP: OX4 (0.0027), and OX41 (0.0225); EBT: OX8 (0.0221), and ART18 (0.0288); EBT + control L-TRP: W3/25 (0.0234), and OX41 (0.0176).

either more than twice as large as the values for the vehicle control animals, or equal to those for the vehicle control animals. Hence these data had large standard errors. Compared with rats receiving the vehicle control, those receiving case-associated L-TRP had mild but significant decreases in the proportions of peripheral blood OX19 positive (CD5) total T cells (78.2 vs 81.0%) and W3/25 (CD4) positive T cells (61.7 vs 64.6%). Such changes were not noted in the EBT group, which did not differ statistically in lymphocyte or macrophage markers from the vehicle control group. Animals receiving EBT plus control L-TRP had significantly more OX41 positive cells (macrophages) than did the vehicle control, control L-TRP, or EBT group. Furthermore, those animals receiving control L-TRP showed mild but significant differences, compared with vehicle control animals, in the proportions of CD4 positive (fewer) and CD8 positive (more) cells, but these animals did not show increases in the markers OX4 (Ia) and ART18 (high affinity IL2R), which would indicate immune activation. There were no significant differences between animals receiving case-associated L-TRP or EBT plus control L-TRP. These immunological changes did not appear to be related to pneumonias or to other pulmonary pathology, which could be nonspecific immune stimulants, because no significant differences were found when the data were analyzed with pulmonary pathology as a confounding variable (data not shown).

Only a partial phenotyping (OX41, OX4, and ART18) was performed on the 1-wk acute experimental groups. Compared with the vehicle control animals, all animals receiving L-TRP or EBT showed significantly increased proportions of OX41 (monocyte/macrophages) positive cells in their peripheral blood (vehicle control 4.4±1.2, control L-TRP 5.8±0.5, case-associated L-TRP 22.4±7.4, and EBT 6.5±0.6 [mean percentage±SEM]). Animals receiving case-associated L-TRP showed the highest percentage of OX4 positive (Ia) cells (23.0±4.9%) of any of the animals, but because of the marked variability of levels within this group these data were not statistically significant. The EBT-treated animals had a mild but significant elevation in the proportions of ART18 positive (IL2R) (4.7%), compared with either the vehicle control (4.3%) or control L-TRP (4.1%) animals.

Phenotyping was also performed on spleen cells with few significant differences noted among any of the treatment groups. However, animals receiving case-associated L-TRP did show a significantly increased proportion of splenic macrophages expressing OX41, compared with animals receiving control L-TRP (8.7 vs 4.9%), and a higher OX4/Ia expression, compared with those animals receiving EBT (15.7 vs 13.5%). The only significant difference in the 6-wk treatment study was that animals receiving case-associated L-TRP showed an increased proportion of OX4 positive (Ia) splenocytes (14.7%), compared with animals receiving the vehicle control (12.2%). Dual-labeling studies to determine which population of cells was associated with the increased IL2R levels (CD4, CD8 or both) were not performed because of logistical considerations.

Discussion

Several impurities that are predictive of EMS–case-associated lots of L-TRP have been identified chemically by HPLC (6–8). This report demonstrates the pathological effects of EBT, one such impurity in EMS–case-associated L-TRP, and extends our earlier findings of the pathological effects of case-associated L-TRP (9). The current study indicates that, EBT alone causes myofascial thickening that is significantly greater than the myofascia observed in vehicle control rats. When combined with control L-TRP, EBT also causes immune cell activation in the peripheral blood. The marked potency of this impurity is evidenced by the significant pathological effects caused by a dose of EBT that was 50-fold lower than the dose of case-associated L-TRP. Our observations, however, do not rule out the possibility that other impurities in the case-associated L-TRP

may also contribute to some of the features of the syndrome. Supporting this hypothesis are our findings that chronic treatment of rats with case-associated L-TRP resulted in more immune cell activation than with EBT alone, with EBT plus control L-TRP, or with control L-TRP. This study also strongly suggests that control L-TRP alone plays an important role in this and possibly other fibrosing illnesses, because it is associated with mild but significant myofascial thickening and alterations in peripheral mononuclear cell phenotypes, as well as with significant pancreatic pathology.

Both our previous study and the current 6-wk study show that implicated L-TRP causes an approximate twofold increase in thickness of the fascia over the gastrocnemius muscle. Thus, we have duplicated the most significant quantitative finding of our previous report. It is unclear why mild to moderate inflammatory infiltrates in the fascia or perimysium were not observed in the current investigation as was reported previously (9). Although these studies were performed under similar circumstances, there were a number of important methodological factors that differed in the two studies. First, different lots of both control and implicated L-TRP were used in each study. Chromatographic analyses of the implicated L-TRP samples used in these two studies reveals a number of as yet unidentified peaks that differ between the two samples, although there are similar concentrations of EBT and the recently identified peak 5 (11, 12) in these samples. Other methodological differences include treatment with a higher dosage of L-TRP for a slightly longer time in the current study compared to the first study; and 5-μm formalin-fixed and paraffin-embedded sections of skeletal muscle were analyzed in the current study compared to 10-μm frozen sections in the previous study. These differences in methodology may have also contributed to our ability to detect small but significant differences in fascial thickness in animals receiving control L-TRP that did not reach significance in our previous study. It is also uncertain why there was such variability in the myofascial thickness in animals treated for only 1 or 2 wk, although these younger animals had much more fat in their fascia, which made analyses more difficult.

All animals ingesting L-TRP or EBT for 6 wk also developed pancreatic changes. The pancreas is a metabolically active organ involved in protein synthesis for secretion, with a high affinity for α-amino acids and specific receptor mechanisms for the transport of amino acids, including L-TRP (13, 14). Various dietary components may influence the absorption of proteins and amino acids. In this respect it is important to point out that L-TRP was taken in bolus form by patients who developed EMS and, therefore, it was given as a bolus dose to the rats in our study. In humans, increased concentrations of various amino acids, including L-TRP in the gastrointestinal system, are associated with increased release of cholecystokinin, which can stimulate pancreatic enzyme production. In dogs, L-TRP was found to be the most potent essential amino acid for pancreatic enzyme stimulation (15), and intraduodenal perfusion of L-TRP produced a dose-dependent increase in plasma cholecystokinin-like reactivity (16). The acinar hyperplasia seen in rats taking L-TRP or EBT could result from increased cholecystokinin. (In this respect it is similar to the pancreatic pathology seen with trypsin inhibitors [reviewed in (17)], in which focal fibrosis and acinar hyperplasia are also noted.) The cumulative dose of L-TRP, or possibly the duration of exposure, also appears to be important. 1 wk of treatment resulted in only mild acinar or inflammatory changes in some of the animals. Since these changes were seen in all the groups, they could be related in part to an inflammatory reaction to the gavage procedure to which the animals later adapt. After 2 wk of treatment, both L-TRP groups showed a significantly increased incidence of pancreatic pathology, compared with the vehicle control or EBT groups. After 6 wk of treatment, these acinar changes were associated with significant periductal and perivascular fibrosis. These dose-related changes are consistent with the disease predictors reported by Kamb et al. (18), who showed that the cumulative and daily doses of L-TRP were independent predictors for determining which individuals in their patient population would develop EMS. The prevalence of pancreatic involvement in patients with EMS is unknown. Chronic pancreatic inflammation has been noted at autopsy in at least one patient with EMS (described in [18, 19]), and acute pancreatitis was a presenting feature in another patient with EMS (20).

It is interesting that animals receiving case-associated L-TRP showed alterations in peripheral mononuclear cell phenotypes, suggesting immune activation, consistent with changes that have been reported in humans. Rats receiving the case-associated L-TRP for 6 wk showed the greatest number of changes in the peripheral circulation (increased proportions of monocyte/macrophages, CD8 positive, Ia positive, and IL-2R positive cells), compared with the other treatment groups, but rats receiving the control L-TRP also showed small but significant differences in the proportions of CD8 positive cells, compared with rats receiving vehicle control. Animals receiving EBT alone did not show these differences, but when EBT was combined with control L-TRP, the results were essentially the same as in animals treated with case-associated L-TRP. Thus while EBT alone is associated with changes in the thickness of the myofascia, it was not sufficient by itself to cause immune activation. There also appeared to be dose-related changes in immunological parameters, e.g., after 1 wk of treatment only the proportions of monocyte/macrophages were higher in animals receiving case-associated L-TRP. After 6 wk of treatment, animals receiving case-associated L-TRP showed increased proportions of monocyte/macrophages and CD8 positive lymphocytes in the peripheral blood, as well as higher percentages of cells with the activational markers, Ia and IL2R. Although changes in the circulating mononuclear cell phenotypes have not been studied systematically in patients with EMS, our findings in rats are consistent with the available literature on humans. Strongwater et al. (21) noted evidence of activated CD8 positive cells with increased expression of CD45RO in two out of three patients with EMS. Although Seidman et al. (22) reported a predominance of CD4 positive cells in the muscle biopsies of patients with EMS, a quantitative study by Emslie-Smith et al. (23) showed that the infiltrates in the muscles of EMS patients were composed mainly of macrophages and activated CD8 positive cells (23), which is suggestive of a specific T cell–mediated component in EMS. In addition, Campagna et al. (24) have recently reported increased CD8 positive cells in the bronchial alveolar lavage of two patients with pulmonary manifestation of EMS.

In summary, Lewis rats develop myofascial thickening and immune cell changes that are most prominent after administration of case-associated L-TRP, but are also present in animals treated with control L-TRP and/or EBT. Additionally, animals receiving L-TRP or EBT developed pancreatic fibrosis. Taken

together, these findings support previous suggestions that the etiology of L-TRP–associated EMS is multifactorial, and may require multiple agents, such as tryptophan itself and some other agent in the tryptophan acting in concert in a susceptible host (2, 9, 19, 25–27). Our study implicates EBT as one compound found in case-associated L-TRP that can cause some pathological changes in Lewis rats that are similar to some pathological features of L-TRP–associated EMS. The present observations also support previous literature postulating a role for tryptophan and its metabolites in fibrosing illness such as scleroderma, eosinophilic fasciitis, and carcinoid syndrome (28–36). Finally, these results support an immune pathogenesis for L-TRP–associated EMS, with EBT and case-associated L-TRP serving as important etiologic agents, and L-TRP itself a contributing factor to the syndrome.

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