Toxic Oil Timeline

Diagnosing Effects Decades Later

Two decades after contaminated cooking oil caused widespread sickness in Spain, patients with so-called toxic oil syndrome (TOS) continue to report neurologic symptoms. Accurately assessing these symptoms—which include headache, insomnia, and muscle weakness—has been challenging, because the symptoms are numerous and tend to be vague. Standard clinical examinations have detected few or no problems, and patients have sometimes been accused of exaggerating or malingering. This month, a team led by Manuel Posada de la Paz of the Instituto de Salud Carlos III in Madrid provides the first quantitative evidence that TOS patients do indeed experience neurologic abnormalities [***EHP* 111:1326–1334**].

In May 1981, Spanish health officials were puzzled and alarmed by the emergence of a severe, possibly novel respiratory illness. The outbreak, which occurred in Madrid and the northwestern Spanish provinces, affected 10,000 people and claimed at least 80 lives in the first month. Initial symptoms included fluid in the lungs, shortness of breath, fever, headache, rash, muscle pain, and a proliferation of eosinophils (a type of white blood cell). An infectious agent seemed implicated, but by mid-June epidemiologic studies revealed the cause to be cooking oil derived from aniline-denatured rapeseed oil. This oil had been produced for industrial uses but was illegally refined to remove the aniline and sold as pure olive oil. The Spanish government acted quickly to remove all suspect oil from the market, but TOS eventually affected at least 20,000 people and caused more than 300 deaths.

Even after the cause was identified, the crisis continued. TOS comprises three phases: the early acute phase, an intermediate phase involving neurologic complaints and intense muscle pain, and, for approximately 60% of victims, a chronic phase marked by motor and sensory nerve disorders and muscle pain and cramping. Researchers strongly suspect that unknown toxic compounds created during refining triggered autoimmune disease.

A slippery slope. Research shows that the health effects of exposure to contaminated cooking oil persist decades after the initial event.

In general, the difficulty in assessing TOS-related neurologic symptoms arises from the vagueness and subtlety of those symptoms. A possible solution rests with quantitative neurologic testing methods that have been developed since the late 1980s and used to detect subtle nerve damage associated with other diseases. These neurobehavioral tests include computerized examination of the central, peripheral, and autonomic nervous systems and use of a "vibrotactile threshold" as a measure of large myelinated nerve fiber function. This study was the first time such tests have been used with TOS patients.

The researchers recruited a random sample of TOS patients who lived in the Alcorcón locality of Madrid province in 1981 and assembled an age- and sex-matched referent group from the same area. All participants completed a questionnaire covering neurologic symptoms, environmental exposures, demographics, and other factors. Participants also underwent clinical examination and completed neurobehavioral testing.

Analysis of the neurobehavioral data revealed significant differences between the groups. The TOS group had higher incidences of muscle cramps and spasms; abnormal sensations such as numbness, burning, and paresthesias; loss of strength; diminished sensitivity to stimulus (hypoesthesias); poor coordination; headache; sleep disturbances; and memory loss. Female TOS patients over 35 were most affected, and some group differences (such as hypoesthesias) were significant only for women. The standard clinical neurologic examination did not reveal any differences aside from increased upper-limb pain and diminished lower-limb sensation in the TOS group. Questionnaire data uncovered no significant differences in neurologic history between the groups.

The researchers suggest that quantitative neurologic testing could help track symptom development over time, not only with TOS but in other hard-to-document neurologic diseases as well. **–Julia R. Barrett**

Setting the Stage for Illness

Mercury Exposure and Autoimmune Disease

The current scientific literature abounds with studies of the strongly suspected link between exposure to inorganic mercury (iHg) and autoimmune disease, a family of often debilitating and sometimes fatal conditions. Although no human association has been documented, the connection is well known in animal models. A great deal of work continues to characterize the complex physiologic mechanisms involved and thereby shed light on the role of environmental mercury exposures in the etiology of these illnesses. Now a team of Maryland investigators has found that even brief, low-level environmental mercury exposure may increase susceptibility to autoimmune disease in mice [***EHP* 111:1273–1277**].

iHg experiments often use mice bred for susceptibility to various autoimmune diseases. In this study, however, the team used healthy, genetically nonsusceptible mice. The researchers injected treatment groups of 6- to 8-week-old female B6D2F_1 mice with iHg doses of 20 or 200 micrograms per kilogram dissolved in water. The mice were dosed every other day for 15 days, for a total of 8 doses. Control animals were injected with an equal total volume of sodium chloride. Five days after cessation of the iHg injections, both case and control mice were intravenously administered spleen cells from another mouse strain to induce chronic graft-versus-host disease (GVHD), a well-established murine model of acquired autoimmunity.

This study involved very low exposures compared to those commonly used in studies of iHg immunotoxicity (typically 500–2,000 micrograms per kilogram). These low doses helped avoid confounding

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of the subsequent results by the toxic effects of iHg exposure itself or by directly causing iHg-associated autoimmune disease.

The dose of parental donor cells was set just above the threshold for consistent induction of chronic GVHD, and under normal conditions would be expected to induce a mild case of the lupuslike condition, as it did in the controls. In the case mice, however, the scientists determined that the iHg pretreatment clearly accelerated and exacerbated the course of the disease.

Unlike the control mice, the iHg-exposed mice experienced glomerulonephritis (an inflammatory kidney disease) and elevated urine protein, evidence of accelerated GVHD. The glomerulonephritis, in turn, resulted in accelerated mortality in the iHg-treated groups. Upon reexamination 2–3 months after disease induction, autoantibodies characteristic of chronic GVHD were found to have become significantly elevated in surviving iHg-treated mice, but no markers characteristic of iHg-associated autoimmunity were seen. These results imply that the iHg treatment affected the acquired autoimmune disease itself—that the disease was not caused by delayed effects of the iHg exposure, but that its course was worsened by the exposure.

The results of the study, the first of its kind, support the hypothesis that low-level environmental exposure to mercury is a potential factor in the development of autoimmune disease in humans. Disturbingly, these results further suggest that "low-level exposure . . . may lower the threshold for disease development in susceptible individuals who later encounter the appropriate infectious or toxic triggers of disease." If these findings are confirmed by replication and further research, the implications regarding safe thresholds for environmental mercury exposure could be profound. **–Ernie Hood**

ALS and Lead

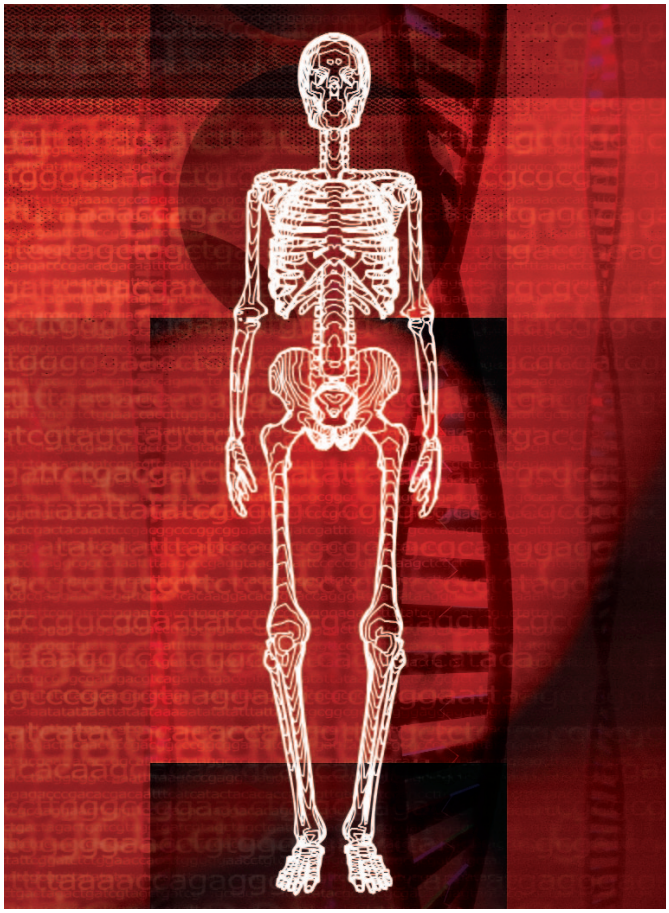
The Polymorphism Possibility

Although the etiology of amyotrophic lateral sclerosis (ALS), an often fatal neurodegenerative disease, is still largely unexplained, research suggests that lead exposure may be a risk factor. Freya Kamel of the NIEHS earlier led a group that identified a potential role of lead exposure in the etiology of ALS. Now she and her colleagues have taken that work a step further, examining the possible association of ALS risk with specific genetic polymorphisms known to affect lead toxicokinetics [***EHP* 111:1335–1339**].

The researchers used data from a case–control study conducted in New England between 1993 and 1996 that involved more than 100 ALS patients and 38 control subjects. The study participants completed questionnaires on demographic and lifestyle characteristics, and were invited to provide tissue samples for measurement of blood and bone lead concentrations. For this analysis, DNA genotyping was performed using blood samples from the original study.

In many adults with no known recent environmental exposure (including most of the subjects in the study), internal lead exposure—that is, the migration of stored lead from bone into the blood—is the major source of blood lead. The researchers speculated that polymorphisms of two genes—*ALAD* (which codes for ∂-aminolevulinic acid dehydratase, an enzyme involved in heme synthesis in red blood cells) and *VDR* (which codes for the vitamin D receptor)—might confer increased susceptibility to ALS through their previously confirmed impact on lead retention or mobilization in bone and blood.

Kamel and colleagues found that the variant allele (*ALAD 2*) of the polymorphism denoted as *ALAD K59N* was positively associated with an approximate twofold increase in risk of ALS after adjustment for age, sex, region, education, and physical activity. In the course of their analysis, they also identified a previously unknown polymorphism, denoted as *ALAD IVS2+299G>A*. The variant allele of that polymorphism (*ALAD I2-2*) was found, after similar adjustment, to be negatively associated with ALS risk. Both alleles were positively associated with decreased bone lead concentrations, and neither affected the relationship of blood or bone lead to ALS. No ALS risk associations were found with the alleles of the *VDR* polymorphism, nor did it appear to be associated with blood or bone lead concentrations.



Origins of disease? A gene polymorphism that promotes the migration of lead from bone to blood and the retention of lead in blood may increase the risk of ALS.

The researchers theorize that although *ALAD* alleles did not modify the relationship of ALS to lead in this cross-sectional study, genetic susceptibility conferred by these polymorphisms might still affect risk through a mechanism related to internal lead exposure. *ALAD 2* appears to promote retention of lead in blood and migration of lead from bone to blood. The current findings are consistent with the hypothesis that this increased retention of lead in blood relative to bone increases its availability to target tissues and hence its toxicity. The authors speculate that "alterations in lead toxicokinetics conferred by the presence of the *ALAD 2* allele may subtly increase exposure to lead throughout a person's lifetime, thereby elevating risk."

The authors point out that the study is limited by a low participation rate of control subjects (41%) in providing tissue samples, although a much higher percentage completed the questionnaire, leading to concerns about selection bias and contributing to imprecision in the statistical evaluation of relationships. They conclude that because the study is small and the observation unique, further research is necessary to confirm or refute the hypothesis. Considering that the frequency of the *ALAD 2* allele is approximately 10% in Caucasian populations, if this study's conclusions are confirmed, it will be an important contribution to identifying a large number of people who could be at elevated risk for developing a devastating, incurable disease. **–Ernie Hood**