Please note that comments (mark-ups and yellow highlights) made to this document were made by the petitioner and not by the WTC Health Program.

Form Approved
OMB No. 0920-0929
Exp. Date 04/30/2015

Petition for the Addition of a New WTC-Related Health Condition for Coverage under the World Trade Center (WTC) Health Program

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

9/11 WTC Health Program

General Instructions

Any interested party may petition the WTC Program Administrator to add a condition to the List of WTC-Related Health Conditions (List) in 42 C.F.R. Part 88 (see http://www.cdc.gov/wtc/faq.html#hlthcond for the complete list).

Please use this form to petition the Administrator to add a health condition (any recognized medical condition requiring treatment or medication) to the List. Please use a separate form for each health condition.

Use of this petition *form* is voluntary, but any petition must include all of the information identified below, as required by 42 C.F.R. Part 88. Petitions that do not provide the required information will not be considered by the WTC Program Administrator. Additional supporting materials may be submitted and are encouraged.

Please note, however, the petition and all supporting materials submitted to the WTC Health Program are part of the public record and may be subject to public disclosure. Personal information will be redacted prior to public disclosure.

Please TYPE or PRINT all information clearly on the form.

If you need more space to provide the required information, please attach additional pages to this form.

Mail or email this form to: World Trade Center Health Program
395 E. Street, S.W., Suite 9200
Washington, D.C. 20201
WTC@cdc.gov

A. Interested Party Information

A1. Do you represent an organization (are you submitting this petition on behalf of an organization)?
☐ Yes (Go to A2) ☒ No (Go to A3)

A2. Organization Information:

Name of organization

A3. Name of Individual Petitioner or Organization Representative:

First name Last name

Position, if representative of organization

A4. Mailing Address:

Street

City State Zip code

A5. Telephone Number:

A6. Email Address:

B. Proposed WTC-Related Health Condition Information

B1. Health Condition Information:

PRIMARY BILIARY CIRRHOSIS

Name of health condition you wish to petition to add to the List of covered conditions

If the name of the condition is not known, please provide a description of the condition or the name of the diagnosis provided by a physician or other healthcare provider.

C. Basis for Proposing that the Condition Be Added to the List of WTC-Related Health Conditions

C1. Describe the reasons the WTC Program Administrator should consider the addition of this health condition. Explain how the health condition you are proposing relates to the exposures that may have occurred from the September 11, 2001, terrorist attacks. Your explanation must include a medical basis for the relationship/association between the 9/11 exposure and the proposed health condition. The medical basis may be demonstrated by reference to a peer-reviewed, published, epidemiologic study about the health condition among 9/11 exposed populations or to clinical case reports of health conditions in WTC responders or survivors. First-hand accounts or anecdotal evidence may not be sufficient to establish medical basis. If you need more space, please attach additional pages to this form.

PLEASE SEE ATTACHED COVER LETTER ALONG WITH SCIENTIFIC + MEDICAL EVIDENCE.

D. Signature of Petitioner

Sign your name below to indicate that you are petitioning the WTC Program Administrator to consider adding a health condition to the list of WTC-related health conditions identified in 42 C.F.R. Part 88.

October 15, 2014

Signature Date

Privacy Act Statement

In accordance with the Privacy Act of 1974, as amended (5 U.S.C. § 552a), you are hereby notified of the following:

Title I of the James Zadroga 9/11 Health and Compensation Act of 2010 amended the Public Health Service Act (PHS Act) to establish the World Trade Center (WTC) Health Program. Sections 3311, 3312, and 3321 of Title XXXIII of the PHS Act require that the WTC Program Administrator develop regulations to implement portions of the WTC Health Program established within the Department of Health and Human Services (HHS). The WTC Health Program is administered by the Director of the National Institute for Occupational Safety and Health (NIOSH), within the Centers for Disease Control and Prevention (CDC). The information provided with this form and supporting documentation will be used by the WTC Program Administrator to consider the disposition of a petitioned-for health condition. Disclosure of this information is voluntary.

Records containing information in identifiable form become part of an existing NIOSH system of records under the Privacy Act, 09-20-0147, "Occupational Health Epidemiological Studies and EEOICPA Program Records and WTC Health Program Records, HHS/CDC/NIOSH." These records are treated in a confidential manner, unless otherwise compelled by law.

Information submitted to WTC Health Program which may be considered "protected health information" pursuant to the Health Insurance Portability and Accountability Act of 1996 (HIPAA) (Pub. L. 104–191; 42 U.S.C. § 1320d) and the HIPAA Privacy, Security, Breach Notification, and Enforcement Rules (45 C.F.R. pts. 160, 162, and 164) will be maintained in accordance with all applicable laws.

NIOSH may disclose information in identifiable form only insofar as such disclosure is permitted pursuant to the HIPAA Privacy Rule; this may include disclosure to the WTC Health Program Scientific/Technical Advisory Committee (STAC), which may be asked to consider the petition and issue a recommendation to the WTC Program Administrator. Information in identifiable form will be redacted from submitted petition forms and supporting documentation that become a part of the public record (e.g. in conjunction with STAC consideration or a rulemaking).

October 15, 2014

John Howard, MD
Director
National Institute for Occupational Safety and Health
395 E Street SW, Suite 9200
Patriots Plaza Building
Washington, DC 20201

Dear Dr. Howard:

With great respect, this 9/11 first responder files a petition pursuant to Sec. 3312(a)(6) of the Zadroga Act initiating your review of the scientific and medical evidence associating toxic exposure at the World Trade Center with triggering Primary Biliary Cirrhosis in susceptible individuals. I ask that you consider establishing Primary Biliary Cirrhosis as a covered condition under the Zadroga Act.

It is my understanding that autoimmune disorders are currently being investigated by your research funding agreement with Albert Einstein College of Medicine under Mayris Webber, DrPH. According to Dr. Webber, this study entitled Post-9/11 Incidence of Systemic Autoimmune Diseases in the FDNY Cohort does not include Primary Biliary Cirrhosis.

Enclosed are several recent medical studies linking the specific toxins at 9/11 to triggering an autoimmune response, along with specifics regarding Primary Biliary Cirrhosis.

It has come to my attention that Mount Sinai Hospital is treating 9/11 workers with liver conditions. Data on the number of 9/11 workers being treated or their specific liver conditions was not available.

Dr. Howard, you have saved my life. Your team at LHI, who I am in contact with several times a week, has assisted me with the greatest medical care in the United States. It was my honor and pleasure to serve in the . and for the Salvation Army.

As I learned in later years, the contamination in the serving 5,000 meals each shift morning and lunch is where God put me as a missionary. I am watching my year old son grow.

My husband who was a first responder at the Site, , is doing what is necessary to take care of our family. Every time I turn the corner and regain my composure, there is a new 9/11 diagnosis.

The Ursidiol prescribed that was $42 per month is $370 this month and $555 a month during my prescription gap. It comes out to around $2,000 yearly.

Dr. Howard, it was my honor and pleasure to serve under the umbrella of the Salvation Army and to assist the United States in my duties . My husband and I cannot afford the medication. I will become very sick if the great Dr. Howard does not assist this first responder. I want to grow and watch my son bloom into a fine man.

Respectfully,

Asbestos Revisited: A New Autoimmune Disease?

Published: Jul 29, 2014

By Nancy Walsh, Senior Staff Writer, MedPage Today
Reviewed by Zalman S. Agus, MD; Emeritus Professor, Perelman School of Medicine at the University of Pennsylvania and Dorothy Caputo, MA, BSN, RN, Nurse Planner

save A A

In the small town of Libby in northwestern Montana, prospectors in 1916 discovered an unusual mineral known as vermiculite that appeared to be resistant to fire after initial exposure to high heat.

The early owners of the mine called their product Zonolite, and for the next half century they dug it out of the Libby mountain and shipped it across the continent for use as insulation and in various commercial products.

Unfortunately, the mine and its product also contained asbestos, and by the 1980s, hundreds of the miners who worked at

Action Points

- This review summarizes

Zonolite mountain – and their family members – had sickened and died of asbestos-related diseases at rates 40 times higher than the U.S. as a whole.

epidemiological, animal model, and in vitro data related to asbestos exposures and autoimmunity.

- Note that there appears to be a body of evidence supporting an association between asbestos exposure and autoantibodies indicative of systemic autoimmunity.

#1

Little was known outside of Libby about the cluster of diseases until 1999, when *Seattle Post-Intelligencer* reporter Andrew Schneider published a series of stories called "Uncivil Action: A Town Left to Die," which began by saying "First, it killed some miners. Then it killed wives and children, slipping into their homes on the dusty clothing of hard-working men. Now the mine is closed, but in Libby, the killing goes on."

Then in a 2004 book about the case, *An Air that Kills: How the Asbestos Poisoning of Libby, Montana, Uncovered a National Scandal*, Schneider and co-author David McCumber wrote, "The sickening of Libby was such a gradual thing. Like a person with asbestos-scarred lungs that are slowly losing their capacity, a town that is slowly dispatching its miners to the graveyard, slowly increasing its per-capita use of oxygen tanks, slowly increasing its quotient of widows with shortness of breath, does not immediately realize what is happening."

Schneider and McCumber's book details the heartbreaking and outrageous story of the efforts by the residents of Libby to bring attention and recompense to their plight, particularly after the mine was purchased in 1963 by the W.R. Grace company -- the same company implicated in the childhood leukemia cluster in Woburn, Mass., chronicled in the 1996 book *A Civil Action*, by Jonathan Harr and in a film by the same name starring John Travolta.

The mine closed in 1990, and W.R. Grace has since faced hundreds of thousands of lawsuits for asbestos-related illness, most often cancer and asbestosis. (The company was acquitted in 2009 of knowingly harming the people of Libby and of covering up its knowledge of the health hazards from the mine.)

But now, a group of researchers from Idaho State University and the Center for Environmental Health Sciences at the University of Montana are suggesting that a further health concern should be added to the list of woes faced by the Libby residents: autoimmune disease, including an as-yet undescribed autoimmune condition affecting the lungs.

Asbestos and the Immune System

(AT 9/11) #4

It's long been known that exposure to another silicate dust, crystalline silica, is associated with the development of autoimmunity, but this had not been recognized for asbestos.

The term asbestos refers to a group of mineral fibers that are classified as "serpentine" or

"amphibole," and most studies of occupational exposure have focused on the serpentine fiber chrysotile. In contrast, the Libby asbestos contained a variety of amphibole fibers, including winchite, richterite, and tremolite.

One of the Idaho researchers, Jean C. Pfau, PhD, explained that the various types of asbestos fibers appear to have quite different effects on the immune system.

"Exposure to the common chrysotile asbestos most often has been associated with cancer, possibly because it suppresses the immune response and the ability of the immune system to destroy the cancer," she said.

"But the amphibole asbestos in Libby doesn't seem to do that. Instead, it appears to activate the immune system leading to the production of autoantibodies," she told *MedPage Today.*

Many different types of autoantibodies have been identified in both mouse and human studies, including antinuclear antibodies and rheumatoid factor, along with increases in serum immunoglobulins and the deposition of immune complexes typical of lupus.
(AMA) #1
One potential mechanism by which amphibole exposure could induce an immune response, Pfau's group explained recently online in *Autoimmune Diseases,* is through a T_H-17 response seen in amphibole-exposed mice, but not in animals exposed to chrysotile.

"The T_H-17 response is characterized by high levels of IL-17, triggered or maintained by other cytokines such as IL-6, IL-23, and TGF-beta. TH-17 responses have been implicated in a variety of diseases, including rheumatoid arthritis, systemic sclerosis, and systemic lupus erythematosus," they wrote.

The Montana Study

In the early 2000s, the CDC conducted an extensive screening program in Libby in an attempt to quantify the extent of the health disaster, questioning more than 7,000 residents of the town about potential exposures. Among their findings was the observation that 494 individuals reported ever having been diagnosed with lupus, rheumatoid arthritis, or systemic sclerosis. This represented 6.7% of the population, which far exceeded the expected prevalence of less than 1% for these conditions, they noted.

To examine this more closely, Pfau and colleagues collected serum samples from local residents, comparing their autoantibody profiles with matched controls from Missoula, an area of Montana where no asbestos had been mined.

In a study published in *Environmental Health Perspectives,* they found that, among 50 serum samples from Libby residents, the frequency of ANAs was 28.6% higher than in the Missoula samples (P=0.006). They also reported that the mean fluorescence intensity of the ANAs was significantly higher (2.34 versus 1.76, P=0.02).

The researchers also found that 24% of the Libby samples contained antibodies to extractable nuclear proteins such as Sm, SS-a, and SS-b, which are commonly found in

patients with systemic lupus erythematosus. Only 4% of the Missoula samples contained these antibodies.

The odds ratios among Libby residents ages 65 and older who had worked at the mine were 2.14 (95% CI 0.90-5.10) for systemic autoimmunity and 3.23 (95% CI 1.31-7.96) for rheumatoid arthritis specifically.

Moreover, while the number of cases of lupus (30) was too small to calculate an odds ratio, the researchers estimated that individuals who had had multiple possible exposures, such as through their job, recreation, and using vermiculite in gardening, had a more than fourfold risk (OR 4.45, 95% CI 1.24-16).

Now, however, Pfau's group is focusing on a specific subgroup of patients in Libby who have developed an unusual very severe and progressive pulmonary illness. Many succumb to infections.

"These patients are in terrible pain and lose their lung function because their lungs aren't elastic enough for them to breathe in and out, as though there's a wrapping of scar tissue around their lungs," she said.

This appears similar to what they found following amphibole exposure in their earlier mouse studies: the production of autoantibodies was followed by an increase in scarring in the connective tissues surrounding the lungs.

"We are proposing that this is a novel autoimmune disease, and we're now working on the data that might actually show cause and effect, rather than just an association," she said.

An Enduring Legacy

In Schneider and McCumber's book, they reported, "W.R. Grace shipping papers and invoices collected by the EPA and the Justice Department show that *9,780,000,000 pounds* (italics mine) of vermiculite ore was mined from 1960 to 1990 and shipped to 563 different addresses in the United States and another 187 in six Canadian provinces."

It was used as insulation in millions of homes, and among the many other construction projects that utilized Zonolite for insulation was the World Trade Center. When the towers fell on 9/11, the airborne debris that covered lower Manhattan and blew across the region contained an unknown quantity of fibers that originated on that mountainside in Montana.

And Libby isn't the only source of potential amphibole contamination. Recently, another group of mineral experts reported high levels in samples of soil and airborne dust obtained in the vicinity of Las Vegas.

"That heightens the public health problem, or pending crisis if you will," Pfau said. "A lot of people think asbestos is an outdated occupational exposure that's not occurring anymore. But that's not true. There are a lot of exposures happening right now, and we want physicians to be aware and watching for this," she said.

- I SPOKE TO DR. HER RESEARCH IS SPECIFICALLY AUTO-IMMUNE RHEUMATOLOGICAL CONDITIONS WHICH DO NOT INCLUDE PBC - (AUTOIMMUNE ONLY)

Pfau and colleagues reported no financial conflicts.

8 Comments

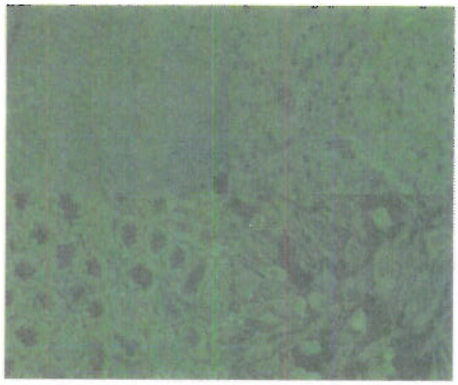
Primary source: Autoimmune Diseases
Source reference: Pfau J, et al "Autoimmunity and asbestos exposure" *Autoimmune Dis* 2014; DOI: 10.1155/2014/782045.

#1

Last edited 5 months ago by Virion123

Anti-mitochondrial antibody

Anti-mitochondrial antibodies (AMA) are autoantibodies, consisting of immunoglobulins formed against mitochondria,[1] primarily mitochondria in cells of the liver. The presence of AMAs in the blood or serum of a person is indicative of several autoimmune diseases such as primary biliary cirrhosis (PBC) (a scarring of liver tissue, confined primarily to the bile duct drainage system of the liver). It is present in about 95% of cases.[2]



Immunofluorescence staining pattern of AMA shown on stomach (top left), liver (top right), kidney (bottom left) and hep-20-10 cells (bottom right).

Primary biliary cirrhosis is seen primarily in middle-aged women, and in those afflicted with other autoimmune diseases. PBC is an autoimmune disorder, a condition in which the human body's immune defense system mistakenly attacks the body's own cells, or in this case parts of the cells.

Cause of AMAs is postulated that xenobiotic-induced and/or oxidative modification of mitochondrial autoantigens is a critical step leading to loss of tolerance. In acute liver failure AMA are found against all major liver antigens.[3]

- Pyruvate dehydrogenase, E2 subunits
- 2-Oxo-glutarate dehydrogenase

- Branched-chain 2-oxo-acid dehydrogenase

Anti-cardiolipin antibodies are another type of AMA, cardiolipin is found on the inner mitochondrial membrane.

Antigens

Correlation with non-mitochondrial antigens

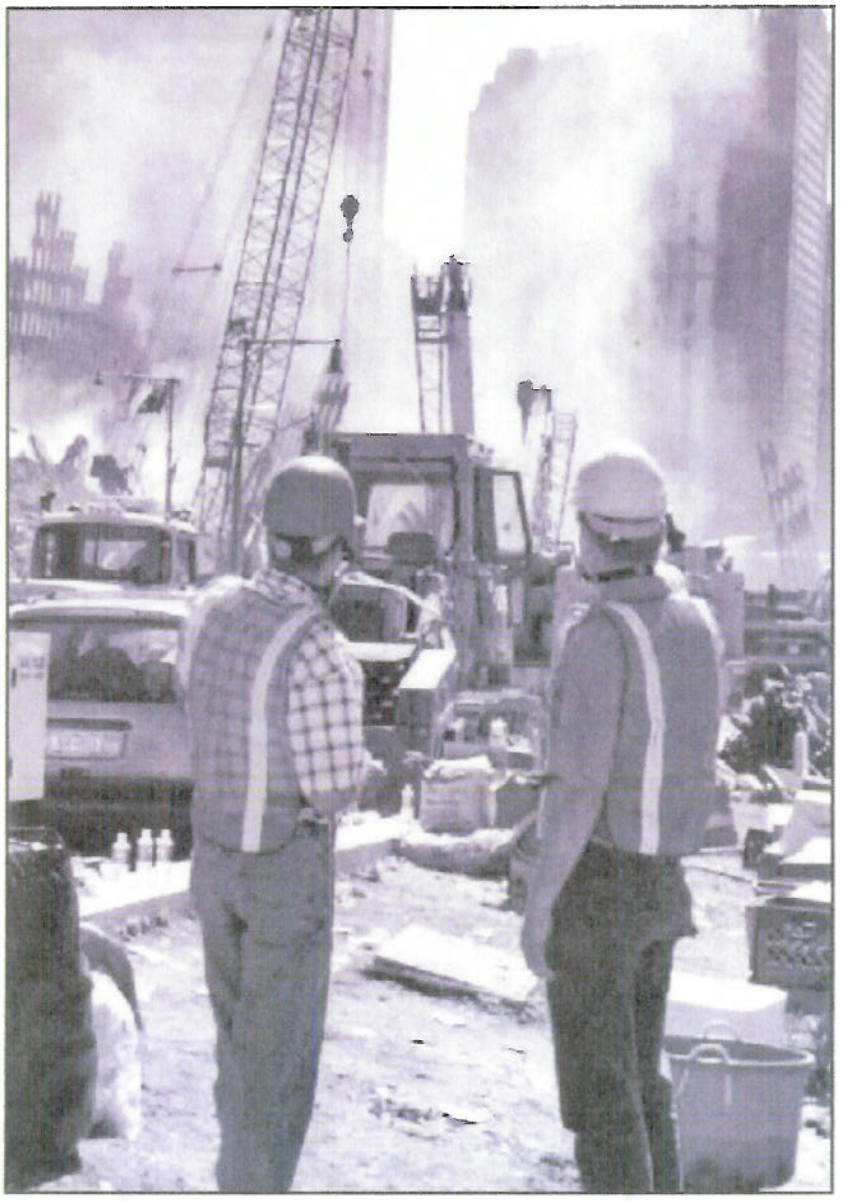
See also

References

Read in another language

September 11
Worker Protection Task Force
Interim Report

March 4, 2008



September 11th Worker Protection Task Force

Introduction

> Many public employees, including police, fire, correction, sanitation and civilians rendered rescue, recovery and cleanup at the former World Trade Center site and other designated location.... [T]he State must recognize the services that these individuals provided not only to the victims and their families, but to all citizens of the City and State of New York and the United States of America. As a result, it is only fitting that they be protected when a disability ensues as a consequence of their selfless acts of bravery working at the World Trade Center site and other sites.

Sponsors' Memorandum in Support of Legislation (A6281A, enacted as Laws of 2005, Chapter 104, amended by Laws of 2005, Chapter 93, hereinafter referred to as the "World Trade Center disability law").

Charter

The September 11th Worker Protection Task Force ("Task Force") was created by the September 11th Worker Protection Task Force Act, which was enacted as part of the World Trade Center disability law. Laws of 2005, Chapter 104, Part B, as amended, Laws of 2005, Chapter 93, section 14.

The World Trade Center disability law amended the New York State Retirement and Social Security Law and the New York City Administrative Code to provide that any injury or illness directly related to the terrorist attack on September 11, 2001, be presumptively eligible for an accidental disability. There are 19 members of the Task Force who are appointed as follows:

- Six members by the Governor
- Three members by the Temporary President of the Senate, two of whom shall be representatives from the organizations representing workers at the World Trade Center site and one of who shall be a representative of a recognized health organization with appropriate expertise;
- Three members by the Speaker of the Assembly, two of whom shall be representatives from the organizations representing workers at the World Trade Center site and one of who shall be a representative of a recognized health organization with appropriate expertise;

- The State comptroller or his or her representative;
- The Comptroller of the City of New York or his or her representative;
- The Mayor of the City of New York or his or her representative;
- The Commissioner of the State Department of Health or his or her representative;
- The Commissioner of the State Department of Labor or his or her representative;
- The Director of the State Division of the Budget or his or her representative; and
- The Commissioner of the State Department of Civil Service or his or her representative.

Task Force Members

The members of the Task Force are as follows:

- Dr. Thomas K. **Aldrich**, Pulmonary Medical Division, Montefiore Medical Center, **Chair**
- Lou **Matarazzo**, Executive Director, Detectives Endowment Association, **Vice Chair**

- Laura L. **Anglin**, Director, New York State Division of the Budget
- Michael **Bloomberg**, Mayor, New York City
- Stephen J. **Cassidy**, President, Uniformed Firefighters Association
- Dr. Richard F. **Daines**, Commissioner, New York State Department of Health
- Thomas **DiNapoli**, New York State Comptroller
- Nancy G. **Groenwegen**, Commissioner, New York State Department of Civil Service
- Dr. Stephen **Levin**, Mt. Sinai-Irving J. Selikoff Center for Occupational and Environmental Medicine
- Patrick J. **Lynch**, President, New York City PBA
- John J. **McDonnell**, President, New York City Uniformed Firefighters
- Peter D. **Meringolo**, Chairman, New York State Public Employees Conference
- Thomas G. **Osimitz**, Ph.D, Science Strategies LLC
- Dr. Jay **Poliner**, Poliner and Associates
- Dr. David **Prezant**, Chief Medical Officer, Office of Medical Affairs, New York City Fire Department
- Lillian **Roberts**, Executive Director, District Council 37, AFSCME, AFL-CIO

- David J. **Rosenzwieg**, President, Uniform Fire Dispatch Benevolent Association
- M. Patricia **Smith**, Commissioner, New York State Department of Labor
- William **Thompson**, New York City Comptroller

Individuals who regularly participated in the Task Force as representatives for certain members included:

- Pico **Ben-Amotz**, Esq. for M. Patricia Smith, Commissioner, New York State Department of Labor
- John **Burke** for Laura L. Anglin, Director, New York State Division of the Budget
- Lee **Clarke** for Lillian Roberts, Executive Director, District Council 37, AFSCME, AFL-CIO
- Robert **Coughlin**, Esq. for Thomas DiNapoli, New York State Comptroller
- Anthony **Crowell**, Esq. for Michael Bloomberg, Mayor, New York City
- Dr. Richard **Ciulla** for Nancy G. Groenwegen, Commissioner, New York State Department of Civil Service
- Lewis **Finkelman**, Esq. for William Thompson, New York City Comptroller
- Brian **Geller**, Esq. for Michael Bloomberg, Mayor, New York City
- Joey Kara **Koch**, Esq. for Michael Bloomberg, Mayor, New York City
- Dr. Matthew P. **Mauer** for Dr. Richard F. Daines, Commissioner, New York State Department of Health
- Christopher J. **McGrath**, Esq. for Patrick J. Lynch
- William **Romaka** for Stephen J. Cassidy, President, Uniformed Firefighters Association
- Richard **Simon**, Esq. for William Thompson, New York City Comptroller

Mission

The purpose of the World Trade Center disability law was to establish presumptive eligibility for accidental disability for the "public employees, including police, fire, correction, sanitation and civilians" who "rendered rescue, recovery and clean up at the former world trade center site and other designated locations" so that they can "be protected when a disability ensues." Sponsor's Memo in support of A6281A.

The Task Force was created in recognition of "health issues and concerns of the workers who participated in the rescue, recovery and clean up of the World Trade Center and related areas". September 11th Worker Protection Task Force Act at section 2 (Laws of 2005, Chapter 104, Part B, section 2)

The Task Force is required to submit annual reports on or before June 1 to the governor, the temporary president of the senate and the speaker of the assembly that address (a) the progress being made in fulfilling the duties of the Task Force and in developing recommendations; and (b) recommend strategies or actions for ongoing monitoring and treatment of individuals.

The Task Force has the following duties relating to worker who participated in the World Trade Center rescue, recovery and cleanup:

a) to obtain from the department of health and the New York city department of health, such departments' review of statistical and qualitative data on the prevalence and incidence of sickness, illness and disability of such workers;

(b) to obtain from other sources reviews of statistical and qualitative data on the prevalence and incidence of sickness, illness and disability of such workers;

(c) assess based upon evidence presented, the nature, scope and magnitude of the health impacts caused by exposure to air and elements;

(d) measure the adverse health effects of exposure on such workers;

(e) to consult with any organization, health institution, governmental agency or person including, but not limited to, the department of health, the department of environmental conservation, the federal environmental protection agency, the New York committee for occupational safety and health and the occupational safety and health administration;

(f) to identify and examine the limitations of any existing laws, regulations, programs, and services with regard to coverage, extent of disability,

process for determination, adequacy of coverage and treatment of specific types of disabilities and to undertake any recommendations;

(g) to receive and to consider reports and testimony from individuals, the health department, community-based organizations, voluntary health organizations, and other public and private organizations statewide to learn more about the diagnosis, care, and treatment of such workers at these designated sites; and

(h) to identify federal funding sources to assist state and local governments in paying costs associated with disability benefits under [the World Trade Center disability law].

The chair of the Task Force is empowered to establish committees for the purpose of making special studies pursuant to the above-referenced duties and may appoint non-Task Force members to serve on each committee as resource persons, who shall be voting members of the committees to which they are appointed.

Summary

The World Trade Center disability law presumes that individuals who meet certain qualifying criteria and were involved in September 11th related operations in the line of duty may have incurred injuries or developed diseases that disabled them. The Task Force reported in its first annual report, dated June 1, 2007, that it was reviewing and examining evidence about adverse health effects and the need to compensate responders properly, that due to certain qualifying criteria, the majority of these individuals are or were members of the uniformed services, that there are severe health impacts suffered by responders, that the pension and disability systems may not be configured properly to deal with these aftereffects, that there may be a need for legislative amendments and that the Task Force will issue findings on at least an annual basis.

The Task Force met nine times during the eight month period following its June 1, 2007 report to finalize an initial set of recommendations that could be reported prior to the second annual report, so that the legislature and governor could have the benefit of those recommendations earlier in the legislative session. Those discussions built on the information and testimony previously received by the Task Force, including materials provided by the New York City Employees' Retirement System on March 1, 2007, which are attached as **Appendix A**.

During that time the chair established a committee of doctors to further study and report on the health consequences of the collapse of the World Trade Center consisting of Task Force chair, Dr. Aldrich, and members, Drs. Prezant and Levin, as well as two psychiatric specialists who were not members of the Task Force, Drs. Katz and Sharma, each of whom are more fully identified in the

prevalent at the WTC site on the morning of 9/11 would be a competent cause of subsequent mental health disturbances.

4. The Impact of Geography on Mental Health Disease. As in the pre-9/11 disaster literature, a range of factors beyond duration of work at Ground Zero appear to create a composite risk for development of PTSD and other disorders in WTC responders. [48] The impact of the WTC terrorist attacks were felt throughout NYC and mental health was affected even in those who did not participate in the rescue and recovery effort. In a random survey of NYC residents, it was found that the rates of PTSD and depression were significantly higher among those who participated in rescue and recovery work at the WTC than among those who did not.[53] In a study of staff members at two Lower Manhattan high schools and two Lower Manhattan colleges, 24-33% of the cohort reported major depressive symptoms, and 15-25% demonstrated symptoms that were consistent with PTSD.[54] Overall, more research must be done in this area to address the full mental health effects impacting both WTC workers as well as other exposed groups.

E. Potentially Late Emerging Diseases

1. Epidemiology:

Because of the various carcinogenic and/or bioreactive compounds that were found either in the WTC dust, or as combustion products from WTC fires, there remains the potential for late emerging systemic diseases, such as cancers and autoimmune syndromes. Many combustion-derived products are known carcinogens, and some, including dioxins, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and various other carcinogenic volatile

3. Cardiac disease: Cardiac diseases are frequently split into 3 areas that sometimes may have overlap - ischemia or coronary artery disease, cardiomyopathy and arrhythmias. Surveys of emergency room presentation of patients with coronary syndromes and perhaps arrhythmias suggested small increases in the two months after 9/11;[56,57] whether those increases will persist or worsen in the years to come is unknown. It is thought that the same psychological effects that cause PTSD put people at risk of cardiac sequelae, particularly those with pre-existing coronary artery disease.[58]

4. **Rheumatologic, autoimmune, hepatic and renal diseases:** Other late emerging diseases include rheumatologic or autoimmune diseases, liver failure, and renal failure and cardiac disease. Anecdotal reports have repeatedly raised concerns about rheumatologic autoimmune diseases, and this is an area of intense scrutiny – not only sarcoidosis, but also polymyositis, lupus, etc. Initially, there was concern that potential toxic exposures would lead to liver and kidney problems. To date, we have not seen an increase in these problems, but we (2007) continue to monitor liver and kidney function via blood/urine tests during the WTC Medical Monitoring exams for both the FDNY and non-FDNY cohorts. WTC MONITORING EXAMS - ELEVATED LFT (LIVER FUNCTION TESTS)

5. **Developmental disorders:** Finally, one could consider the category of late emerging WTC-related diseases to include effects on children exposed indirectly while in-utero (children of workers and residents who were pregnant at the time of the collapse or during rescue/recovery work), directly as residents, or while attending schools in the area. To date, there are 2 studies on birth outcomes among term deliveries.[59,60] Term infants of women living within 2 miles of WTC during the month after 9/11 showed significant decrements in birth weight (149 gm) and

#3

USGS
science for a changing world

Environmental Health - Toxic Substances

BTEX

Definitions

BTEX - "BTEX is the term used for benzene, toluene, ethylbenzene, and xylene-volatile aromatic compounds typically found in petroleum product, such as gasoline and diesel fuel." - Environmental Protection Agency, 2010

BTEX (benzene, toluene, ethylbenzene, and xylene) - "Volatile, monocyclic aromatic compounds present in coal tar, petroleum products, and various organic chemical product formulations (Cohen and Mercer, 1993). These are the most soluble of the major gasoline compounds and, therefore, are common indicators of gasoline contamination." - Wilson and Moore, 1998

BTEX (benzene, toluene, ethylbenzene, and xylene) - "BTEX is a group of chemicals which is quantified by one analytical method...Toluene, ethylbenzene, and the three isomers of xylene are colourless liquids, immiscible with water but miscible with organic solvents. They have a characteristic strong odour and are highly flammable." - European Environment Agency, 2010

Related Definitions

Volatile Organic Compounds (VOCs)

USGS Toxic Substances Hydrology Program Research on BTEX and other VOCs

- Crude Oil Contamination in the Shallow Subsurface - Bemidji, Minnesota
- Processes Affecting the Natural Attenuation of Fuels in Ground Water
  - Fuel Oxygenates - Laurel Bay, South Carolina
  - Gasoline - Galloway Township, New Jersey
- Fate of Landfill Leachate - Norman Municipal Landfill, Norman, Oklahoma

USGS Toxic Substances Hydrology Program Remediation of BTEX and other VOC-Related Activities

- Biodegradation of Charcoal Production Wastes, Kingsford, Michigan
- Oil Recovery Performance Monitoring, Bemidji, MN
- Oxygen-Release Compound Remediation Tests, Laurel Bay, SC
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- Vapor Extraction Optimization, Galloway Township, NJ

USGS Information on VOCs

- NAWQA National Synthesis--Volatile Organic Compounds
- Search the Toxic Substances Hydrology Program's Bibliography for "BTEX"

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BTEX Toxicological Information from the Agency for Toxic Substances and Disease Registry

- Interaction Profile for Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX), Draft, For Public Comment
- ToxFAQs for Benzene
- Toxicological Profile for Benzene
- ToxFAQs for Ethylbenzene
- Toxicological Profile for Ethylbenzene
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Review Article

Silicon, a Possible Link between Environmental Exposure and Autoimmune Diseases: The Case of Rheumatoid Arthritis

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Abstract

Silicon is one of the most common chemicals on earth. Several compounds such as silica, asbestos, silicone or, nanoparticles are built from tetrahedral units with silicon as the central atom. Despite these, structural similarities, they have rarely been analyzed as a group. These compounds generate significant biological alterations that include immune hyperactivation, production of the reactive species of oxygen and tissue injury. These pathological processes may trigger autoimmune responses and lead to the development of rheumatoid arthritis. Populations at risk include those that constantly work in industrial process, mining, and agriculture as well as those that undergo silicone implants. Herein a review on the main features of these compounds and how they may induce autoimmune responses is presented.

1. Introduction

Rheumatoid arthritis (RA) is a common autoimmune disease (AD), characterized by synovial inflammation, autoantibody production, cartilage and bone destruction, and other systemic complications including cardiovascular, pulmonary, and psychological disability. The etiology is unknown although it involves a complex interplay among genetic and epigenetic factors as well as environmental exposure [1].

The influence of several environmental stressors has been broadly described in processes that may trigger autoimmune responses which lead to RA. Habitual smoking and certain previous infections (i.e., *Porphyromonas gingivalis*, *Epstein-Barr* virus, cytomegalovirus, *Proteus* sp., and *Escherichia coli)* are the most significant associations that have been found for this disease [1, 2].

In the case of a particular class of chemical compounds, long exposure to them has been related to RA, and, in spite of their similar biophysical and biochemical properties, they have rarely been analyzed as a group. This is the case with the silicon-derived compounds (silica, asbestos, silicone, and nanoparticles; for details see Table 1). All of these compounds are built from tetrahedral units with silicon as the central atom and are basically extended networks based on Si–O–Si bonds [3]. In human tissues, silicon is associated with glycosaminoglycans that covalently attach to core proteins to form proteoglycans, which are part of the connective tissue matrix [4].

Table 1: Characteristics and effects of silicon-derived compounds.

The goal of this paper is to discuss the chemical features of these kinds of compounds and to describe the biological and immunological alterations that are generated *in vivo*. These alterations may trigger autoimmune responses and lead to autoimmune diseases such as RA. At the same time, another goal is to indicate the different sources of exposure and the population at risk from this class of compound.

2. Asbestos

Asbestos are a series of silicate minerals that produce thin fibers when they are crushed; however, this feature covers a large number of distinct minerals. That is why there is an inappropriate and incomplete definition of "asbestos" which makes their classification difficult [5]. The negative

effects of asbestos on health were recognized in the early twentieth century. Miners and mining communities are the most vulnerable populations but are better prepared to limit their exposure to asbestos than homeowners who are unknowingly breathing asbestos. Today, it is difficult to associate documented exposure to current symptoms and demonstrate that the diseases in both residents and miners are caused by asbestos due to the large range of periods of exposure (from 15 to 40 years) [6].

Chemical and physical properties of asbestos are related to its carcinogenicity, fibrogenecity, and toxicity. Asbestos is mainly absorbed through air passageways, and the deposition of inhaled fibers in the lung is determined by their length, width, shape, density, and by the anatomy of the respiratory tract [7]. Approximately 20% of elongate minerals inhaled are retained in the respiratory tract, primarily in the tracheobronchial and alveolar areas [8]. The Libby community (Montana) is the community that best depicts the high prevalence of pulmonary disease resulting from occupational and environmental exposure to asbestos. This town has recently been the focus of national attention about the danger of particle silica and asbestos [8].

As it was mentioned before by Aust et al. [9], there are a number of factors thought to induce pathological responses to asbestos. These include levels and intensity of the dose, frequency of exposure, durability of the dust in the biological system, toxicity of a given dust, and individual susceptibility to these variables [9]. Although those pulmonary manifestations of asbestos exposure are well documented, the nonpulmonary outcomes are less understood. One of these manifestations is malign mesothelioma. The relationship of asbestos with immune diseases (specially the risk of developing systemic autoimmune diseases) has been assessed, but it is less conclusive than the relationship with other diseases, and more tests are necessary [10].

2.1. Biological Responses to Asbestos

The ability of asbestos to persist over time in the body (mainly in the respiratory tract) is a feature that makes these kinds of compounds a risk for miners and the population associated with them [7]. Asbestos exposure is associated with pulmonary interstitial fibrosis due to accumulation and deposition of inflammatory cells within the lung with subsequent destruction of the lung airspaces. Thus, chemotactic peptides, proinflammatory cytokines, and growth factors produced by lung fibroblast, lung epithelial, and alveolar macrophages are important mediators in the immunological responses against this exposure [11]. For instance, asbestos stimulates the transcription of interleukin-8 (IL-8), which is the major neutrophil chemoattractant in the lung, and the transcription of transforming growth factor-β-1 (TGFβ-1), an important mediator of hematopoietic differentiation, cellular chemoattraction, and stimulation of fibroblast and myofibroblast [11, 12]. Likewise, Uppal et al. [13] found that activated peripheral blood mononuclear cells (PBMCs) of RA patients showed higher levels of IL-8 [13]. In addition, polymorphism in *TGFB1* has been related to bone-erosive damage in these patients [14]. This may indicate the relationship between the outcomes of exposure to asbestos and RA. [13, 14] The results of Song et al. [15] in which TGFβ-1 promotes the differentiation of synovial fibroblast to myofibroblast seem to support the above. This is the first step in the process that ends in tissue fibrosis [15]. Pulmonary fibrosis and synovial fibrosis caused by TGFβ-1 may be a link between pulmonary manifestations and an influence on autoimmunity.

The experimental evidence suggests that exposure to asbestos plays a direct role in the activation of NALP3 inflammasome, the release of interleukin 1 beta (IL-1β), and inflammatory perpetuation [16]. The relationship between inflammasome activation and IL-1 production has been well documented, and in this context, it is worth noting that IL-1β is present in the synovial tissue of animal models and patients with RA, and its ectopic transfer results in a more aggressive disease [17].

In an *in vitro* model with the T-cell line MT-2, it was possible to determine that a lengthy exposure to asbestos is able to alter the expression of more than 139 genes including chemokine receptor 3 (*CXCR3*) and interferon gamma (*IFNG*), which demonstrates that asbestos influences the responses mediated by the Th1 cell population [18, 19]. Interestingly, asbestos exposure mediates the transcription of multiple inflammatory cytokines through the activation of the protein kinase C (PCK) pathway [20]. Even PCK −/− deficient mice exposed to asbestos present a reduction in the clinical manifestations produced by asbestos [20].

Furthermore, asbestos exposure impairs the cytotoxic activities of natural killer (NK) cells and alters the expression of NK-cell activating receptors. This is preceded by the dysfunctional activities of the extracellular-signal-regulated kinase (ERK) phosphorylation pathway [21, 22]. It should be noted that the impaired function of NK cells and the decrease in their activating receptors have also been observed in patients with RA [23].

Asbestos also is a potent stimulator of reactive oxygen species (ROS) production due to the chemical properties of its fibers (particles rich in iron), which can induce the formation of hydroxyl radicals (-OH), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and subsequent ROS release on the part of inflammatory cells (alveolar macrophages and neutrophils). The most important feature is that asbestos fibers cause mitochondrial dysfunction in alveolar epithelial cells (AECs) through iron-catalyzed ROS and final apoptosis of this cellular group [24].

Studies *in vivo* have shown that crocidolite (a particular kind of asbestos) induces a significant increase in mutation frequency, especially transversion of type G-T. This is very probably due to the formation of premutagenic DNA bases such as 8-hydroxydeoxyguanosine (8-OHdG), where free radicals play a significant role in chemical changes on nitrogenous bases [25]. In brief, different studies have provided evidence about the mutagenicity mediated by ROS, which is, in turn, produced by environmental exposure to asbestos and silica particles [26]. The fact that ROS production plays a vital role in the main immune process that leads to an inflammatory process in RA should also be highlighted [27].

2.2. Asbestos and RA

In 2006, Noonan and colleagues [28] published a nested case-control study in which 7,307 residents of Libby (Montana) participated. The results showed that this population presented a 65% increase in the risk of developing RA and a 54% increase in the risk of other systemic ADs. Moreover, the OR calculated for the association between asbestos exposure and RA was 3.23. Noteworthy, this population had been exposed to asbestos for over 70 years through mining [28]. In addition, Pfau et al. [29] found that the serum of individuals evaluated in this particular population showed a higher frequency of antinuclear autoantibodies (ANAs), extractable nuclear antigen autoantibodies (ENAs), and a higher serum IgA level compared to other populations with similar geographic and demographic characteristics such as Missoula in the state of Montana [29]. Olsson, in turn, showed that miners who were exposed to asbestos present a higher risk of developing RA [30].

However, despite the fact that Salazar et al. [31] found alterations in the titer of ANAs when Lewis rats were exposed to asbestos, they failed to show correlations with other indicators of RA induction in these mice such as onset, joint inflammation, or RA serum biomarkers (rheumatoid factor (RF) or anti-CCP autoantibodies) [31]. In another case, Pfau et al. [32], with C57BL/6 mice, was able to demonstrate that exposure to asbestos not only increased the levels of ANAs (mainly anti-dsDNA) but also caused glomerulonephritis to develop with a marked complex deposition in the kidneys [32]. Finally, antifibroblast autoantibodies (AFA) were detected in this strain of mice. AFA autoantibodies alter the fibroblast phenotype and stimulate it to differentiate toward myofibroblast and production of type I collagen [33].

The experimental evidence shows that, in spite of the strong and toxic effects on the immune system, the relationship between asbestos and autoimmunity remains unclear. Therefore, and because the epidemiology data suggest a possible association, further research on this issue is warranted.

3. Silica

Silica or silicon oxide is one silicon atom combined with two atoms of oxygen (SiO_2) naturally occurring as quartz or sand. There are multiple crystalline forms and one amorphous form of silica. The continuous inhalation of the crystalline forms of silica has been associated with the development of silicosis, a pulmonary disease characterized by lung pneumoconiosis, diffuse fibrosis, alveolar proteinosis, and loss of pulmonary function [34]. The risk of exposure to these compounds is very high. The majority of activities similar to mining, agriculture, and construction release silica dust, which becomes airborne and puts workers in a position in which they are dangerously exposed [35]. An interesting retroprospective study undergone in a cohort of Chinese workers heavily exposed to silica who were followed for 43 years reported that the main causes of death for 74,040 individuals were related to respiratory diseases, lung cancer, and cardiovascular diseases [36]. However, silica has also been associated with the risk of developing autoimmune diseases, and this is supported by epidemiologic and experimental data.

3.1. Biological Response to Silica

The bioassimilation of silica particles occurs when the particles are coated by phospholipids and surfactant proteins perhaps as a protective mechanism. The cell/particle contact between alveolar macrophages (AM) and silica is the first step in recognition and internalization of silica in the body. After this occurs, it is followed by a marked recruitment of neutrophils and other inflammatory cells through the production of chemokines such as monocyte chemoattractant protein-1 (MCP-1) [34]. The exposure to silica that enters through respiratory passageways causes serious and progressive pulmonary toxicity even after the exposure ceases. The biological effects of silica include direct ones on several pathways such as inflammatory responses, cell-to-cell signaling and interaction, cellular movement that finally leads to cancer and inflammatory and respiratory diseases [37]. Moreover, crystalline silica has been observed to induce more intense responses from gene expression and the cytokine and chemokine secretion than amorphous silica [38].

The toxicity and tissue damage generated by silica in the body involve the production of ROS and nitric oxide (NO). This effect is independent of the length of time the exposure lasts [39], and it is followed by the activation of caspase 3 and caspase 9 with subsequent apoptosis of AM [40]. The effects of ROS produced by silica extend to the ability to produce lipid peroxidation, disrupt lipid rafts, activate protein tyrosine kinase, and to the subsequent translocation of transcription factors such as the nuclear factor kappa B (NF-κB) or the nuclear factor of activated T cells (NAFT) to the nucleus. This leads to the production of several proinflammatory cytokines such as IL-1β, IL-8, tumor necrosis factor alpha (TNF-α), and interleukin-6 (IL-6) [41, 42].

Moreover, the clearance of silica particles by macrophages leads to NALP3 inflammasome activation, cytokine production, and immune cell recruitment to the affected tissue [16, 43].

3.2. Silica and RA

In 1953, Caplan [44] described the occurrence of multiple peripheral lung nodules in coal workers that had RA. This disease was termed Caplan's syndrome. Also called rheumatoid pneumoconiosis (RP), Caplan's syndrome is defined as the combination of multiple well-knit pulmonary nodules which are predominantly on the periphery of the lungs and which are produced by exposure of patients with RA to inorganic silica [44, 45]. The evidence showed that these conditions were highly prevalent in miners with RA. Nevertheless, miners with radiographic signs of pneumoconiosis but without the history and symptoms of RA presented positive levels of RF [46]. As a result, they may possibly develop RA later.

The association between silica and autoimmunity has been assessed (Table 2). Several studies demonstrate that silica exacerbates the development of ADs in genetically susceptible mice models. For instance, Lupus-prone mice exposed to silica showed an increase in ANAs, pulmonary fibrosis, glomerulonephritis accompanied by proteinuria, and circulation and deposition of immune complexes in the kidney [67]. The apoptosis of AM may be a trigger mechanism for autoantibody production and immune complex deposition in this class of mice [68, 69].

Table 2: Epidemiological evidence about the relationship between silicon-derived compounds and autoimmune diseases.

Furthermore, mice which have been exposed have higher levels of CD4+ T helper cells, B1 and B cells, and a decrease in regulatory T (Treg) cells followed by an alteration in immunoglobulin levels and an increase in the production of TNF-α [70]. Brown Norway rats that were injected with sodium silicate showed higher levels of ANA and ENAs [71]. Further, in a group of workers who had been exposed to silica, the authors observed that two molecules involved in self-tolerance the cytotoxict-lymphocyteantigen4 (CTLA-4) and programmed death-1 (PD-1) were significantly

reduced [72]. The expression of CTLA-4 in Treg cells of patients with RA is significantly reduced and correlates with the abnormal function of Treg cells in this disease [73].

PBMCs and serum from patients with silicosis without clinical symptoms of ADs present higher levels of soluble Fas (an alternative splice of CD95) than membrane Fas [74, 75]. Likewise, decoy receptor 3 (DcR3), which also inhibits interaction between membrane Fas and Fas ligand (FasL) thus affecting apoptosis activation, is overexpressed in these patients [76]. It has been observed that RA patients with active arthritis present higher levels of soluble Fas, and this correlates with markers of the disease activity [77]. The increase in synovial inflammatory cell infiltration in RA has also been associated with the elevated expression of DCR3 [78]. These results suggest that autoreactive cells may escape from apoptosis control for a long time thus leading to autoantibody production and triggering autoimmune responses.

Furthermore, patients with silicosis and other ADs show increased levels of autoantibodies against the death domain of Fas. It is possible that anti-Fas autoantibodies stimulate Fas-mediated apoptosis thus showing another face of Fas in diseases produced by silica exposure [79].

Both silica and asbestos can act like superantigens and stimulate polyclonal activation of T cells, which is a mechanism involved in pathogenesis of RA, SLE, and SSc. In the same context, patients with silicosis present significant levels of anti-topoisomerase I, anti-caspase-8, and anti-desmoglein autoantibodies [80–82]. Patients with silicosis also present a reduction in number and function of Treg cells which may be due to activated T cells substituting for them in response to silica exposure [83]. Added to that, patients with RA also present a marked decrease in number and function of this T cell subset [84]. This reduction is mediated partly by Fas which leads the Treg cells to an accelerated apoptosis [83]. Two related compounds (silica and asbestos) generate important molecular and immunological alterations that can function as enhancers of autoimmune responses in RA (Figure 1). Undoubtedly these silicates represent an environmental risk factor for the susceptible population.

Figure 1: Shared mechanism and biological consequences of exposure to silicon-derived compounds. Figures were downloaded from: (1) natural resources; (2) North east online; (3) Arizona Center for Aesthetic Plastic Surgery; (4) AZoM.com Pty Ltd; (5). Amethyst Galleries, Inc.

4. Silica Nanoparticles

Silica nanoparticles (NP) are nanosized structures of silicon dioxide (SiO_2) and are widely utilized in artificial bones, artificial teeth, interventional catheters, and drug delivery systems. Furthermore, they are used in industries (i.e., paint, catalyst, and textile design) [85]. The cytotoxicity assays showed that the size and porosity of some nanomaterials are an important variable in stimulating inflammatory responses and promote apoptosis [86]. For instance, it has been demonstrated that several classes of NP induce cytotoxic effects such as cell membrane damage, reduction of metabolic activity, generation and release of ROS, apoptosis, and cytokine production in murine macrophages [87, 88].

Strikingly, NPs are able to promote citrullination of proteins such as cytokeratins and plectins through the activation of peptidylarginine deiminase (PAD) [89]. The citrullination of proteins has been related to modifications of antigenicity and production of autoantibodies against these citrullinated proteins [90]. Anti-CCP autoantibodies present high sensitivity and specificity in the diagnosis of RA [91].

Just like silica and asbestos, NP induces the activation of the NRLP3 inflammasome with the release of IL-1β and perpetuation of the inflammatory responses as observed in RA [17, 92]. These results suggest a potential mechanism of immune system activation that could possibly lead to RA.

5. Silicone

Silicones are a family of silicon oxide polymers that vary in composition based on the length of the polymer and the organic group side chain. When the polymer is short, silicone is a low-viscosity fluid and when the polymer is long, the silicone is a viscous semisolid. The main use of silicone is in esthetic surgery for breast implants, which after decades of research is considered the ideal material for augmentation mammoplasty. For over 20 years, there have been multiple published reports associating silicone breast implants with autoimmune diseases (Table 2) such as RA, scleroderma, morphea, SLE and CREST syndrome [93].

The experimental approaches show that MRL mice −/− implanted with silicone showed increased levels of anti-dsDNA and a modest elevation of RF. Some cytokines such as IL-1 and IL-2 were also elevated [94]. In a murine model of Type II collagen-induced arthritis, the implantation of silicone did not exert any effect on the incidence or severity of the disease. Autoantibodies against silicone-bound proteins were present in the serum of these mice although their pathological significance is unknown. Nevertheless, the long-term implantation of diverse forms of silicone significantly increases the incidence of this animal model of arthritis [95]. Similarly, the genetic background is important in this susceptibility given that the injection of silicone in two different strains of mice—the New Zealand Black (NZB) and BALB/cAnPt (BALB/c)—results in the exacerbation of ADs in one while in the other it does not [96].

It should be noted epidemiological studies have not reported an association between autoimmune diseases, such as RA, and silicone implants. This is also true even with respect to serological markers (autoantibodies) of the disease [86, 97, 98].

6. Autoimmune/Inflammatory Syndrome Induced by Adjuvants

A recently denominated autoimmune/inflammatory syndrome induced by adjuvant (ASIA) was defined (for complete review see references [99, 100]). As it was described previously by Shoenfeld and Agmon-Levin [101] this syndrome includes four particular medical conditions, defined by hyperactive immune responses. The major diagnostic criteria are the clinical manifestations such as arthralgia and/or arthritis, neurological manifestations, unrefreshing sleep or sleep disturbances, chronic fatigue, cognitive impairment and memory loss, myalgia, muscle weakness, myositis pyrexia, and dry mouth after a systemic exposure to external stimuli, for example, infections, vaccines, silicone, and adjuvants. There are minor criteria in which specific HLA (HLA DQB1 and HLADRB1) are highlighted and AIDs such as multiple sclerosis (MS) and systemic sclerosis (SSc) are involved [101].

As an adjuvant, silicone is capable of inducing autoimmune-like conditions (e.g., the Gulf war syndrome (GWS), siliconosis, postvaccination phenomena, and the macrophagic myofasciitis syndrome (MMF)). This could be the case for symptoms such as arthralgia and myalgia that are more common in individuals exposed to silicone implants. Siliconosis is one of the most characteristic diseases because of its potential as an adjuvant in the immunization process [99].

Over the last year, a few case reports have related the association of breast implants with autoimmune or autoinflammatory diseases [64, 100, 102]. There are reports that patients with siliconosis began experiencing connective tissue disease (CTD) or immunological syndromes (similar to Sjögren's syndrome (SS), MS, SSc, RA, and others). There seems to be a relationship between siliconosis and CTD. Although siliconosis does not fulfill any diagnostic criteria for a defined CTD, it must be noted that silicosis or asbestosis like siliconosis shared strong immunological and adjuvant responses that could lead to ADs. However there are discrepancies on this issue as found in the meta-analysis study [66].

7. Conclusions and Remarks

Environmental factors belong to the large group of significant mediators in the mosaic of autoimmunity. The long exposures to these factors become a risk for specific populations. In this context, silica, asbestos, silicone, or nanoparticles not only generate various immunological alterations but are also extensively in contact with people (Table 1). They may be mediators together with the genetic background in the mechanism that leads to autoimmune diseases such as the case of RA. Furthermore, these compounds are derived from the same chemical group. All of them contain silicon, which is one of the most common elements on earth, and despite their similarities, it is very rare for them to be seen as group. The epidemiological evidence and experimental approach have revealed the role of these compounds in autoimmunity, especially in RA, and their potential in the activation of the cellular recruitment, Th1-Treg misbalance, inflammasome activation, cytokine production, or ROS release. All these responses have been related to autoimmune diseases for years.

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Air levels of carcinogenic polycyclic aromatic hydrocarbons after the World Trade Center disaster

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The catastrophic collapse of the World Trade Center (WTC) on September 11, 2001, created an immense dust cloud followed by fires that emitted soot into the air of New York City (NYC) well into December. The subsequent cleanup used diesel equipment that further polluted the air until the following June. The particulate air pollutants contained mutagenic and carcinogenic polycyclic aromatic hydrocarbons (PAHs). By using an assay developed for archived samples of fine particles, we measured nine PAHs in 243 samples collected at or near Ground Zero from September 23, 2001, to March 27, 2002. Based on temporal trends of individual PAH levels, we differentiated between fire and diesel sources and predicted PAH levels between 3 and 200 d after the disaster. Predicted PAH air concentrations on September 14, 2001, ranged from 1.3 to 15 ng/m^3; these values are among the highest reported from outdoor sources. We infer that these high initial air concentrations resulted from fires that rapidly diminished over 100 d. Diesel sources predominated for the next 100 d, during which time PAH levels declined slowly to background values. Because elevated PAH levels were transient, any elevation in cancer risk from PAH exposure should be very small among nonoccupationally exposed residents of NYC. However, the high initial levels of PAHs may be associated with reproductive effects observed in the offspring of women who were (or became) pregnant shortly after September 11, 2001. Because no PAH-specific air sampling was conducted, this work provides the only systematic measurements, to our knowledge, of ambient PAHs after the WTC disaster.

The catastrophic collapse of the World Trade Center (WTC) on September 11, 2001, released an estimated 1 million tons of dust and smoke into the air of New York City (NYC) (1). More than 90% of the airborne mass consisted of large particles (>10 μm in diameter), generated from pulverization of concrete, metals, silica, and organic materials in the structures (1, 2). Although these large alkaline particles were irritating to the upper airways (3), they settled quickly from the air and did not penetrate deeply into the lungs of city residents. Thus, the vast majority of the dust generated by the collapse of the WTC posed a relatively small health risk to the general public. However, fine particles [particulate matter (PM)$_{2.5}$, diameters <2.5 μm] were continually emitted from Ground Zero by fires, and thus became widely dispersed throughout greater NYC [based on the deposited dust (1), an estimated 11,000 tons of $PM_{2.5}$ were released]. Given their deep penetration into the lungs, particles in the $PM_{2.5}$ fraction are of potentially great concern to the public health.

In addition to constituents from the pulverized WTC structures, the $PM_{2.5}$ fraction contained soot particles emanating from fires that persisted from September 11 through December 20, 2001. Fires started with the ignition of ≈91,000 liters of jet fuel (from the two commercial aircraft that initiated the collapse of the WTC) and spread to an estimated 100,000 tons of organic debris, 490,000 liters of transformer oil, 380,000 liters of heating and diesel oil, and fuel from several thousand automobiles (stored in subterranean structures of the WTC) (4–6). Soot from the fires contained numerous carcinogens, notably polycyclic aromatic hydrocarbons (PAHs) that are ubiquitous products of incomplete combustion. Even after the fires were extinguished, PAHs were generated by diesel trucks, cranes, generators, and construction equipment that were used to clear 1,500 million kg of rubble from Ground Zero through May 2002 as described in contemporary major media reports (e.g., CNN, USA Today, etc.) and other official reports (4–6).

Since Pott (7) first linked squamous cell carcinomas with exposure to soot among British chimney sweeps in 1775, PAHs have frequently been associated with human cancers of the skin, lungs, and bladder (8). In fact, our modern understanding of the relationship between cancer and the environment is largely conditioned by investigations involving exposures to PAHs (9–11). Several individual PAHs, including benzo(*a*)pyrene, chrysene, indeno(*1,2,3-c,d*)pyrene, and benzo(*b*)fluoranthene have produced carcinogenic, mutagenic, and genotoxic effects in animal experiments (12–15). Air pollution, presumably enriched with PAHs, has been shown to induce heritable (paternal germ-line) mutations in mice (16). More recently, PAHs have been associated with elevated levels of DNA adducts and P53 mutations in humans (17–19). Airborne PAHs have also been implicated in human reproductive effects, notably, DNA adducts and *hypoxanthine-guanine phosphoribosyltransferase* mutations in newborns as well as preterm birth and intrauterine growth restriction (20–22).

Despite the potential importance of PAH exposures to human health and the generation of large quantities of soot, remarkably little is known about air levels of PAHs in NYC after September 11, 2001. Based on analysis of 13 portions of the settled dust, Offenberg *et al.* (23) estimated that the collapse generated between 100 and 1,000 tons of PAHs. Yet, because the settled dust contained relatively little mass from the $PM_{2.5}$ fraction, it is still an open question as to whether the public was exposed to high levels of airborne PAHs in the wake of September 11, 2001. In fact, only five relevant air measurements of PAHs have been reported in $PM_{2.5}$ samples (24) and these measurements were sufficiently large to motivate "... the most serious kind of concern ..." (25).

Materials and Methods

Air Sampling. In response to the WTC disaster, the U.S. Environmental Protection Agency rapidly began collecting air samples at or near Ground Zero to monitor a variety of pollutants. Four of the sites deployed monitors for fine particulate matter ($PM_{2.5}$) by using Teflon membrane filters through which air was drawn at 5–15 l/min. Three of the sites (designated A, C, and K) were at the fence line of Ground Zero, whereas the fourth site

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Abbreviations: WTC, World Trade Center; NYC, New York City; PAH, polycyclic aromatic hydrocarbon; PM, particulate matter.

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and diesel oil, and fuel from several thousand automobiles (stored in subterranean structures of the WTC) (4–6). Soot from the fires contained numerous carcinogens, notably polycyclic

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Fig. 1. Aerial photograph of lower Manhattan taken on September 23, 2001, from an altitude of 3,300 feet. Ground Zero (GZ) of the WTC and four sampling sites are labeled. Site A: Park Place and West Broadway. Site B: 290 East Broadway between Reade Street and Duane Street. Site C: Trinity Place and Cedar Street. Site K: West Street and Albany Street. Photo courtesy of the National Oceanographic and Atmospheric Administration (NOAA).

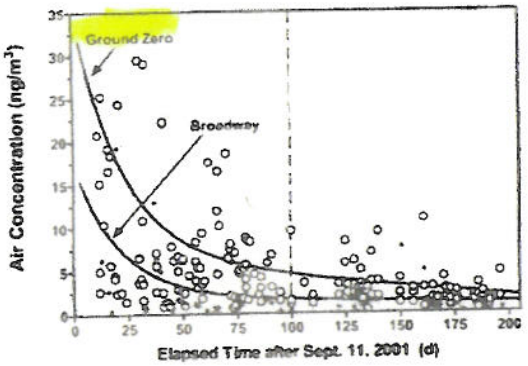

Fig. 2. Scatter plot of PAH levels (sum of nine analytes, ng/m³) versus time after September 11, 2001. Open symbols, mean values for sites A, K, and C at Ground Zero, filled symbols, site B at 290 Broadway; vertical dashed line, December 20, 2001. Curves are calculated for the summed data based on the regression model presented in Eqs. 1 and 2.

PAHs changed significantly during the period of observation. This finding is illustrated in Fig. 3 for the increasing percentage of benzo(*g,h,i*)perylene and the decreasing percentage of benzo(*b*)fluoranthene with respect to all measured PAHs. Such behavior suggests that the predominant source(s) of PAHs changed during the investigation, almost certainly from fires to

(B) was on the 16th floor of an office building at 290 Broadway, ~0.5 km from Ground Zero (see Fig. 1). Samples were collected daily at each site (≈24-h duration) between September 23, 2001, and March 27, 2002. After performing nondestructive assays for particle mass (gravimetric) and metal content (x-ray fluorescence), the U.S. Environmental Protection Agency archived the Teflon filters.

Analysis for PAHs. Because PAH-specific samplers were not deployed, we assayed some of the most hazardous four- to six-ring PAHs in 243 of the archived $PM_{2.5}$ filters described above, by using a method we developed for this purpose (26). Briefly, the Teflon filters were extracted with 10 ml of dichloromethane-containing deuterated PAHs as internal standards. After concentration of the extracts to 50 μl under N_2, 2-μl aliquots were analyzed by GC MS in selective ion-monitoring mode. Results are reported for nine carcinogenic and/or mutagenic PAHs, namely, benz(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*e*)pyrene, benzo(*a*)pyrene, indeno(*1,2,3-c,d*)pyrene, dibenz(*a,h*)anthracene, and benzo(*g,h,i*)perylene.

Results and Discussion

The overall distribution of airborne PAHs in the 243 air samples is illustrated in Fig. 2, which shows the sum of the nine analyte concentrations versus time after September 11, 2001. Despite considerable variability within and between sites, PAH levels were consistently greater at Ground Zero than at 290 Broadway and were much greater during the first 2 months after September 11, 2001, than thereafter. There is also evidence of declining trends in PAHs levels, particularly in the period before December 20, 2001 (day 100, vertical dashed line).

Relative amounts of individual PAHs differ among combustion sources and thereby serve as potential source signatures (27–29). We observed that the relative contributions of several

diesel exhaust. This conjecture is supported by Governor Pataki's declaration that all fires were extinguished by December 20, 2001, but WTC rubble continued to be removed by diesel equipment through May 2002 (from contemporary media reports, e.g., U.S. World News, CNN, or USA Today). This conjecture also consistent with the chronology of events offered by Landrigan *et al.* (30) who identified the primary sources of combustion products between September 14 and December 20, 2001, as "smoldering fires (with occasional flare-ups)" and diesel exhaust.

The trends in PAH levels, illustrated in Figs. 2 and 3, can be explained by three underlying processes. First, after the initial collapse, explosions, and fierce jet-fuel fires of the initial two days, PAHs were continually generated by smoldering fires as the

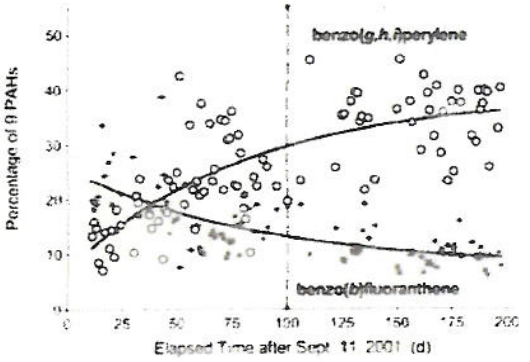

Fig. 3. Percentages of benzo(*g,h,i*)perylene (open symbols) and benzo(*b*)fluoranthene (filled symbols) to the total of nine PAHs (averaged over all sites) versus time after September 11, 2001. Vertical dashed line represents December 20, 2001, the date that all fires were declared extinguished. Curves represent least-squares fits of a single-compartment model to the data.

Table 1. Predicted air concentrations and 95% confidence intervals (in parentheses) of nine PAHs (ng/m³) measured at Ground Zero and 290 Broadway on t_j = 3, 100 and 200 d after September 11, 2001

Compound	Ground Zero (n = 170)			290 Broadway (n = 73)			Los Angeles (1998–2002)	Teplice* (Winter 1993–1994)
	t_j = 3	t_j = 100	t_j = 200	t_j = 3	t_j = 100	t_j = 200		
Benz(*a*)anthracene	1.3 (0.10, 2.8)	0.17 (0.14, 0.20)	0.16 (0.13, 0.19)	0.40 (0.00, 0.92)	0.03 (0.02, 0.04)	0.02 (0.01, 0.03)	—	5.8 (5.1, 6.5)
Chrysene	1.5 (0.24, 2.4)	0.16 (0.14, 0.19)	0.15 (0.12, 0.18)	0.37 (0.00, 0.79)	0.04 (0.03, 0.05)	0.03 (0.01, 0.04)	—	7.5† (6.6, 8.4)
Benzo(*b*)fluoranthene	15 (0.82, 25)	0.53 (0.43, 0.63)	0.25 (0.14, 0.35)	5.6 (0.00, 13)	0.36 (0.25, 0.46)	0.07 (0.02, 0.12)	0.17 (0.14, 0.19)	6.2 (5.4, 6.9)
Benzo(*k*)fluoranthene	4.8 (0.72, 9.0)	0.31 (0.26, 0.36)	0.23 (0.17, 0.29)	1.7 (0.00, 3.6)	0.12 (0.09, 0.16)	0.05 (0.02, 0.07)	0.07 (0.05, 0.08)	4.0‡ (3.5, 4.5)
Benzo(*e*)pyrene	7.0 (1.6, 12)	0.42 (0.35, 0.49)	0.30 (0.22, 0.38)	4.1 (0.00, 8.2)	0.21 (0.15, 0.27)	0.09 (0.05, 0.13)	—	4.7 (4.2, 5.3)
Benzo(*a*)pyrene	2.2 (0.07, 4.4)	0.44 (0.36, 0.52)	0.34 (0.25, 0.43)	1.6 (0.00, 2.4)	0.17 (0.12, 0.22)	0.07 (0.03, 0.11)	0.11 (0.04, 0.18)	6.0 (5.3, 6.8)
Indeno(1,2,3-*c,d*)pyrene	4.0 (0.06, 7.9)	0.57 (0.47, 0.68)	0.31 (0.19, 0.43)	1.5 (0.00, 3.2)	0.39 (0.28, 0.50)	0.13 (0.06, 0.21)	0.23 (0.19, 0.27)	6.0 (5.3, 6.7)
Dibenz(*a,h*)anthracene	1.3 (0.00, 2.9)	0.10 (0.08, 0.12)	0.03 (0.01, 0.06)	0.69 (0.00, 1.7)	0.08 (0.05, 0.11)	0.01 (0.00, 0.03)	0.03 (0.02, 0.03)	2.1 (1.9, 2.4)
Benzo(*g,h,i*)perylene	4.1 (0.00, 8.3)	1.1 (0.93, 1.3)	0.85 (0.60, 1.1)	1.3 (0.00, 3.1)	0.68 (0.49, 0.87)	0.40 (0.23, 0.58)	0.50 (0.42, 0.57)	5.3 (4.7, 6.0)

Air concentrations in Los Angeles, CA (California Air Resources Board data), and Teplice, Czech Republic (21, 22), are shown for comparison.
*Confidence intervals estimated by imputing variances from Dejmek *et al.* (22).
†Chrysene/triphenylene.
‡Benzo(*k* + *j*)fluoranthene.

available fuel was consumed; this finding suggests a first-order process leading to an exponential decline in PAH emissions over time. Second, PAHs were generated from diesel-fueled activity related to removal of debris from Ground Zero; this result would lead to a quasi-linear decline in PAH emissions as diesel sources diminished from mid-September 2001 to May 28, 2002. Third, PAHs were continuously generated by background sources, notably vehicular engine exhausts in NYC; this result would lead to a near-constant rate of PAH emission throughout the period of observation. With these processes in mind, we constructed the following statistical model to characterize PAH levels in our samples:

$$C_{j,s} = \beta_{0,s} e^{-\alpha_s t_j} + \beta_{1,s}\left(1 - \frac{t_j}{T}\right) + \beta_{2,s} + \varepsilon_{j,s} \text{ for } 0 < t_j \le t_{0,s},$$

differences were observed in estimates of the time constant α_s (for the fire-derived PAHs) at the different sites, all final models assumed a common time constant $\alpha_{1,\ldots,9} = 0.0483\ d^{-1}$ obtained as the estimated mean value for the nine PAHs (SE = 0.0092 d^{-1}); this result corresponds to a half-life of 14.4 d. Given this decay rate, fires contributed insignificant quantities of PAH to the air of NYC after 100 d had elapsed from September 11, 2001. Likewise, no significant differences were detected in the linear (diesel-related) slopes $\beta_{1,s}$ across sites for a given PAH and common values were used for each analyte in the final model.

The estimated model parameters and SE were used to predict air levels and confidence intervals for each PAH at t_j = 3 (September 14, 2001), t_j = 100 (December 28, 2001, fires officially extinguished), and t_j = 200, (March 29, 2002, final day of the study). Table 1 summarizes these predictions for the nine PAHs and presents comparison values from downtown Los

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and

$$C_{j,s} = \beta_{1,s}\left(1 - \frac{t_j}{T}\right) + \beta_{2,s} + \varepsilon_{j,s} \text{ for } t_{0,s} < t_j \le T. \quad [2]$$

where $C_{j,s}$ represents the level of a given PAH on the jth day (time t_j after September 11, 2001) at site s. The first three terms in Eq. 1 refer, respectively, to the PAH contributions from fires, WTC-derived diesel exhausts (where T = 289 d represents May 28, 2002), and background city contributions. The term $\varepsilon_{j,s}$ represents all random errors arising from sources, changes in wind speed and direction, and assays, whereas $t_{0,s}$ is the time at which the fire contribution became negligible for a given site. (The derivation of this model and detailed results are given in *Supporting Text*, which is published as supporting information on the PNAS web site). Although our model includes t_j < 12 d (our first observation), we recognize that the nature of the fire-related source changed from combustion of jet fuel during the first two days to smoldering fires thereafter. Thus, in what follows, we restrict our predictions of PAH levels to begin at day 3 when smoldering fires were the primary combustion sources (30).

The above model was applied to each of the nine PAHs by means of nonlinear segmented regression. After preliminary analyses indicated that there were no statistical differences in PAH levels at the three Ground Zero sites (A, C, and K), data from these sites were combined and models were rerun. Observed and predicted levels of the individual PAHs in our samples were similar in appearance to the plots representing the sum of these nine analytes shown in Fig. 2. Because no significant differences were observed in estimates of the time constant α_s (for the fire-derived PAHs) at the different sites, all final models assumed a common time constant $\alpha_{j,s} = 0.0483\ d^{-1}$ obtained as the estimated mean value for the nine PAHs (SE = 0.0092 d^{-1}); this result corresponds to a half-life of 14.4 d. Given this decay rate, fires contributed insignificant quantities of PAH to the air of NYC after 100 d had elapsed from September 11, 2001. Likewise, no significant differences were detected in the linear (diesel-related) slopes $\beta_{1,s}$ across sites for a given PAH and common values were used for each analyte in the final model.

The estimated model parameters and SE were used to predict air levels and confidence intervals for each PAH at t_j = 3 (September 14, 2001), t_j = 100 (December 28, 2001, fires officially extinguished), and t_j = 200, (March 29, 2002, final day of the study). Table 1 summarizes these predictions for the nine PAHs, and presents comparison values from downtown Los Angeles (mean values for 1998–2002) (furnished by the California Air Resources Board, Sacramento, CA) and from Teplice, in the coal-burning region of the Czech Republic (winter 1993–1994; ref. 31). Our results indicate that, in the immediate aftermath of the WTC disaster (t_j = 3), PAH concentrations at Ground Zero were 10- to 214-fold greater (median = 65-fold) than the background values observed when t = 200 d at 290 Broadway. Indeed, predicted PAH concentrations at t_j = 3 in lower Manhattan were in the range of those observed in Teplice in winter, when some of the highest outdoor PAH concentrations in the world have been reported. However, air levels rapidly declined with the dissipation of fires during the first 100 days and slowly declined thereafter as diesel equipment was phased out, approaching Los Angeles mean values after 200 days. Nonetheless, even then (t_j = 200 d) the contributions of diesel sources close to Ground Zero produced PAH levels that were 2- to 8-fold greater (median = 4-fold) than those at 290 Broadway.

Conclusion

Our retrospective analysis of PAHs in archived $PM_{2.5}$ filters provides an important insight into the environmental implications of the WTC disaster. Without this opportune investigation, inferences about air levels of PAHs would have been restricted to 13 settled dust samples (23) and 5 $PM_{2.5}$ samples (24), which were insufficient for modeling PAH sources and trends.

Given the rapid decline of PAH levels in the aftermath of September 11, 2001, we consider it unlikely that the long-term risks of cancer arising from PAHs would have been significantly elevated above those from background PAH exposures in NYC over 70 years among city residents. Indeed, employing standard methods, we estimate only a 10^{-8} increase in lifetime cancer risk

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at Ground Zero from WTC-related PAHs in our samples (details are given in *Supporting Text*, specifically in *Risk Estimates* published as supporting information on the PNAS web site). We temper this observation with knowledge that workers engaged in the cleanup efforts could have been exposed to much higher levels of PAHs than those in our samples and, thus, could bear higher cancer risks. Also, our conclusion regarding cancer risks from PAHs cannot be extended to other carcinogenic substances that were also released to the air after the WTC disaster.

However, because PAH levels were very high during several weeks after the WTC disaster, the potential for adverse reproductive effects cannot be ruled out among the offspring of women who were (or became) pregnant during that period. In fact, a recent report that levels of aromatic DNA adducts were greater in the blood of newborns than of their PAH-exposed mothers in Krakow, Poland (20) suggests that the fetus may be at particular risk to PAH damage. Certainly PAH exposures in early gestation (especially the first month of pregnancy) have been implicated as a source for reproductive effects in the Czech Republic (21, 22), and preliminary results point to a 2-fold elevation of intrauterine growth restriction among the offspring of women residing close to the WTC during those fateful weeks after September 11, 2001 (32). By carefully mapping PAH levels in space and time after the WTC disaster, it may be possible to determine the nature and extent of links between PAH exposure and developmental effects in the local population.

Finally, we caution that the transient nature of exposures to high levels of PAHs from the WTC disaster is only indicative of outdoor air, where massive dilution rapidly reduced the air concentrations. However, dust and soot from the WTC disaster also deeply penetrated residential and commercial buildings, leaving thick layers of residue (33–35). Human indoor activities and contaminated central ventilation, heating, and cooling systems can continually resuspend settled dusts into the air; therefore, indoor air may represent a continuing source of exposure to PAHs (and other particle-related pollutants) that should be considered.

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ORIGINAL

Air levels of carcinogenic polycyclic aromatic hydrocarbons after the World Trade Center disaster

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The catastrophic collapse of the World Trade Center (WTC) on September 11, 2001, created an immense dust cloud followed by fires that emitted soot into the air of New York City (NYC) well into December. The subsequent cleanup used diesel equipment that further polluted the air until the following June. The particulate air pollutants contained mutagenic and carcinogenic polycyclic aromatic hydrocarbons (PAHs). By using an assay developed for archived samples of fine particles, we measured nine PAHs in 243 samples collected at or near Ground Zero from September 23, 2001, to March 27, 2002. Based on temporal trends of individual PAH levels, we differentiated between fire and diesel sources and predicted PAH levels between 3 and 200 d after the disaster. Predicted PAH air concentrations on September 14, 2001, ranged from 1.3 to 15 ng/m³; these values are among the highest reported from outdoor sources. We infer that these high initial air concentrations resulted from fires that rapidly diminished over 100 d. Diesel sources predominated for the next 100 d, during which time PAH levels declined slowly to background values. Because elevated PAH levels were transient, any elevation in cancer risk from PAH exposure should be very small among nonoccupationally exposed residents of NYC. However, the high initial levels of PAHs may be associated with reproductive effects observed in the offspring of women who were (or became) pregnant shortly after September 11, 2001. Because no PAH-specific air sampling was conducted, this work provides the only systematic measurements, to our knowledge, of ambient PAHs after the WTC disaster.

The catastrophic collapse of the World Trade Center (WTC) on September 11, 2001, released an estimated 1 million tons of dust and smoke into the air of New York City (NYC) (1). More than 90% of the airborne mass consisted of large particles (>10 μm in diameter), generated from pulverization of concrete, metals, silica, and organic materials in the structures (1, 2). Although these large alkaline particles were irritating to the upper airways (3), they settled quickly from the air and did not penetrate deeply into the lungs of city residents. Thus, the vast majority of the dust generated by the collapse of the WTC posed a relatively small health risk to the general public. However, fine particles [particulate matter $(PM)_{2.5}$, diameters <2.5 μm] were continually emitted from Ground Zero by fires, and thus became widely dispersed throughout greater NYC [based on the deposited dust (1), an estimated 11,000 tons of $PM_{2.5}$ were released]. Given their deep penetration into the lungs, particles in the $PM_{2.5}$ fraction are of potentially great concern to the public health.

In addition to constituents from the pulverized WTC structures, the $PM_{2.5}$ fraction contained soot particles emanating from fires that persisted from September 11 through December 20, 2001. Fires started with the ignition of ~91,000 liters of jet fuel (from the two commercial aircraft that initiated the collapse of the WTC) and spread to an estimated 100,000 tons of organic debris, 490,000 liters of transformer oil, 380,000 liters of heating and diesel oil, and fuel from several thousand automobiles (stored in subterranean structures of the WTC) (4–6). Soot from the fires contained numerous carcinogens, notably polycyclic aromatic hydrocarbons (PAHs) that are ubiquitous products of incomplete combustion. Even after the fires were extinguished, PAHs were generated by diesel trucks, cranes, generators, and construction equipment that were used to clear 1,500 million kg of rubble from Ground Zero through May 2002 as described in contemporary major media reports (e.g., CNN, USA Today, etc.) and other official reports (4–6).

Since Pott (7) first linked squamous cell carcinomas with exposure to soot among British chimney sweeps in 1775, PAHs have frequently been associated with human cancers of the skin, lungs, and bladder (8). In fact, our modern understanding of the relationship between cancer and the environment is largely conditioned by investigations involving exposures to PAHs (9–11). Several individual PAHs, including benzo(*a*)pyrene, chrysene, indeno(*1,2,3-c,d*)pyrene, and benzo(*b*)fluoranthene have produced carcinogenic, mutagenic, and genotoxic effects in animal experiments (12–15). Air pollution, presumably enriched with PAHs, has been shown to induce heritable (paternal germ-line) mutations in mice (16). More recently, PAHs have been associated with elevated levels of DNA adducts and P53 mutations in humans (17–19). Airborne PAHs have also been implicated in human reproductive effects, notably, DNA adducts and *hypoxanthine-guanine phosphoribosyltransferase* mutations in newborns as well as preterm birth and intrauterine growth restriction (20–22).

Despite the potential importance of PAH exposures to human health and the generation of large quantities of soot, remarkably little is known about air levels of PAHs in NYC after September 11, 2001. Based on analysis of 13 portions of the settled dust, Offenberg *et al.* (23) estimated that the collapse generated between 100 and 1,000 tons of PAHs. Yet, because the settled dust contained relatively little mass from the $PM_{2.5}$ fraction, it is still an open question as to whether the public was exposed to high levels of airborne PAHs in the wake of September 11, 2001. In fact, only five relevant air measurements of PAHs have been reported in $PM_{2.5}$ samples (24) and these measurements were sufficiently large to motivate "… the most serious kind of concern … " (25).

Materials and Methods

Air Sampling. In response to the WTC disaster, the U.S. Environmental Protection Agency rapidly began collecting air samples at or near Ground Zero to monitor a variety of pollutants. Four of the sites deployed monitors for fine particulate matter $(PM_{2.5})$ by using Teflon membrane filters through which air was drawn at 5–15 l/min. Three of the sites (designated A, C, and K) were at the fence line of Ground Zero, whereas the fourth site

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Abbreviations: WTC, World Trade Center; NYC, New York City; PAH, polycyclic aromatic hydrocarbon; PM, particulate matter.

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ENVIRONMENTAL SCIENCES

Fig. 1. Aerial photograph of lower Manhattan taken on September 23, 2001, from an altitude of 3,300 feet. Ground Zero (GZ) of the WTC and four sampling sites are labeled. Site A: Park Place and West Broadway. Site B: 290 East Broadway between Reade Street and Duane Street. Site C: Trinity Place and Cedar Street. Site K: West Street and Albany Street. Photo courtesy of the National Oceanographic and Atmospheric Administration (NOAA).

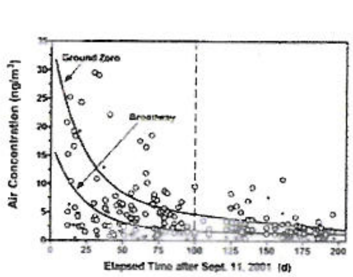

Fig. 2. Scatter plot of PAH levels (sum of nine analytes, ng/m³) versus time after September 11, 2001. Open symbols, mean values for sites A, K, and C at Ground Zero; filled symbols, site B at 290 Broadway; vertical dashed line, December 20, 2001. Curves are calculated for the summed data based on the regression model presented in Eqs. 1 and 2.

PAHs changed significantly during the period of observation. This finding is illustrated in Fig. 3 for the increasing percentage of benzo(*g,h,i*)perylene and the decreasing percentage of benzo(*b*)fluoranthene with respect to all measured PAHs. Such behavior suggests that the predominant source(s) of PAHs changed during the investigation, almost certainly from fires to

(B) was on the 16th floor of an office building at 290 Broadway, ~0.5 km from Ground Zero (see Fig. 1). Samples were collected daily at each site (~24-h duration) between September 23, 2001, and March 27, 2002. After performing nondestructive assays for particle mass (gravimetric) and metal content (x-ray fluorescence), the U.S. Environmental Protection Agency archived the Teflon filters.

Analysis for PAHs. Because PAH-specific samplers were not deployed, we assayed some of the most hazardous four- to six-ring PAHs in 243 of the archived $PM_{2.5}$ filters described above, by using a method we developed for this purpose (26). Briefly, the Teflon filters were extracted with 10 ml of dichloromethane-containing deuterated PAHs as internal standards. After concentration of the extracts to 50 µl under N_2, 2-µl aliquots were analyzed by GC MS in selective ion-monitoring mode. Results are reported for nine carcinogenic and/or mutagenic PAHs, namely, benz(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*e*)pyrene, benzo(*a*)pyrene, indeno(*1,2,3-c,d*)pyrene, dibenz(*a,h*)anthracene, and benzo-(*g,h,i*)perylene.

Results and Discussion

The overall distribution of airborne PAHs in the 243 air samples is illustrated in Fig. 2, which shows the sum of the nine analyte concentrations versus time after September 11, 2001. Despite considerable variability within and between sites, PAH levels were consistently greater at Ground Zero than at 290 Broadway and were much greater during the first 2 months after September 11, 2001, than thereafter. There is also evidence of declining trends in PAHs levels, particularly in the period before December 20, 2001 (day 100, vertical dashed line).

Relative amounts of individual PAHs differ among combustion sources and thereby serve as potential source signatures (27–29). We observed that the relative contributions of several diesel exhaust. This conjecture is supported by Governor Pataki's declaration that all fires were extinguished by December 20, 2001, but WTC rubble continued to be removed by diesel equipment through May 2002 (from contemporary media reports, e.g., U.S. World News, CNN, or USA Today). This conjecture also consistent with the chronology of events offered by Landrigan *et al.* (30) who identified the primary sources of combustion products between September 14 and December 20, 2001, as "smoldering fires (with occasional flare-ups)" and diesel exhaust.

The trends in PAH levels, illustrated in Figs. 2 and 3, can be explained by three underlying processes. First, after the initial collapse, explosions, and fierce jet-fuel fires of the initial two days, PAHs were continually generated by smoldering fires as the available fuel was consumed; this finding suggests a first-order process leading to an exponential decline in PAH emissions over time. Second, PAHs were generated from diesel-fueled activity related to removal of debris from Ground Zero; this result would lead to a quasi-linear decline in PAH emissions as diesel sources diminished from mid-September 2001 to May 28, 2002. Third, PAHs were continuously generated by background sources, notably vehicular engine exhausts in NYC; this result would lead to a near-constant rate of PAH emission throughout the period of observation. With these processes in mind, we constructed the following statistical model to characterize PAH levels in our samples:

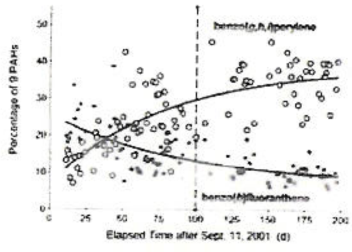

Fig. 3. Percentages of benzo(g,h,i)perylene (open symbols) and benzo(b)-fluoranthene (filled symbols) to the total of nine PAHs (averaged over all sites) versus time after September 11, 2001. Vertical dashed line represents December 20, 2001, the date that all fires were declared extinguished. Curves represent least-squares fits of a single compartment model to the data.

Table 1. Predicted air concentrations and 95% confidence intervals (in parentheses) of nine PAHs (ng/m³) measured at Ground Zero and 290 Broadway on t_j = 3, 100 and 200 d after September 11, 2001

Compound	Ground Zero (n = 170) t_j = 3	Ground Zero t_j = 100	Ground Zero t_j = 200	290 Broadway (n = 73) t_j = 3	290 Broadway t_j = 100	290 Broadway t_j = 200	Los Angeles (1998–2002)	Teplice* (Winter 1993–1994)
Benz(a)anthracene	1.3 (0.18, 2.4)	0.17 (0.14, 0.20)	0.16 (0.13, 0.19)	0.40 (0.00, 0.92)	0.03 (0.02, 0.04)	0.02 (0.01, 0.03)	—	5.8 (5.1, 6.5)
Chrysene	1.5 (0.24, 2.4)	0.16 (0.14, 0.19)	0.15 (0.12, 0.18)	0.37 (0.00, 0.79)	0.04 (0.03, 0.05)	0.03 (0.01, 0.04)	—	7.5† (6.6, 8.4)
Benzo(b)fluoranthene	15 (0.82, 29)	0.53 (0.43, 0.63)	0.25 (0.14, 0.35)	5.6 (0.00, 13)	0.36 (0.25, 0.46)	0.07 (0.02, 0.12)	0.17 (0.14, 0.19)	6.2 (5.4, 6.9)
Benzo(k)fluoranthene	4.8 (0.72, 9.0)	0.31 (0.26, 0.36)	0.23 (0.17, 0.29)	1.7 (0.00, 3.6)	0.12 (0.09, 0.16)	0.05 (0.02, 0.07)	0.07 (0.05, 0.08)	4.0‡ (3.5, 4.5)
Benzo(e)pyrene	7.0 (1.6, 12)	0.42 (0.35, 0.49)	0.30 (0.22, 0.38)	4.1 (0.00, 8.2)	0.21 (0.15, 0.27)	0.09 (0.05, 0.13)	—	4.7 (4.2, 5.3)
Benzo(a)pyrene	2.2 (0.07, 4.4)	0.44 (0.36, 0.52)	0.34 (0.25, 0.43)	1.0 (0.00, 2.4)	0.17 (0.12, 0.23)	0.07 (0.03, 0.11)	0.11 (0.04, 0.18)	6.0 (5.3, 6.8)
Indeno(1,2,3-c,d)pyrene	4.0 (0.06, 7.9)	0.57 (0.47, 0.68)	0.31 (0.19, 0.43)	1.5 (0.00, 3.2)	0.39 (0.28, 0.50)	0.13 (0.06, 0.21)	0.23 (0.19, 0.27)	6.0 (5.3, 6.7)
Dibenz(a,h)anthracene	1.3 (0.00, 2.9)	0.10 (0.08, 0.12)	0.03 (0.01, 0.06)	0.69 (0.00, 1.7)	0.08 (0.06, 0.11)	0.01 (0.00, 0.03)	0.03 (0.02, 0.03)	2.1 (1.9, 2.4)
Benzo(g,h,i)perylene	4.1 (0.00, 8.8)	1.1 (0.93, 1.3)	0.85 (0.60, 1.1)	1.3 (0.00, 3.1)	0.68 (0.49, 0.87)	0.40 (0.23, 0.58)	0.50 (0.42, 0.57)	5.3 (4.7, 6.0)

Air concentrations in Los Angeles, CA (California Air Resources Board data), and Teplice, Czech Republic (21, 22), are shown for comparison.
*Confidence intervals estimated by imputing variances from Dejmek *et al.* (22).
†Chrysene/triphenylene.
‡Benzo(*b*–*j*)fluoranthene.

$$C_{j,s} = \beta_{0,s}e^{-\alpha_s t_j} + \beta_{1,s}\left(1-\frac{t_j}{T}\right) + \beta_{2,s} + \varepsilon_{j,s} \text{ for } 0 < t_j \le t_{0,s}, \qquad [1]$$

and

$$C_{j,s} = \beta_{1,s}\left(1-\frac{t_j}{T}\right) + \beta_{2,s} + \varepsilon_{j,s} \text{ for } t_{0,s} < t_j \le T, \qquad [2]$$

where $C_{j,s}$ represents the level of a given PAH on the *j*th day (time t_j after September 11, 2001) at site *s*. The first three terms in Eq. 1 refer, respectively, to the PAH contributions from fires, WTC-derived diesel exhausts (where T = 289 d represents May 28, 2002), and background city contributions. The term $\varepsilon_{j,s}$ represents all random errors arising from sources, changes in wind speed and direction, and assays, whereas $t_{0,j,s}$ is the time at which the fire contribution became negligible for a given site. (The derivation of this model and detailed results are given in *Supporting Text*, which is published as supporting information on the PNAS web site). Although our model includes t_j < 12 d (our first observation), we recognize that the nature of the fire-related source changed from combustion of jet fuel during the first two days to smoldering fires thereafter. Thus, in what follows, we restrict our predictions of PAH levels to begin at day 3 when smoldering fires were the primary combustion sources (30).

The above model was applied to each of the nine PAHs by means of nonlinear segmented regression. After preliminary analyses indicated that there were no statistical differences in PAH levels at the three Ground Zero sites (A, C, and K), data from these sites were combined and models were rerun. Observed and predicted levels of the individual PAHs in our samples were similar in appearance to the plots representing the sum of these nine analytes shown in Fig. 2. Because no significant differences were observed in estimates of the time constant α_s (for the fire-derived PAHs) at the different sites, all final models assumed a common time constant α_{j-s} = 0.0483 d^{-1} obtained as the estimated mean value for the nine PAHs (SE = 0.0092 d^{-1}); this result corresponds to a half-life of 14.4 d. Given this decay rate, fires contributed insignificant quantities of PAH to the air of NYC after 100 d had elapsed from September 11, 2001. Likewise, no significant differences were detected in the linear (diesel-related) slopes $\beta_{1,s}$ across sites for a given PAH and common values were used for each analyte in the final model.

The estimated model parameters and SE were used to predict air levels and confidence intervals for each PAH at t_j = 3 (September 14, 2001), t_j = 100 (December 28, 2001, fires officially extinguished), and t_j = 200, (March 29, 2002, final day of the study). Table 1 summarizes these predictions for the nine PAHs, and presents comparison values from downtown Los Angeles (mean values for 1998–2002) (furnished by the California Air Resources Board, Sacramento, CA) and from Teplice, in the coal-burning region of the Czech Republic (winter 1993–1994; ref. 31). Our results indicate that, in the immediate aftermath of the WTC disaster (t_j = 3), PAH concentrations at Ground Zero were 10- to 214-fold greater (median = 65-fold) than the background values observed when t = 200 d at 290 Broadway. Indeed, predicted PAH concentrations at t_j = 3 in lower Manhattan were in the range of those observed in Teplice in winter, when some of the highest outdoor PAH concentrations in the world have been reported. However, air levels rapidly declined with the dissipation of fires during the first 100 days and slowly declined thereafter as diesel equipment was phased out, approaching Los Angeles mean values after 200 days. Nonetheless, even then (t_j = 200 d) the contributions of diesel sources close to Ground Zero produced PAH levels that were 2- to 8-fold greater (median = 4-fold) than those at 290 Broadway.

Conclusion

Our retrospective analysis of PAHs in archived $PM_{2.5}$ filters provides an important insight into the environmental implications of the WTC disaster. Without this opportune investigation, inferences about air levels of PAHs would have been restricted to 13 settled dust samples (23) and 5 $PM_{2.5}$ samples (24), which were insufficient for modeling PAH sources and trends.

Given the rapid decline of PAH levels in the aftermath of September 11, 2001, we consider it unlikely that the long-term risks of cancer arising from PAHs would have been significantly elevated above those from background PAH exposures in NYC over 70 years among city residents. Indeed, employing standard methods, we estimate only a 10^{-8} increase in lifetime cancer risk

at Ground Zero from WTC-related PAHs in our samples (details are given in *Supporting Text*, specifically in *Risk Estimates* published as supporting information on the PNAS web site). We temper this observation with knowledge that workers engaged in the cleanup efforts could have been exposed to much higher levels of PAHs than those in our samples and, thus, could bear higher cancer risks. Also, our conclusion regarding cancer risks from PAHs cannot be extended to other carcinogenic substances that were also released to the air after the WTC disaster.

However, because PAH levels were very high during several weeks after the WTC disaster, the potential for adverse reproductive effects cannot be ruled out among the offspring of women who were (or became) pregnant during that period. In fact, a recent report that levels of aromatic DNA adducts were greater in the blood of newborns than of their PAH-exposed mothers in Krakow, Poland (20) suggests that the fetus may be at particular risk to PAH damage. Certainly PAH exposures in early gestation (especially the first month of pregnancy) have been implicated as a source for reproductive effects in the Czech Republic (21, 22), and preliminary results point to a 2-fold elevation of intrauterine growth restriction among the offspring of women residing close to the WTC during those fateful weeks after September 11, 2001 (32). By carefully mapping PAH levels in space and time after the WTC disaster, it may be possible to determine the nature and extent of links between PAH exposure and developmental effects in the local population.

Finally, we caution that the transient nature of exposures to high levels of PAHs from the WTC disaster is only indicative of outdoor air, where massive dilution rapidly reduced the air concentrations. However, dust and soot from the WTC disaster also deeply penetrated residential and commercial buildings, leaving thick layers of residue (33–35). Human indoor activities and contaminated central ventilation, heating, and cooling systems can continually resuspend settled dusts into the air; therefore, indoor air may represent a continuing source of exposure to PAHs (and other particle-related pollutants) that should be considered.

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Dr. Odin is an associate professor in the Division of Liver Diseases and the Recanati/Miller Transplantation Institute with a clinical background in gastroenterology and liver disease. He is co-director of the Mount Sinai Autoimmune Liver Disease Program and director of the Center for Advanced Medicine Liver Disease Practice. He has research interest in liver autoimmunity and toxicology. His current research studies are focused on novel treatments for primary biliary cirrhosis, primary sclerosing cholangitis and the effect of drugs and toxins on the liver. He cares for individuals with all liver diseases including those with liver transplants. His research is supported by the NIH and he has received past support and awards from the American Gastroenterolofical Association and the American Association for the Study of Liver Disease. He also receives support from pharmaceutical companies for participation in clinical trials examining treatments for viral hepatitis.

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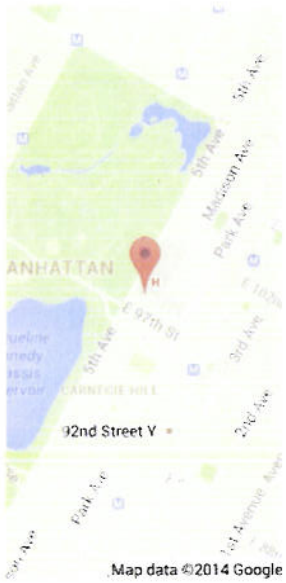

* SPOKE TO ADMIN ASST
- THEY ARE TREATING 9/11 WORKERS FOR LIVER DISEASES
- SHE DID NOT KNOW HOW MANY OR WHICH LIVER CONDITIONS.

Dr. Odin's liver research focuses on Autoimmune Liver Disease, Toxicity, Fatty Liver Disease, and Viral Hepatitis. Clinical trials and studies are being conducted or planned for primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, drug or toxin induced liver injury, hepatitis B and C, and non-alcoholic fatty liver disease. Some of the research is investigator initiated while other studies are supported by the National Institutes of Health or Pharmaceutical Companies.

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Clinical Trials

- Evaluation of Tenofovir DF vs. Adefovir Dipivoxil
- A Phase 2b Randomized, Double-Blind, Placebo-Controlled Trial Comparing 24, or 48 Weeks of GS-9190 in Combination with Peginterferon Alfa 2a and Ribavirin, to 48 weeks of Peginterferon Alfa 2a and Ribavirin for the Treatment of Genotype-1 Chronic
- Tenofovir DF vs. Adefovir Dipivoxil Chronic Hepatitis

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Diesel Oil Vapor Inhalation: A Health Risk for Liver Function

Rezaee M*, Rezaee E , Ahmadi R, Mehdi-Araghi A, and Mostofi A

***Abstract*—Studies show that inhalation of oil gas vapor may impose serious health risks for individuals exposed to such vapor. The main aim of this study was to determine the effects of diesel oil vapor inhalation on SGOT and SGPT as indices of liver function. Male Wistar rats were randomly divided into control animals, and rats that exposed to diesel oil vapor for 1 hour/day, for 2 hours/day and for 3 hours/day. After a period of 6 weeks, blood samples were collected and level of SGOT and SGPT was measured by spectrophotometry method. Data were statistically analyzed and compared between groups using ANOVA. Our findings indicated that SGOT was significantly decreased in rats exposed to diesel oil for 2h/day and 3h/day ($P<0.01$ and $P<0.05$, respectively). SGPT was non-significantly decreased in rats exposed to diesel oil vapor compared with control animals. Our finding indicates that exposure to diesel oil vapor can bring about decreased SGPT level, indicating the health risk caused by exposure to diesel oil inhalation, in particular, to liver.**

***Keyword*— Diesel oil, Health Risk, Liver, Rat.**

I. Introduction

DIESEL oil is a fuel obtained from petroleum distillation that is used in diesel engines. Diesel fuel is a mixture of hydrocarbons obtained by distillation of crude oil [1]. Diesel fuel specifications differ for various fuel grades and in different countries. Diesel fuel contains toxic constituents, including benzene, toluene, ethylbenzene, and xylenes (collectively known as "BTEX" compounds). The Department of Health and Human Services, the International Agency for Research on Cancer, and EPA have determined that benzene is a human carcinogen.[2] Chronic exposure to toluene, ethylbenzene, or xylenes also can damage the central nervous system, liver, and kidneys[3]. Diesel vapors and also gasoline vapor can irritate eyes, nose, throat and lungs. Excessive short-term exposure can lead to dizziness, drowsiness, loss of coordination, blood pressure elevation, headaches, nausea, asphyxiation and lung damage. Breathing diesel vapors for long periods of time can cause kidney damage and reduce the clotting ability of blood [4]-[7]. Neurotoxic effects of fume oil inhalation has been also established [8]. Traffic congestion increases vehicle emissions and degrades ambient air quality, and recent studies have shown excess morbidity and mortality for drivers, commuters and individuals living near major roadways [9].

Serum glutamic oxaloacetic transaminase (SGOT), is an enzyme is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health. Serum glutamic pyruvic transaminase (SGPT) is found in serum and in various bodily tissues, but is most commonly associated with the liver [10]. Alteration in SGOT or SGPT levels is prevalent in various conditions including liver damages [11].

This study was designed to evaluate the effects of diesel oil vapour inhalation on SGOT and SGPT levels as indices of liver function.

II. Material and Methods

A. Animals

Adult Wistar rats weighting 200±30g were purchased and raised in our colony from an original stock of Pasteur institute (Tehran, Iran).The temperature was at 23±2 ^{0}C and animals kept under a schedule of 12h light:12h darkness (light on at: 08: 00 a.m.) with free access to water and standard laboratory chow.

B. Protocol of Study

Male Wistar rats were randomly divided into control animals, and rats that exposed to diesel oil vapor for 1 hour/day, for 2 hours/day and for 3 hours/day. After a period of 6 weeks, blood samples were collected in appropriate tubes by cardiac puncture technique 24h after the last treatment. After collection, the blood samples left to clot at room temperature for 15 minutes and then centrifuged at 2500 r.p.m for 15 minutes. The serum layer was then separated and aliquoted into small test tubes and stored at -20 ^{0}C until enzyme activity determination. SGOT and SGPT levels were measured by spectrophotometry method. All animal experiments were carried out in accordance with the guidelines of Institutional Animal Ethics Committee.

C. Statistical Analysis

All values are presented as mean ± S.E.M. Statistical significance was evaluated by one-way analysis of variance

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(ANOVA) using SPSS 19. Differences with P<0.05 were considered significant

III. Results

Table 1 shows SGOT and SGPT levels in control and rats exposed to diesel oil vapor.

TABLE I
SGOT AND SGPT IN CONTROL AND EXPERIMENTAL GROUPS.

Groups	SGOT (UU/L)	P	SGPT (UU/L)	P
Control	227.4±40.8	-	101.6±29.5	-
Diesel Oil Vapor Receiving (1 h/day)	187.2±24.8	NS	102.4±19.3	NS
Diesel Oil Vapor Receiving (2 h/day)	140.6±20.1	<0.01	86.6±10.4	NS
Diesel Oil Vapor Receiving (3 h/day)	161.2±32.1	<0.05	81.2±6.3	NS

The data are indicated as mean ± SEM . P values are expressed in comparison with control group. N.S. represents non- significant difference.

Our findings indicate that SGPT was non-significantly decreased in rats exposed to diesel oil vapor compared to control animals. SGOT was significantly decreased in rats exposed to diesel oil for 2h/day and 3h/day (P<0.01 and P<0.05, respectively); however, there was not significant decrease in SGOT between rats exposed to diesel oil for 1h/day and control animals.

IV. Discussion

Our study indicated that diesel oil vapor inhalation results in reduced SGOT levels. In line with our finding, other research findings also indicate that breathing the evaporative and refueling emissions bring about serious toxic risks [12] which in turn, may give rise to damages in tissues including liver to reduce SGOT and SGPT. The reports also indicate that ingestion of diesel and biodiesels can cause mild hepatic peroxisomal proliferation [13]. The impacts of fume oil exposure on Na^+, K^+-ATPase, superoxide dismutase, and monoamine neurotransmitters dopamine , norepinephrine and serotonin were also estabilished [14], indicating that diesel oil inhalation impose cellular and molecular alterations by which alteration in tissues including liver tissue and reducing of liver enzymes is explainable.

V. Conclusion

We have shown that exposure to diesel oil vapor can bring about enhanced SGOT level, indicating the health risk , in particular to liver, caused by exposure to diesel oil inhalation.

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Smyk et al.

Review Article

PBC triggers in water reservoirs, coal mining areas and waste disposal sites: From Newcastle to New York

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Abstract. Various environmental factors have been proposed as triggers of primary biliary cirrhosis (PBC), a progressive autoimmune cholestatic liver disease which is characterised by the destruction of the small intrahepatic bile ducts. Support for their pathogenic role in PBC is provided by epidemiological studies reporting familial clustering and clusters of the disease within a given geographical area. The seminal study by Triger reporting that the great majority of PBC cases in the English city of Sheffield drank water from a specific water reservoir, has been followed by studies reporting disease 'hot spots' within a restricted geographic region of the former coal mining area of Newcastle. The New York study reporting an increased risk and significant clustering of PBC cases near toxic federal waste disposal sites has added strength to the notion that environmental factors, possibly in the form of infectious agents or toxic/chemical environmental factors in areas of contaminated land, water or polluted air may play a key role in the development of the disease. This review discusses the findings of reports investigating environmental factors which may contribute to the cause of primary biliary cirrhosis.

Keywords: Autoimmunity, disease, bile ducts, cholestasis, liver, immunity, tolerance, mimicry, cross-reactivity, environment

List of abbreviations

PBC	primary biliary cirrhosis
AMA	anti-mitochondrial antibody
BEC	biliary epithelial cell
OADC	oxo-acid dehydrogenase complex
PDC	pyruvate dehydrogenase complex

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1. Introduction

The aetiology of primary biliary cirrhosis (PBC), an immune mediated and potentially fatal cholestatic liver disease, remains unsolved [1–3]. Genetic, infectious and environmental factors have been considered important for the development of the disease [4–10]. Support for the implication of environmental factors has been provided by case-control or large epidemiological studies [11–22]. Immunological investigations in biological material obtained from patients at early stages of the disease, and studies in experimental models resembling the human disease have revealed the pathogenic potential of specific environmental agents [10,23,24]. This review will discuss the findings of epidemiologi-

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cal, clinical and experimental studies investigating environmental factors which may contribute to the development of primary biliary cirrhosis.

2. General considerations

In looking for the causes of PBC, a major issue is the relative contribution of genetics and environment [1, 25,26]. The *tabula rasa* (Latin for blank slate) view has swung back and forth over time with regard to the relative contribution of nurture (i.e. environment) *vs* nature (i.e. genes) in the development of PBC. The proponents of the thesis that environmental factors dominate the pathogenic processes determining the disease's induction have based their arguments on the findings of a series of studies failing to identify a strong genetic basis. Thus, several independent studies have shown over the years a weak association of specific HLA class I or II genes with disease susceptibility [4,27]. This is different from other autoimmune diseases affecting the liver, as in the case of autoimmune hepatitis which shows a strong association with the HLA DR3 and DR4, or other autoimmune diseases like insulin dependent diabetes, multiple sclerosis and rheumatoid arthritis [28–32]. The contribution of a single non-HLA gene that could predispose to the development of most PBC cases is virtually absent [4,7,8,29].

3. Migration studies and PBC

Evidence in support of environmental factors contributing to the risk of developing PBC, is based on the observation that the risk in migrants is adjusted to, and correlates with the risk of the population into which they move [15,33–35]. An example of this can be found with British migrants to Australia [33,34]. In the UK, the PBC rate is 150–240 cases/million compared to a much lower rate of 19 cases/million in Australia [33]. British migrants to Australia show a 3–5 times decrease in risk of developing PBC (47 cases/million) [36]. The opposite trend is seen in migrants from India to the UK, whose rate increases from 1–4 cases/million in the Indian subcontinent, to 14 cases/million in those who migrate to the UK [35]. These findings added support to the notion that affected individuals within a familial or regional group that has a high PBC prevalence, are exposed to a common environmental factor which triggers or contributes to the development of PBC. The interpretation of the epidemiological studies needs to be treated with caution due to methodological constraints and the significant intra- and inter-variability between studies. Nevertheless, environmental factors such as viruses, bacteria, chemicals, drugs, or xenobiotics may indeed play a role in the development of the disease [1, 3,9,23,37–50]. The expectation is that these environmental factors would be absent in regions or populations of low PBC prevalence, or that exposure to them is minimal. On the other hand, regions with a high incidence of PBC would have an increased exposure to these environmental factors via a common media, such as air, soil, or water.

4. Familial and non-familial clustering of cases with PBC

Evidence in support of environmental and genetic factors in relation to risk for developing PBC has been developed from familial studies [51–53]. One study of familial PBC based in the Japanese city of Hiroshima found that the relative risk of a first degree relative was 50 to 100 times higher than compared to the general population [54]. This study involved 18 cases of PBC which were derived from eight different families in which more than one family member had a history of PBC. It frequently included mothers and daughters (4 families), sisters (1 family), and intriguingly also included brothers and sisters (2 families) and a mother-daughter-son (1 family) [54]. A subsequent study has also estimated that the prevalence of PBC among atomic bomb survivors in Nagasaki was much higher (792 per million women) than that reported for the general population in Japan, suggesting that environmental factors, including radiation exposure, may predispose to PBC [55]. Another study from the North-East of England also showed an increase in relative risk of developing PBC, with siblings showing an increased risk of 10.5 [53]. This level of risk is similar to that found in other autoimmune diseases.

Two shared factors between affected individuals should be examined when looking at familial clustering of a disease: genes and environment. These two factors are likely to play a role individually, or combined in disease clusters. Although genetics are more commonly associated with a familial disease, commonly stated evidence in support of environmental factors comes from the timing of disease onset, in that the disease tends to occur at the same time or within a short period of time from the diagnosis of the first case. However, this is not entirely correct. When a patient is diagnosed with

PBC, screening of relatives for autoantibodies and liver biochemical profiles is advised. It is often this screening that detects PBC cases which may be asymptomatic at the time. That is to say, that early screening often detects relatives (usually mothers, sisters, or daughters) with PBC, as opposed to symptomatic clinical presentation. Further evidence in support of an environmental factor in these familial clusters is found when examining unrelated, but affected individuals who share the same environment. Douglas and Finlayson report a mother and daughter affected by PBC, as well as a close family friend [52]. In that case, the mother and family friend developed PBC within 21 months of the daughter's death from PBC [52]. Another PBC cluster was reported in six women living in two cities in Alaska, where three of the women worked together as phone operators for several years [51]. Yet another report involves two women in a German town, both with a 20 year history of deranged liver function tests, who developed PBC within two years of each other [56]. These two women were found to share common genetic factors, as well as environmental. Genetically, they shared alleles A2, B51, DR4, DR8 and DR53 [56]. Environmentally, they shared the same desk at school from ages six to ten, and were lifelong friends [56].

5. Nature vs nurture: The twins study

To disentangle the effects of genes (and indirectly of the environment), Selmi et al. performed a twins study [6]. These investigators identified 16 pairs of twins within a 1400-family cohort, including 8 sets each of monozygotic (all female) and dizygotic twins (4 female and 4 female-male pairs). In 5 of 8 sets of the monozygotic twins, both individuals had PBC (0.63 concordance). The argument that in cases that genes were the only risk factors, someone would expect that all monozygotic pairs would develop the disease is not valid. In fact, the concordance rate of PBC in identical twins is among the highest reported for any autoimmune disease.

Among the 8 dizygotic twin pairs, however, none were concordant for PBC [6]. Interestingly, the age at onset of disease was similar in 4 of the 5 concordant monozygotic twin pairs. In the fifth pair, the diagnosis of PBC was made with a 5 year gap. In one case, the twins had variant clinical behaviour, with one listed for liver transplantation 8 years after presentation and the other showing no sings of advanced liver disease for more than 13 years of follow-up [6]. Patients with PBC can have a prolonged sub-clinical period of the disease [3,57]. Thus, the possibility that the 3 disease-free women (aged 55, 60 and 61 at the time of the report) of the discordant for PBC monozygotic pairs may develop full-blown disease can not be excluded [57]. The fact that the disease affected only one of the dizygotic twins is of interest. Four of them were men and PBC in men is infrequent. The overall discordance for PBC in 11 (3 monozygotic and 8 dizygotic pair sets) is puzzling. Such findings are against the role of environment in the development of PBC. If environmental factors played a role in PBC development, one would expect some concordance in the non-genetically identical dizygotic twins who share environmental factors. This would be especially true in female-female twin pairs, but could be questionable in male-female pairs due to the low preponderance of PBC in males. Dizygotic pairs do not share the same genes and therefore this discordance is more in support of genetic involvement.

6. The Triger who triggered the water-contaminant hypothesis as a PBC trigger

Perhaps the most significant contribution to the hypothesis of an environmental trigger of PBC came from a 1980 study by David Triger, a 38 year old physician and Senior Lecturer in Medicine at Royal Hallamshire Hospital [21]. This study, published in the British Medical Journal, noted a high prevalence of PBC in the city of Sheffield in Northern England (a total of 34 cases, giving a prevalence of 54 cases/million) [21]. Triger suggested a potential environmental cause of this increased prevalence, possibly due to the heavy industrialisation of the city with coal mines and steel smelting factories. Clusters of PBC were found in districts of the city with prevalence rates too high to be attributed to chance [21]. Examination of the HLA subtypes and careful questioning ruled out the possibility that these clusters were familial. Atmospheric pollution was implicated due to the large scale smelting works, but this was ruled out as a potential trigger, as surrounding areas did not report clusters of PBC. Triger demonstrated that 30 (88%) of all PBC cases received their water supply from a single reservoir, known as the Rivelin reservoir (this was one of six reservoirs supplying the city at the time) [21]. The population supplied by this reservoir showed a prevalence of PBC ten times higher than the population supplied by other reservoirs. The remaining 4 PBC cases received their water supply from the remaining five reservoirs, which provided 60% of the

cities water. In order for the water supply to be implicated, it would need to be established that affected individuals had a lengthy exposure to a common water supply. Triger notes that 26 of the 34 patients lived in Sheffield their entire lives, with the remaining eight having lived there for an average of 49 years before presenting with PBC [21]. The average amount of time in the same house was 20 years, with the mean period of time spent in the area supplied by the same reservoir was 42 years.

With the above data in mind, Triger set out to examine the cities water supply for aluminium and other metals. Aluminium was selected based on a previous report which noted its high concentrations in Sheffield's water, and implicated this with cases of bone disease and encephalopathy due to haemodialysis for chronic renal failure. Triger found high aluminium concentrations in three reservoirs, one of which was the Rivelin reservoir. This finding pointed away from aluminium as a potential trigger/cause for PBC, as the other two reservoirs did not supply a population with a high PBC prevalence [21]. No other significant findings were found in relation to zinc, copper, and several other metals. The only significant finding was that the Rivelin reservoir has particularly soft water (total and non-carbonate), and lower concentrations of fluoride. It remained unclear as to whether these findings had any significance in the pathogenesis of PBC. In addition to the reservoirs, Triger also examined the water delivery system, as contamination along this route was also a possibility [21]. This was performed by analysing random water samples from the taps of patients and controls homes. A 300 fold variation in copper concentrations was found from one house to another, in the patient and control groups. The study by Triger, reporting a clustering of cases and their temporal association with a specific water supply system has formulated a series of subsequent studies aiming at investigating whether clusters of PBC do really exist, and whether there is a waterborne risk factor which can be identified. Another study of the epidemiology of PBC noted an urban clustering of this disease in the Greater Nottingham area, UK but no specific risk factor has been identified on analysis of water [58]. Neither the Sheffield nor the Nottingham studies have published subsequent reports in their series and the possibility that clustering may have been coincidental has been considered. This has been strengthened by studies of patients with PBC in Ontario, Canada which were unable to identify clusters of the disease [59].

7. Triggers of PBC: The Newcastle 'hot spots'

Epidemiological studies of PBC have been done extensively in the city of Newcastle, perhaps more so than in any other population [11,12,14,16,17]. Much like Sheffield, Newcastle was a heavily industrialised city, especially in regards to coal mining. Prince et al. noted a high prevalence of PBC in the North-East of England, with 32.2 new cases per million, and a prevalence of 251 cases per million (based on data from 1994) [12]. In women over the age of 40, the PBC prevalence was 940 per million. The study included 770 patients with PBC, and showed a significant non-random spatial distribution of cases. Three well defined clusters of high PBC prevalence were found [12]. Ten cases were noted in one cluster, which is located in a suburb of Newcastle and North Tyneside, with an area of 1.4 km^2 giving a case density of 7.1/km^2. The remaining two consisted of eight cases in a 0.75 km^2 area (10.7 cases per km^2), and six cases in a 1 km^2 area [12]. Familial clustering was ruled out, and there did not appear to be any other commonality between the clusters. This study noted that there was no association with the water supply, which came from a variety of sources, but unlike the study by Triger, the Newcatle group did not test the water supply [12]. The clustering of patients with PBC has led the Newcastle group to suggest that an unidentified environmental factor may explain the higher prevalence and clustering of PBC in former industrial and/or coal mining areas. As these regions are no longer heavily industrialised, one would expect the incidence of PBC to decrease, as the exposure to these environmental triggers is reduced or absent. However, this is not the case, and there is still a significant prevalence of PBC in these regions. If an environmental trigger related to industry and/or coal mining exists, it could be hypothesised that regions which are still currently involved in coal mining (such as in parts of Canada, Arizona, and China) will show a higher prevalence of PBC in the future. Whether or not this is the case remains to be seen.

Another factor in favour of an environmental trigger is the prevalence of men with PBC in the clusters, compared to the general (non-clustered) prevalence of PBC. As the coal mining and steel working industries were heavily employed by men in these regions, there would therefore be a higher rate of exposure to environmental factors, and therefore a higher rate of PBC in men than is normally found. Although the studies by Prince et al and Triger note an overwhelmingly higher preponderance of females affected with PBC, both

studies include affected men, at a rate higher than that of the general (non-clustered) PBC population. In other words, the prevalence of PBC among males remains low, but is higher than expected in some clusters.

Emerging data from the Newcastle group may help us to better understand the relative contribution of environmental factors in the disease pathogenesis [11]. McNally et al. analyzed for space-time clustering using population-based data from northeast England over a defined period of seven years (from 1987–2003) [11]. Space-clustering is observed when excess cases of a given disease can be identified within restricted geographical areas at limited periods of time. Analysis of their data suggests space-clustering of PBC which can not be attributed to variations in population density.

These findings indicate the possible involvement of a geographically widespread, but transient, causative agent and are consistent with 'either a very short "lag time" between exposure to an etiologically relevant agent and subsequent diagnosis or a longer, but relatively constant, "lag time" between exposure and diagnosis' [11].

8. From Newcastle to New York

In 2006, Ala et al. published a study evaluating the relationship of environmental factors with PBC [22]. This study showed that the prevalence of PBC patients listed for transplantation was increased near superfund toxic waste sites in New York State. Additionally, a statistically significant PBC patient cluster, including both patients not listed for transplantation and those listed for transplantation, was identified in Staten Island near a superfund waste site contaminated with volatile aromatic hydrocarbons and trichloroethylene [22]. The precise mechanism(s) by which the proximity to such sites may increase exposure to these toxins is not clear. The Sheffield study speculated that differences in regional water supplies might account for clustering. According to Ala et al., 85% of ground water near superfund toxic waste sites in New York State is contaminated, but the residents do not rely on groundwater as a water supply [22]. This has led them to suggest that inhalation of volatile organic compounds (e.g., benzene) and particle bound chlorinated hydrocarbons released into the air from these sites, is a more plausible method of exposure. This view is supported by large epidemiological studies of risk factors for PBC performed in the UK and USA, reporting that cigarette smoking (another source of benzene) is associated with PBC [11,18,19]. Of relevance to the New York study, Amano et al. reported that halogenated forms of benzene may mimic the lipoylated PBC autoantigen epitope, and indicated that air-borne volatile organic compounds such as benzene may indeed contribute to the immunopathogenesis of PBC [23,24].

The higher prevalence of cases with primary sclerosing cholangitis (an immune mediated cholestatic disease which does not overlap with PBC) near toxic waste sites suggests that exposure to toxins is not specific for PBC [22].

9. Xenobiotics vs Xeno(P)b(C)iotics

Although specific environmental compounds causing PBC have not been clearly identified, xenobiotics are now emerging as compounds that could possibly narrow the gap between environmental exposure and PBC pathogenesis [1,26]. This is not overly surprising, as xenobiotics have been linked with autoimmune disease, so much that specific xenobiotics are linked to specific autoimmune diseases. Xenobiotics are capable of altering or forming complexes with self or non-self proteins. This induces a change in the proteins molecular structure, which may cause an immune response, and subsequent cross-recognition with mitochondrial autoepitopes. This process is similar to that of molecular mimicry both in concept, as well as the transient presence of the triggering molecule/organism [60,61]. The list of exogenous compounds is long and complex, but includes common items such as detergents and cosmetics, as well as polluting materials [62]. Being the primary detoxification organ, the hepatocytes and the biliary epithelial cells are prime targets of chemical compounds. The working hypothesis is that modifications of the lipoylated major mitochondrial autoantigen could trigger the production of autoantibodies. A series of recent studies from the group of Eric Gershwin have provided data to support this view; their findings are reviewed elsewhere [1,10,23,24,63]. Hence, it has been shown that that 2-nonynoic acid is recognized by PBC sera with high affinity, and indicate that this compound is able to induce cholangiopathy in immunised mice [1, 10,23,24,63]. This xenobiotic is found in cosmetics and nail polish, which may give a possible clue as to the female preponderance of the disease.

10. Novosphingobium aromaticivorans: The missing environmental link?

The same group of investigators has provided sero-

#2 * Pleil et al.

#3 USGS

logical and molecular data suggesting that a ubiquitous xenobiotic-metabolizing Gram-negative bacterium, *Novosphingobium aromaticivorans*, is a potential trigger for the induction of PBC [46]. This bacterium contains two proteins which share amino acid sequences to the immunodominant epitope of PDC-E2 [46]. It is also capable of metabolizing organic compounds such as oestrogens, which is intriguing given the female preponderance of PBC [64]. *N. aromaticivorans* was found in 25% of human faecal samples tested. Contributing to this is the study by Mattner et al, who found that *N. aromaticivorans* was capable of inducing PBC-specific anti-mitochondrial antibodies in mice following immunization [65]. These mice were also shown to have PBC-like bile duct lesions [65].

11. PBC triggering agents may be everywhere – Concluding remarks

Thirty years after the Sheffield study, the agent contained in the Rivelin reservoir which could account for the development of PBC remains unknown. Much effort has been put into identifying chemicals or other compounds that are contained in the water, but none have looked for an infectious component. This would need to be ubiquitous in the environment (soil, water) and pathogenic to susceptible hosts. The idea that exposure to certain environmental factor(s), not harmful per se, could result in the breakdown of immunological tolerance and the induction of disease-specific destruction, cannot be discouraged. The number of studies investigating the role of specific environmental triggers has increased considerably, and may lead to a more conclusive result in the not too distant future. There is no doubt that the triggering agents of PBC can be everywhere, from water reservoirs and coal mining areas, to superfund waste disposal sites, from Sheffield and Newcastle to New York. We may not yet know them, but we hope that we will find them.

12. In Memoriam

Professor David R. Triger, DPhil, FRCP. 1941–1993.

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Infectious agents and xenobiotics in the etiology of primary biliary cirrhosis

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Abstract. Primary biliary cirrhosis (PBC)is a chronic autoimmune cholestatic liver disease that manifests a latitudinal gradient in prevalence and incidence. The mechanisms leading to the initiation and perpetuation of PBC remain largely enigmatic, although it is established that a combination of genetic predisposition and environmental stimulation is required. PBC is also characterized by a high concordance rate in monozygotic twins and is considered a model autoimmune disease because of several features common to other conditions and the relatively homogeneous serological and biochemical features. From a diagnostic standpoint, PBC is characterized by the highest specificity of serum autoantibodies directed at mitochondrial proteins. Several risk factors have been suggested to be associated with PBC, including exposure to infectious agents and chemical xenobiotics that will be critically discussed in the present review article.

1. Introduction

Primary biliary cirrhosis (PBC) is a rare organ-specific autoimmune disease characterized by an immune-mediated destruction of small- and medium-size bile ducts with resulting chronic cholestasis and ultimately liver cirrhosis [1]. One major paradox in PBC pathogenesis is common to several autoimmune diseases and is based on the observation that, although the autoimmune attack is directed against ubiquitous mitochondrial antigens belonging to the family of 2-oxoacid dehydrogenase complexes (2-OADC), the disease specifically involves biliary epithelial cells, i.e. cholangiocytes [2]. Among components of 2-OADC, the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2) constitute the predominant autoantigen recognized by the vast majority of patient sera [3]. As for other autoimmune diseases, genetic and environmental factors are involved in PBC pathogenesis, as well represented in monozygotic twins who fail to demonstrate a complete concordance rate [4] or the reported significant genetic associations encountered in subgroups of patients [5]. The immunologic scenario of the disease is based on both the innate and adaptive arms of immunity [6] while the latter includes both humoral and cellular responses [7]. Highly disease-specific serum antimitochondrial autoantibodies (AMA) are directed against components of the 2-oxoacid dehydrogenase complex (2-OADC) and are detected in the vast majority of patients [8]. Similarly, liver infiltrating autoreactive T cells are found in patients with PBC irrespective of their AMA status [9]. These cells recognize antigens overlapping with AMA specificities and are thought to play the major role in biliary cell destruction through direct cytotoxicity (CD8+ cells) and as a result of cytokine production (CD4+) [7,10], as well established in liver immunity [11,12].

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2. The immunobiology of the biliary epithelium

Biliary epithelial cells are actively involved in the peculiar immunity of the liver [13] as they represent a specialized epithelium expressing a plethora of factors contributing to antimicrobial defense. Overall, cholangiocytes represent the first line of defense for the biliary system against microbes derived from the gut through the portal vein and the extrahepatic bile ducts [14], but they are also a possible target of immune mediated in-

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jury as in the cases of PBC, primary sclerosing cholangitis [15], or graft-versus-host liver disease [16], along with autoimmune hepatitis overlap syndromes [17].

Indeed, cholangiocytes express receptors for pathogen-associated molecular pattern (PAMP) represented by the majority of toll like receptors [18,19], cytokines, such as IL-6 and IL-8 [20], chemokines, such as MCP-1 [20], growth factors, such as TGF-β, CTGF, PDGF, endothelin-1 [19], and immunoactive peptides, such as defensin β [21]. Further, cholangiocytes can cross-talk with other immune cells [22] in the liver through the expression of specific adhesion molecules, such as LFA-3 and CD40 [23] and, unlike other epithelial cells, they also feature as antigen presenting cells expressing HLA class II [24–26] and co-stimulatory molecules, such as CD80 and CD86 [23,27]. More pertinent to PBC pathogenesis, biliary epithelial cells are unique in secreting immunoglobulins A of the secretory type (sIgA) through transcytosis in the biliary lumen [28,29] which may in turn cause the organ-specific immune-mediated injury and justify the detection of IgA-AMA in the bile and saliva of patients [30] or the general pro-inflammatory milieu [31].

The mitochondrial antigens recognized by both B and T cell autoimmune responses in PBC are ubiquitously expressed in nucleated cells and highly conserved in phylogenesis [32]. It has been proven that mitochondrial antigens are not cryptic to the immune system and that the immune system is normally tolerant to mitochondrial self-antigens, even if responsive against bacterial homologues. During spontaneous or induced apoptosis, various cell types (virtually all) express mitochondrial antigens on the intact plasma membrane and within the apoptotic blebs [33,34] which are then capable to initiate the immune response by presenting the AMA antigens [35]. This latter process is specific to cholangiocytes and suggests why the disease recurs following liver transplantation [36,37]. Indeed, AMA react, though weakly, against biliary epithelial cells of normal subjects [38], and specific autoreactive T [39, 40] and B cells, and serum AMA [41] have been found in the serum of non-PBC subjects. Nevertheless, the immune system starts an autoimmune attack against cholangiocytes only in PBC patients and this appears to be irrespective of whether the biliary epithelium is derived from a patient with PBC or a control subject [42]. Accordingly, liver infiltrating autoreactive T cells were found only in PBC patients [43], irrespective of the AMA status [44]. Genetic predisposition is evoked to explain the breakdown of tolerance to 2-OADC antigens in PBC [8,45] but additional factors are advocated, including the peculiar processing and presentation of antigens within cholangiocytes and the occurrence of an environmental trigger which represents the major issue to be discussed in the present article.

Mitochondrial antigens undergo cell-specific processing that are thought to contribute to PBC organ specificity [46,47]. Of seminal importance is the observation that the lysine-lipoylated domains (common immunogenic core of 2-OADC autoantigens, including PDC-E2) derived from cholangiocytes retain their immunogenicity following apoptosis [48]. The lack of putative post-translational modifications alters protein degradation leading to the accumulation and exposure of a great amount of self-reactive antigens, as hypothesized in other organ-specific autoimmune diseases [49].

As previously stated, the AMA immunodominant epitope is represented by the inner lipoylated domain of PDC-E2 while other epitopes are localized within the outer lipoylated domain of the same complex, the E2 subunit of the branched chain 2-oxoacid dehydrogenase complex (BCOADC-E2), and the E2 subunit of the 2-oxoglutarate dehydrogenase [50] complex (OGDC-E2). They all share a common motif in the N-terminal region containing lysine-lipoylated domains [47]. In most cell types lysine-lipoylated sequences are oxidized by glutathiones when released from mitochondria during apoptosis [46] as the oxidated forms are not immunogenic and are not recognized by serum AMA as the glutathionylation masks the autoantibody recognition site [46] via potential mechanisms [51]. Conversely, cholangiocytes and cells from other epithelia fail to covalently link glutathione to lysine-lipoyl groups during apoptosis [46] with consequent accumulation and exposure of potentially self-reactive antigens as the reduced forms of PDC-E2 fail to undergo normal protease degradation. In cholangiocytes the cleavage of the immunodominant PDC-E2 epitope has not been detected *in vivo* either during apoptosis [46,48] or during phagocytosis [52]. Moreover, some authors reported an enhanced expression of 2-OADC proteins, with particular luminal concentration, in cholangiocytes from patients with PBC compared to healthy subjects and patients with other chronic inflammatory biliary disease such as primary sclerosing cholangitis [38]. This abnormal expression can be explained by different mechanisms; one possibility is that self-antigens are presented by cholangiocytes complexed to HLA molecules. Nevertheless, HLA class II have a prevalent intrahepatic basolateral, rather than luminal, surface expression on cholangiocytes [24–27,53] and are weakly expressed in the early stages of disease [27]. Different hypotheses

state that self-antigens are exposed on the cell membrane during cholangiocyte apoptosis [33,46] or during the rearrangement of lipid rafts as seen after toll like receptor activation by microbe infection [54] or after the ingestion of apoptotic cholangiocytes by other cholangiocytes [52]. Cholangiocyte phagocytosis of neighboring apoptotic cholangiocytes is not specific to PBC, being observed *in vitro* in cultured cholangiocyte lines and in other chronic liver diseases, but it has been suggested that this phenomenon can be involved in the presenting process of mitochondrial antigen observed in PBC.

3. The epidemiology of PBC suggests a crucial role for the environment

Studying the epidemiology of complex diseases is commonly utilized as a proof of concept to determine the impact of the environment on their development, although these observations rarely lead to the identification of specific factors. In the case of PBC, it has been observed that prevalence and incidence rates are higher in Northern European countries (particularly the United Kingdom and Scandinavian countries) and the Northern United States (Minnesota) compared to other areas such as Mediterranean countries. However, these observations might be the result of different methodologies for case finding in epidemiological studies, rather than reflect a true difference in the prevalence of cases and, similar to clusters [55], could ultimately be anecdotal. More importantly, no solid population-based study has been proposed thus far and all estimates are based on the identification of cases already diagnosed [56]. This is in part due to the lack of a sensitive non-invasive marker of disease with the exception of serum AMA which, may appear decades before the disease and cannot ben detected in up to 10% of PBC cases. It has also been hypothesized that the incidence of PBC in the developed world might be increasing, but this hypothesis is potentially burdened by additional types of bias as observed in other conditions [57]. Finally, it should be noted that population-based studies on PBC must necessarily rely on AMA detection using indirect immunofluorescence, an assay that fails to determine antibody positivity in up to 15% of PBC cases. Following numerous studies in which PBC incidence and prevalence have been addressed in different populations, more recent data were reported to support a possible role for genetics as well as environmental factors in determining disease susceptibility, as supported by increasing incidence rates. Once again, however, the impact of an increased disease awareness among physicians and of the more widespread use of AMA determination cannot be overlooked when comparing different time frames. A population-based study performed in Australia has identified 84 cases of PBC from the region of Victoria using rigorous case-finding methods that included all three major diagnostic criteria and reported a PBC prevalence of 19.1 per million, among the lowest rates ever reported [58]. However, a subsequent study from the same region reported different data on 249 cases with a cumulative incidence almost 10-fold higher than that reported earlier [59]. Importantly, prevalence rates were significantly higher for British, Italian, and Greek immigrants, compared to the Victorian-born population (which clearly recognizes a British background in most cases) thus implying the importance of environmental priming possibly occurring early in life. In the United States, a large study performed on the population of Olmsted county, Minnesota [60] estimated the age- and sex-adjusted PBC prevalence to be 27 per million, among the highest ever reported, and the calculation was based on data from a computerized State index of diagnoses for inpatients and outpatients. Data from Canada have provided intriguing observations on subjects from British Columbia of Native Canadian ancestry for whom PBC has strikingly high prevalence and incidence rates [61]. Whether these ethnic differences reflect solely the effects of genetics or include a role for migratory fluxes still needs to be determined while anecdotal data are fascinating in suggesting that pollutants may enhance the incidence of PBC in specific areas [62]. The role of genetics in PBC appears well established, yet data from association studies in candidate genes including human leukocyte antigens [63] have been inconclusive or limited to specific geographical areas [56] and a genome-wide study is awaited [64].

Identifying risk factors is a critical challenge in directing experimental research, particularly when searching for environmental factors. Familial history and genetic predisposition are by far the most convincing risk factors identified for PBC. However, several non genetic factors have also been proposed to play a role. Our group recently concluded the largest epidemiological study reported thus far which included 1032 patients from 20 Tertiary Referral Centers in the US representative of all but two States [65]. A similar number of controls were matched to cases for sex, age, and geographical origin (using the random-digit dialing matching system) and we utilized a survey based

on over 180 standardized questions, including subquestions, investigating demographics, personal and family medical history, reproductive history, and lifestyle factors [66]. The multivariate analysis demonstrated that having a first-degree relative with PBC, a history of recurrent urinary tract infections, past smoking, or the use of hormone replacement therapies are significantly associated with an increased risk of having PBC [65]. The frequent use of nail polish also slightly increased the risk of having PBC. A potentially confounding factor in this study was that patients with PBC had a significantly higher family income compared to controls suggesting a role for a better quality of healthcare. Nevertheless, the proposed association were most recently independently confirmed [67].

4. Environmental agents in PBC onset

As previously illustrated, cell type-specific differences in apoptosis and phagocytosis of apoptotic cells seem to contribute to tissue-specific damage in PBC rather than to the breakdown of tolerance to 2-OADC antigens [68] and should thus be considered mechanisms for injury perpetuation rather than initiation. To this regard, environmental factors, such as chemical compounds and/or infectious agents are more likely involved into the breakdown of tolerance through molecular mimicry and cross reactivity mechanisms [69]. This is well supported by the incomplete concordance rate for PBC in monozygotic twins [4] and more generally by the epigenetic differences in healthy monozygotic twins sharing different durations of environmental stimuli [70] that have already been advocated for other autoimmune diseases [71] through different mechanisms [72,73]. A mimotope carried by a microbe or a neo-antigen generated by xenobiotic-modified self-antigen mimicking mitochondrial proteins may activate autoreactive lymphocytes. Then, the process could become self-perpetuating because of the presence of cross reactive unmodified self-antigens on cholangiocytes surface.

PDC-E2 specific autoantibodies and autoreactive CD4+ and CD8+ T cells recognize different overlapping or close epitopes [41,74–77]. These self-epitopes are well-conserved sequences among species with a high degree of similarity between human and microbial sequences. As a result, molecular mimicry between human and microbial epitopes is the base of the cross recognition by B and T cells of microbial proteins by PBC sera that has been repeatedly proposed to support molecular mimicry [78].

Different from other conditions [79], the pathogenetic role of serum AMA is still debated and a recent report has questioned the sequence of the immunodominant B cell epitope and the role of the lysine-lypoilated motif in the PBC B cell response [77], the study of the immunodominant T cell epitope, PDC-E2 163-176, has provided evidence crucial to PBC pathogenesis [40]. This can be summarized in one unifying hypothesis. Based on the reactivity of cloned PDC-E2 163-176-specific T cell lines, it has been demonstrated that the contact residues with T cell receptors (TCRs) are ^{168}EIExDK^{173} and that microbial proteins, whether related to PDC-E2 or not, that have an ExDK sequence can undergo cross recognition by autoreactive T cells. Interestingly, the PDC-E2 peptide was not lipoylated in K^{173} and conservative substitutions in this position did not abrogate T cell response thus suggesting a minor role of the lysine-lipoylated group in T cell recognition. Moreover, E^{170} is crucial to T cell recognition as no substitution was allowed without abrogating the immunoreactivity, possibly secondary to the fact that the CDR3 motif used by TCRs to contact the negative charged glutamic acid (E^{170}) is RGxG or G,S, and/or R i.e. a sequence containing the positive charged arginine (R). These results are of particular relevance considering the proximity of E^{170} to K^{173} and we hypothesize that the glutathionylation of the lysine-lipoyl residue in position 170 can mask or alter the exposure of E^{170} thus abolishing the critical contact residue with CDR3. This mechanism is an immunologic defense in all cell type but cholangiocytes where the reduced form of the group can favor the TCR recognition of the epitope and the role of lipoic acid per se requires further studies [80].

5. Infectious agents: Numerous studies, limited evidence

Epidemiology suggests an involvement of an external trigger in PBC. The diagnosis of PBC at the same time or within a short period of time among members of the same family [81], the reported cases of non familial clustering [55,62,82], and the concordance rates among monozygotic twins [4] suggest the need for a common environmental factor. Moreover, it has been described a changing risk of PBC in subjects moving from areas with high risk of PBC to areas with lower risk and vice versa [58,83]. As with other autoimmune diseases, infectious agents have been implicated as pu-

tative triggers in the induction and/or maintenance of PBC. To further support this hypothesis it has been reported that lipopolysaccharide (LPS), a specific component of gram negative bacteria cellular wall, alone or in combination with PDC-E2, can induce portal lymphocyte infiltration and cholangiocyte degeneration mimicking PBC in mice. On the other hand, lipoteichoic acid (LTA), the gram positive cell wall component, has been detected in PBC liver samples around damaged bile ducts and LTA-specific IgA titer has been found to be significantly higher in sera from patients with PBC compared to healthy subjects [84]. In addition, it has been reported that bacterial DNA,containing unmethylated CpG motifs, could trigger PDC-specific Th1 response in mice immunized with PDC [85]. Recently, Th17 cells, i.e. components of mucosal host defense system against infections, but also involved in the pathogenesis of other autoimmune diseases [86], have been found around bile ducts in PBC [87,88]. However, the identity of the pathogen and the exact mechanism potentially triggering the disease remain to be determined despite the large number of studies who attempted to address the issue.

Several mechanisms have been proposed to explain how an infectious agent may trigger or contribute to perpetuate an autoimmune disease in susceptible subjects. Molecular mimicry is by far the most studied having been reported for many microbes [89]. In fact, shared sequences between human and microbial proteins can break the immune tolerance to self proteins by inducing cross reactive antibodies or cross recognition by effector T cells. Moreover, molecular mimicry can lead to epitope spreading accelerating or perpetuating the autoimmune process [90], a phenomenon which has been repeatedly disproven in PBC. Pathogens can also stimulate autoreactive lymphocytes through bystander activation. The release of self-antigens from damaged cells together with microbial antigens (mitochondrial related and unrelated proteins, LPS, LTA) and DNA (unmethylated CpG motifs) causes hyperactivation of antigen presenting cells and innate immune system cells [91] leading to over-processing of self-mimicking microbial antigens and enhanced cytokine production with consequent immune complex formation and expansion of autoreactive cells [92], similar to other experimental settings [93]. Other non-exclusive mechanisms involve superantigen polyclonal activation of T cells, i.e. staphylococcal enterotoxins [94], mouse mammary tumor virus antigens [95] and viral polyclonal activation of B cells, i.e. Epstein Barr virus [96], IgA production, and Th17 differentiation. Of particular interest is the role of IgA which represents the major class of antibodies produced in the mucosal immune system of gastrointestinal and respiratory tract as defense against microbes entering by these routes. Cholangiocytes, having the unique role of secreting IgA into bile through transcytosis, are exposed to IgA antimitochondrial antibodies. It has been experimentally demonstrated that PDC-E2-specific dimeric IgA during transcytosis can activate caspases leading to cholangiocyte apoptosis and contributing to bile duct damage in PBC [48]. In a different fashion, Th17 cells are essential for effective microbial host defense against extracellular bacteria and fungi; moreover, Th17 contribute to virus persistence and chronic inflammation during parasite infections [97], but also to the balance between microbial defense and immune diseases in concert with Treg. It has been suggested that Th17, eventually differentiating after liver microbial exposure, could contribute to the Th1 shift observed in PBC [86–88] along with the defect of T regulatory cells [98] which resembles data from other clinical settings [99].

Numerous specific agents, mainly bacteria, but also viruses, parasites, and fungi, have been investigated as possible agents involved in PBC (recapitulated in Table 1), but most of the studies failed to demonstrate a clear association of a microbial agent with the disease and reported only circumstantial evidence that could not be independently recapitulated. Indeed, most studies supporting the role of infectious agents in the pathogenesis of PBC are based on the linear or conformational mimicry between microbial proteins and human mitochondrial antigens. Notwithstanding a substantial shared sequence homology, in a fewer cases a cross reactivity by 2-OADC-specific autoantibodies and/or T cells has been also demonstrated. This is the case for *Escherichia coli [100,101,103]*, *Novosphingobium aromaticivorans [32,104,105]*, *Salmonella Minnesota [106]*, *Pseudomonas aeruginosa [107]*, *Haemophilus influenzae [103]*, *Yersinia enterocolitica [103]*, *Streptococcus intermedius [108]*, *Lactobacillus delbrueckii [109]*, *Paracoccus denitrificans [110]*, *Mycoplasma [111,112]*, *Mycobacterium gordonae [113]*, *Borrelia burgdorferi [114]*, *Trypanosomes [115]*, *and Ascaridia galli [115]* (Table 1). Moreover, microbial antigens or DNA have been found in liver specimens, gallbladder bile, and fecal samples of patients with PBC as for *N. aromaticivorans [104]*, *Propionibacterium acnes [116]*, and *Epstein Barr virus [117]*. It should be note, however, that these findings were not limited to patients with PBC thus supporting the major role of individual susceptibil-

Table 1
Infectious agents proposed for PBC etiology and the putative evidence available in the literature

Infectious agents		Proposed evidence	References
Gram negative bacteria	*Escherichia coli*	higher frequency of urinary tract infections in patients with PBC presence of anti-bacterial proteins Ab in PBC pts sera molecular mimicry with Ab and CD4 cross reactivity between bacterial mitochondrial homologous proteins and non mitochondrial proteins and PDC-E2 21-226 and 163-176	[40,100–103]
	Salmonella minnesota mutant	Ab cross recognition between mitochondrial antigens and bacterial peptides	[106]
	Salmonella typhimurium	molecular mimicry between product of mutB gene and gp 210 recognized by PBC pts Ab	[100]
	Novosphingobium aromaticivorans	molecular mimicry and Ab cross reactivity between lipoylated microbial proteins and PDC-E2 208-237 autoreactive AMA and chronic T cell-mediated cholangiopathy in a murine model of PBC	[32,104,105,118]
	Helicobacter pylori	molecular mimicry but not Ab or CD4 TCR cross recognition between urease beta and PDC-E2 212-226 exposure of experimental animals to helicobacter species is able to induce hepatobiliary pathology	[119,155]
	Pseudomonas aeruginosa	molecular mimicry and CD8 TCR cross recognition between diaminopimelate decarboxylase and PDC-E2 159-167	[107]
	Haemophilus influenzae	molecular mimicry and Ab cross reactivity between caseinolytic proteases P and PDC-E2 212-226	[103]
	Yersinia enterocolitica	molecular mimicry and Ab cross reactivity between caseinolytic proteases P and PDC-E2 212-226	[103]
Gram positive bacteria	*Streptococcus intermedius*	high anti-bacterial histone-like DNA-binding protein titers and cross reactivity against BECs cytoplasmic proteins	[108]
	Lactobacillus delbrueckii	molecular mimicry and Ab cross recognition between beta galactosidase and PDC-E2 212-226	[109]
	Propionibacterium acnes	DNA in liver granulomas	[116]
	Paracoccus denitrificans	cross reactive Ab with bacterial membrane vesicles	[110]
Intracellular bacteria	*Chlamydia pneumoniae*	presence of anti-chlamydia Ab in PBC sera but not specific antigens or DNA in PBC liver samples	[156]
	Mycoplasma pneumoniae	molecular mimicry and Ab cross reactivity between microbial and human PDC	[112]
	Mycoplasma gallisepticum	molecular mimicry between bacterial surface molecules and PDC-E2	[111]
	Mycobacterium gordonae	molecular mimicry and Ab cross reactivity between microbial hsp65 and PDC-E2 212-226 but not mycobacterial DNA in PBC liver granulomas	[113,157]
Spirochetes	*Borrelia burgdorferi*	molecular mimicry and Ab cross recognition between bacterial p41 flagellin and PDC-E2 208-235	[114]
Parasites	*Trypanosomes*	molecular mimicry and Ab cross reactivity between parasitic and human mitochondrial antigens	[115]
	Ascaridia galli	molecular mimicry and Ab cross reactivity between parasitic and human mitochondrial antigens	[115]
Fungi	*Saccharomyces cerevisiae*	high frequency of anti-saccharomyces cerevisiae Ab in PBC sera	[158]
Viruses	*Mouse mammary tumor virus*	questioned molecular mimicry between viral proteins and mitochondrial antigens	[159,160]
	Epstein Barr virus	high frequency of viral DNA in PBMCs, liver and saliva of PBC cases	[117]

ity in the presence of a common trigger. In a few cases an autoimmune cholangiopathy has been mimicked in animal models after bacterial components exposure as in the most recent case of *N. aromaticivorans [118]* or the less convincing *Helicobacter pylori [119]*. Taken together these studies suggest that multiple agents and multiple mechanisms could be involved in the pathogenesis of PBC. Interestingly, among microbial pathogens *N. aromaticivorans* is supported by the most solid data, considering also its ability to metabolize halogenated compounds [32,104,105,118].

6. Chemical compounds

A different environmental factor proposed to trigger disease onset is constituted by foreign chemicals (i.e. xenobiotics) that can either alter or complex to a defined self or non-self protein, causing a change in its molecular structure that induces an immune response. This fascinating hypothesis has been proposed for numerous autoimmune diseases, particularly based on the observed geoepidemiological gradient [50,120,121] in which definitive confirmation is awaited and should not be considered as exclusive with regard to the role of infectious agents. The hypothesis is supported by a number of epidemiology studies, as previously discussed, as well as by the appearance of autoantibodies in subjects immunized with halothane, an inhalatory anesthetic no longer used, with antibodies crossreacting with lipoylated PDC-E2 [122]. As previously stated, lipoic acid is attached to a limited number of proteins, yet it is a critical component of the PDC-E2 epitope [80]. The PDC-E2 structure exposes lipoic acid at the exterior of the protein complex making it accessible to chemical modification [123]. The role of xenobiotics in PBC is supported by serum reactivity against specific organic compounds with structures similar to lipoic acid [124]; further, two of these compounds (6-bromohexanoate and 2-octynoic acid) are capable of inducing AMA and PBC-like liver lesions in guinea pigs [125] and NOD.1101 [126] or C57BL/6 [127] mice, respectively. The ability of *N. aromaticivorans* to metabolize chemical compounds might link xenobiotics and bacteria in the etiology of PBC, as discussed in the previous paragraph.

The 2-OADC antigens undergo several post-translational modifications endogenously, and such changes may alter the epitope regions of the proteins. Nevertheless, external influences can also contribute to protein alterations and neo-antigen formation [47]. Of note, the liver is constantly exposed to chemicals derived from the gut through the portal circulation to be metabolized, activated, or excreted in the bile. It has been reported that xenobiotics can either alter or complex mitochondrial proteins. In 2001, Long and colleagues first demonstrated that specific organic structures attached to the mitochondrial antigens were recognized by PBC sera with a higher affinity than the native forms of such antigens [128] and indicated that an organic compound may serve as a mimotope for an autoantigen. This provided further evidence for a potential mechanism by which environmental organic compounds can cause PBC. One such halogenated compounds has been shown to induce AMA production in rabbits without requiring the peptide backbone of PDC-E2 [129] but failed to produce liver lesions (possibly in agreement with observations in humans where AMA is present several years prior to the appearance of liver injury) and disappeared when the stimulus was discontinued [130]. A different approach reported the induction of PBC-like liver lesions following longer follow-ups in guinea pigs [125] while a further study reported two new xenobiotic-induced PBC murine models based on the immunization with 2-octynoic acid of NOD.1101 [126] or C57BL/6 [127] mice. These two most recent models share the breakdown of tolerance in the absence of PDC-E2 molecules but fail to manifest the progression to liver cirrhosis. Utilizing a different approach, our group also demonstrated that 2-nonynoic acid is capable of being recognized by PBC sera with high affinity [124]. This is particularly interesting since this non-naturally occurring compound is known to be found in several cosmetic products (including specific nail polish products) and could contribute to the association of PBC with their frequent use [65,67] or possibly the female predominance of the disease [131]. Regarding this issue, several sex-related factors appear to increase the risk of PBC, mostly by means of reproductive life variables. Among these are the role of pregnancies [132], contraceptives, estrogen replacement treatments [65], and recurrent vaginitis [133] but the data provided thus far are inconclusive and the mechanisms remain to be clearly defined. However, the novel hypothesis of sex chromosome-related effects on PBC appears promising [134], and may possibly operate via gene dosage or epigenetic changes [135] which appear to be common to autoimmunity in general [136–139].

7. Conclusions and future views

Currently available data on the pathogenesis and epidemiology of PBC support the role of environmental triggers but fails to identify specific agents that are causative of disease onset. Indeed, while geoepidemiology warrants further investigation [140–145], identified risk factors and experimental data appear to point in the same directions and support a role for infectious agents and/or xenobiotics. However, we cannot rule out that what is currently thought to be caused by an agent found in the environment is in fact a result of epigenetic changes, as suggested in other autoimmune diseases [146]. Similarly, the study of innate immunity, as supported by data in other autoimmune condi-

tions [147] is expected to provide crucial evidence to complete the scenario. Ultimately, over the past few years several animals models have been proposed for PBC, in some cases based on genetic defects [148], in others on specific immunizations [149,150]. It will therefore be necessary for future efforts to gather the available models and unite their major strengths into a single comprehensive model. The development of such a model will allow us to overcome the major obstacles to the study of environmental determinants of PBC i.e. the long latency period prior to disease appearance and possibly control additional confounding factors [151] while identifying new therapeutic agents [152–154].

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Primary biliary cirrhosis: Environmental risk factors

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Abstract. Primary biliary cirrhosis (PBC) is an autoimmune disease of unclear etiology. It is a chronic, progressive condition that causes intrahepatic ductal destruction ultimately leading to symptoms of cholestasis, cirrhosis and liver failure. The disease predominantly affects middle aged Caucasian women. It has a predilection to certain regions and is found in higher incidences in North America and Northern Europe. It also has a genetic predisposition with a concordance rate of 60% among monozygotic twins. Combinations of genetic and environmental factors are proposed in the pathogenesis of this disease with a compelling body of evidence that suggests a role for both these factors. This review will elucidate data on the proposed environmental agents involved the disease's pathogenesis including xenobiotic and microbial exposure and present some of the supporting epidemiologic data.

1. Introduction

Primary biliary cirrhosis (PBC) is a disease of presumed autoimmune etiology that causes inflammation within the portal tracts. This leads to destruction of small and medium sized intrahepatic biliary ducts. Ultimately, this may result in symptoms of cholestasis, cirrhosis and liver failure [1]. The serologic hallmark of the disease is the production of anti- mitochondrial antibodies (AMA), known to react most frequently with the E2 subunit of private dehydrogenase complex among other autoantigens [2]. The disease predominates among women with a 10:1 female to male ratio and is usually diagnosed in middle aged women though it has been diagnosed in patients as young as 15 and as old as 93 [1,3].

The pathogenesis of the disease remains enigmatic since the cause is likely complex and multi-factorial. The current view is that PBC occurs in a genetically susceptible population with external triggers precipitating its development. Considerable evidence points to environmental triggers involved in the development of this disease. In this review, we will focus on observed and circumstantial data pertaining to possible environmental exposures including xenobiotics, and microbes.

2. Genetic predisposition

The prevalence of PBC amongst families with an affected member is estimated to be 1000 times greater than the general population [4,5]. PBC has been weakly associated with the HLA-DRB1*0801 haplotype and a recent study by Hirschfield et al suggests a significant association between HLA class II, IL12A and IL12RB2 loci as well [6]. This is especially interesting as these haplotypes are associated with IL12 immunoregulatory signaling. These findings further affirm the genetic predisposition to this disease.

In 2004, Selmi et al identified 8 pairs of monozygotic twins and 8 pairs of dizygotic twins from a large cohort of patients with PBC. 60% of the monozygotic twins had PBC affecting both siblings while none of the dizygotic twins were dually affected. This concordance rate for the monozygotic twins is one of the highest of all diseases. This study confirms the strong role genetics plays in the pathogenesis of this disease [4]. However, genetics alone are not sufficient to explain the pathogenesis as 3 of the 8 monozygotic twins were not concordant for the disease. There is likely a second trigger.

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Table 1
Selected studies showing geo-clustering of primary biliary cirrhosis [8,9,11–18]

Author(s), Year	Geographic clustering	Number of cases	Site of study	Possible environmental risk factor
Triger, 1980	Yes	34	Sheffield, U.K.	Water
Hamlyn,1983	No	117	Northeast England	Sunlight
Triger, 1984	Yes	552	Western Europe	None
Borda, 1989	Yes	50	Navarra, Spain	None
Danielsson, 1990	Yes	111	Northern Sweden	None
Myszor, 1990	No	347	Northeast England	None
Witt-Sullivan, 1990 [16]	No	225	Ontario, Canada	None
Metcalf, 1997	Yes	160	Newcastle upon Tyne, England	Urban area
Tsuji, 1999	Yes	156	Hiroshima, Japan	Nuclear bomb
Prince, 2001	Yes	770	Northeast England	Urban area
Ala, 2006	Yes	211	New York City	Toxic waste site

3. Disease triggers

PBC demonstrates a large geographic variation in its incidence and prevalence. The incidence of the disease is significantly higher in Northern Europe and North America when compared to Mediterranean and African countries [1]. In addition, disease clustering with and without environmental associations has been reported by several groups (Table 1). Abu- Mouch et al described 4 unique clusters including: a family of 10 siblings with half having PBC, a husband and wife with PBC, a family of two genetically unrelated individuals with PBC, and a cluster of PBC in Alaska [7]. Their data adds to the considerable body of clinical and molecular data suggesting clustering of this disease is associated both with genetic and likely environmental triggers. The epidemiologic and molecular data presented below help support this hypothesis.

4. Epidemiology

The first real suggestion of disease clustering was reported by Triger in 1980 when he found clustering of PBC cases in Sheffield, England. He proposed groundwater contamination as a possible source for this clustering. However, analysis of the suspected water reservoir, showed no significant differences from the other reservoirs in the region [8]. James et al. also proposed water contamination as a possible mechanism of the PBC clustering that his group noted in urban areas of Northeast England [9].

In 2006, we investigated whether our perception of a higher prevalence of PBC from an area known to be developed on landfill sites was accurate. Superfund sites (SFS) are hazardous waste sites designated for clean-up by the Department of Environment and Conservation (DEC). We looked at prevalence rates of PBC near designated SFS in New York City. Data were collected from PBC and PSC patients in the Mount Sinai School of Medicine PBC database (MSSM PBC database). To avoid referral bias, we also collected data from all PBC and PSC patients listed for liver transplantation by the Organ Procurement Transfer Network (OPTN) in the five boroughs of New York City from 1995–2003. In the first part of our study we examined zip codes for all PBC ($n = 99$) and PSC ($n = 73$) patients listed for liver transplant in New York City. Expected prevalence ratios, adjusted for age, gender and race were calculated. Median standardized prevalence ratios in zip codes containing/adjacent to a SFS ($n = 89$) were compared to the zip codes without SFS ($n = 85$). Median prevalence ratios for PBC were significantly higher in clusters associated with SFS compared with PSC (Table 2).

Table 2
Median std prevalence ratio of PBC significantly higher in clusters with SFS

	CLUSTERS WITHOUT SFS	CLUSTERS WITH SFS	P value
PBC	0.51	0.94	0.001
PSC	0.28	0.28	0.572

p: significance values for Mann-Whitney U test, 2-tailed Std prev ratio: observed/std expected prevalence.

In the second part of our study, we applied SaTScan technology (a validated statistical analysis software program looking for disease clusters) to detect specific clusters of PBC near SFS using our OPTN and MSSM PBC databases [10]. Again, an increased prevalence of PBC patients was identified in clusters around SFS. Furthermore, the clusters of PBC in our cohort (MSSM PBC database) correlated with the OPTN data [11]. The major contaminants in these superfund sites were halogenated aromatic hydrocarbons including polychlorethylene and benzene.

Since then many studies continue to show geographic clustering of PBC [8,9,11–18]. McNally et al showed space-time clustering in a population from Northeast England [19]. This phenomenon is interesting as it supports the role for a transient environmental agent in the disease pathogenesis. Space-time clustering is an epidemiologic term used to describe a large number of cases presenting in a small geographic area within a limited time period. When space time clustering is identified, a transient, possibly environmental agent becomes suspect. Using population based data from the northeast regions of England over a 16 year period (1987–2003), space time clustering was noted for PBC. The Knox test and K-function methods used in statistical determination of spatiotemporal patterns, were used in this study to analyze space-time clustering. Variations in population density were adjusted based on nearest neighbor thresholds. Individual space-time clusters were identified using Kulldorff's scan statistic. Using these methods, 1015 cases of highly significant space-time clustering were identified ($P < 0.001$). Cases diagnosed within 1–4 months of each other showed the highest degree of clustering. McNally's identification of space-time clustering using rigorous statistical tools in a stable population without migration is highly suggestive of transient environmental agent involvement in disease pathogenesis.

In the largest epidemiologic study involving PBC, Gershwin et al interviewed 1032 individuals with PBC and a randomly picked control group [20]. Using multivariate analysis, they were able to show multiple risk factors for developing PBC. These included: having a first degree relative with PBC, recurrent urinary tract infections, past smoking history, use of hormone replacement therapy, and the frequent use of nail polish. Of note, an increased frequency of urinary tract infections has previously been reported in PBC [21]. As will be described in the next section, this association may be one related to molecular mimicry.

5. Molecular data

5.1. Xenobiotics

Xenobiotics are small molecular weight foreign chemicals that can complex with the body's own proteins to alter structure and induce an immunogenic response. These small compounds are found ubiquitously in our environment in household detergents, cleaners, food preservatives, pesticides and pollutants among other things. They have been implicated in the pathogenesis of other autoimmune disease. For example, systemic lupus erythematosis can be induced by the drug hydralazine and scleroderma has been linked to silica exposure [22]. In a similar manner, the involvement of xenobiotics in the pathogenesis of PBC has also been studied. Most xenobiotics are processed by the liver.

The liver is the major detoxification organ of the body and the biliary tree is thereby exposed to high levels of xenobiotics during their clearance. Gershwin et al have extensively studied the immunoenicity of modified forms of PDC-E2, the major autoantigen recognized by anti-mitochondrial antibody. They propose loss of tolerance to PDC-E2, secondary to modification of the autoantigen by xenobiotic conjugation, as a mechanism for triggering autoantibody production (AMA) in PBC [23]. Several studies have implicated halogenated compounds as the mimotope that triggers this cascade of events [24].

In one study, rabbits immunized with 6-bromohexonate, a halogenated xenobiotic complex, all developed AMA that reacted with PDC-E2 and inhibited its enzymatic function [25]. Similar results were seen in guinea pig models [26]. Interestingly, in rabbit models, tolerance was recovered and a gradual reduction in autoantibody production was documented following repeated immunization with the xenobiotic [27]. Other examples of possible xenobiotics include halothane. Halothane is an antiquated anesthetic which oxidizes to a reactive intermediate that binds to cellular proteins leading to tri fluoro acetyls (TFAs). These TFAs not only cause an immunogenic response but also cross react with PDC-E2 [28].

2-octynoic acid has also been identified as having possible in vivo activity in modifying PDC-E2. This xenobiotic is widely used in perfumes, makeup and nail polishes possibly suggesting a linkage for the female predominance of the disease [29]. Additionally as mentioned earlier, an epidemiologic study suggested nail polish use as a risk factor for PBC. Despite the many identified immunogenic xenobiotics, identifying potential causative agents is not an easy task as exposures and molecular modifications likely take place long before a diagnosis of PBC is made. The triggering agent may no longer be present at the time of disease manifestation.

5.2. Microbial exposure

A number of microbes have been implicated in the pathogenesis of PBC. DNA of gram positive bacteria

and LTA, an antigenic bacterial cell wall component, have been detected in the bile of PBC patients. It is postulated that the peri- portal inflammation seen in PBC patients may cause increased ductal permeability. This may lead to leakage of bacterial antigens into the ductal system thereby provoking an immunogenic response [30]. The suggested mechanism of action for microbial exposure causing an immunogenic response is molecular mimicry. This refers to the hypothesis that antigens on bacteria and viruses may be similar enough to host antigens to elicit an autoimmune response by host T cells.

Escherichia coli have been suggested as a possible culprit. This is interesting because epidemiologic studies have shown an increased incidence of urinary tract infections in the PBC population [20,21,31]. As E. coli is the most common cause for these infections; this theory is an attractive one. The PDC-E2 auto-antigen is a highly preserved protein sequence in phylogeny and is found in many prokaryotes. Shimoda et al had shown that a peptide derived from E. coli was able to activate a host response against PDC-E2. These observations further suggest the possibility of molecular mimicry in the initiation of this disease [31]. Those that refute the theory have suggested that titers of antibodies against E. coli are often much less than titers against human complex. They are also often not seen in patients with early PBC but rather in those with advanced disease.

Another gram negative bacterium that has been implicated in PBC is the aerobic *Novosphingobium* aromaticivorans. This microbe is found in soil and water and is non-pathogenic in humans. Studies have shown a high degree of homology between the sequence of human PDC-E2 and lipoylated proteins for N. aromaticivorans. The reactivity against PDC-E2 is also 100 to 1000 fold greater than that seen with E. coli antigens. One study showed 100% reactivity with the bacterium in PBC patients compared to none in the control group [32]. Another study demonstrated that autoreactive AMA and T-cell mediated autoimmunity can be induced in the biliary ducts after immunization with this bacterium [33]. However, this was done in murine models. Interestingly, the bacteria also have a role in activating estrogens suggesting a role in the female predominance of PBC [34]. Furthermore, this bacterium is commonly found in soil and water, again linking with the aforementioned epidemiologic studies of PBC.

A role for viruses in the pathogenesis of PBC has also been explored. One group of investigators was able to isolate human beta retrovirus in the peri-hepatic lymph nodes of PBC patients. The retrovirus bore similarities to a murine mammary tumor and human retrovirus cloned from breast cancer tissue [35]. However this study could not be replicated by other groups. There have also been studies of Propionibacterium, Chlamydia and a case report of lactobacillus as causative factors of PBC; these findings have yet to be reproduced [36–38]. Identifying a single causative microbe in the pathogenesis of PBC is unlikely given the natural course of the disease. It is also probable that there are multiple agents capable of inciting the immune mediated response (Table 3).

Table 3
Proposed antigens in pathogenesis of PBC

Xenobiotics:	Benzene
	Polychlorethylene
	6-bromohexonate
	2-octynoic acid
	Halothane
	Other halogenated hydrocarbons
Microbes:	E. coli
	N. Aromaticivorans
	Human β retrovirus
	Chlamydia
	P. acnes
	Lactobacillus
	Mycobacterium

#5

5.3. Current perspective and future direction

Rather than solving the enigma of PBC, we have presented many of the individual pieces of this still unsolved puzzle. Much of our knowledge is based on observational and circumstantial evidence. While some of the pieces seem to form a cohesive theory others may be a "red herring". The current thought remains that the pathogenesis of PBC is both complex and multi-factorial in nature.

Like other autoimmune conditions, the two hit hypothesis has been suggested in which an already genetically susceptible individual faces a secondary insult triggering the cascade of autoimmune events. However, neither the genetic inheritance pattern nor the inciting triggers are yet clear. Though numerous studies have supported a role for environmental agents such as xenobiotics and microbes, further work is needed to elucidate this disease's development. The development of animal models will likely help us move towards putting the pieces of the puzzle together. Clearly, great strides remain to be made in the study of PBC and other autoimmune diseases.

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Primary biliary cirrhosis: Environmental risk factors

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Abstract. Primary biliary cirrhosis (PBC) is an autoimmune disease of unclear etiology. It is a chronic, progressive condition that causes intrahepatic ductal destruction ultimately leading to symptoms of cholestasis, cirrhosis and liver failure. The disease predominantly affects middle aged Caucasian women. It has a predilection to certain regions and is found in higher incidences in North America and Northern Europe. It also has a genetic predisposition with a concordance rate of 60% among monozygotic twins. Combinations of genetic and environmental factors are proposed in the pathogenesis of this disease with a compelling body of evidence that suggests a role for both these factors. This review will elucidate data on the proposed environmental agents involved the disease's pathogenesis including xenobiotic and microbial exposure and present some of the supporting epidemiologic data.

1. Introduction

Primary biliary cirrhosis (PBC) is a disease of presumed autoimmune etiology that causes inflammation within the portal tracts. This leads to destruction of small and medium sized intrahepatic biliary ducts. Ultimately, this may result in symptoms of cholestasis, cirrhosis and liver failure [1]. The serologic hallmark of the disease is the production of anti- mitochondrial antibodies (AMA), known to react most frequently with the E2 subunit of private dehydrogenase complex among other autoantigens [2]. The disease predominates among women with a 10:1 female to male ratio and is usually diagnosed in middle aged women though it has been diagnosed in patients as young as 15 and as old as 93 [1,3].

The pathogenesis of the disease remains enigmatic since the cause is likely complex and multi-factorial. The current view is that PBC occurs in a genetically susceptible population with external triggers precipitating its development. Considerable evidence points to environmental triggers involved in the development of this disease. In this review, we will focus on observed and circumstantial data pertaining to possible environmental exposures including xenobiotics, and microbes.

2. Genetic predisposition

The prevalence of PBC amongst families with an affected member is estimated to be 1000 times greater than the general population [4,5]. PBC has been weakly associated with the HLA-DRB1*0801 haplotype and a recent study by Hirschfield et al suggests a significant association between HLA class II, IL12A and IL12RB2 loci as well [6]. This is especially interesting as these haplotypes are associated with IL12 immunoregulatory signaling. These findings further affirm the genetic predisposition to this disease.

In 2004, Selmi et al identified 8 pairs of monozygotic twins and 8 pairs of dizygotic twins from a large cohort of patients with PBC. 60% of the monozygotic twins had PBC affecting both siblings while none of the dizygotic twins were dually affected. This concordance rate for the monozygotic twins is one of the highest of all diseases. This study confirms the strong role genetics plays in the pathogenesis of this disease [4]. However, genetics alone are not sufficient to explain the pathogenesis as 3 of the 8 monozygotic twins were not concordant for the disease. There is likely a second trigger.

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Table 1
Selected studies showing geo-clustering of primary biliary cirrhosis [8,9,11–18]

Author(s), Year	Geographic clustering	Number of cases	Site of study	Possible environmental risk factor
Triger, 1980	Yes	34	Sheffield, U.K.	Water
Hamlyn,1983	No	117	Northeast England	Sunlight
Triger, 1984	Yes	552	Western Europe	None
Borda, 1989	Yes	50	Navarra, Spain	None
Danielsson, 1990	Yes	111	Northern Sweden	None
Myszor, 1990	No	347	Northeast England	None
Witt-Sullivan, 1990 [16]	No	225	Ontario, Canada	None
Metcalf, 1997	Yes	160	Newcastle upon Tyne, England	Urban area
Tsuji, 1999	Yes	156	Hiroshima, Japan	Nuclear bomb
Prince, 2001	Yes	770	Northeast England	Urban area
Ala, 2006	Yes	211	New York City	Toxic waste site

3. Disease triggers

PBC demonstrates a large geographic variation in its incidence and prevalence. The incidence of the disease is significantly higher in Northern Europe and North America when compared to Mediterranean and African countries [1]. In addition, disease clustering with and without environmental associations has been reported by several groups (Table 1). Abu- Mouch et al described 4 unique clusters including: a family of 10 siblings with half having PBC, a husband and wife with PBC, a family of two genetically unrelated individuals with PBC, and a cluster of PBC in Alaska [7]. Their data adds to the considerable body of clinical and molecular data suggesting clustering of this disease is associated both with genetic and likely environmental triggers. The epidemiologic and molecular data presented below help support this hypothesis.

4. Epidemiology

The first real suggestion of disease clustering was reported by Triger in 1980 when he found clustering of PBC cases in Sheffield, England. He proposed groundwater contamination as a possible source for this clustering. However, analysis of the suspected water reservoir, showed no significant differences from the other reservoirs in the region [8]. James et al. also proposed water contamination as a possible mechanism of the PBC clustering that his group noted in urban areas of Northeast England [9].

In 2006, we investigated whether our perception of a higher prevalence of PBC from an area known to be developed on landfill sites was accurate. Superfund sites (SFS) are hazardous waste sites designated for clean-up by the Department of Environment and Conservation (DEC). We looked at prevalence rates of PBC near designated SFS in New York City. Data were collected from PBC and PSC patients in the Mount Sinai School of Medicine PBC database (MSSM PBC database). To avoid referral bias, we also collected data from all PBC and PSC patients listed for liver transplantation by the Organ Procurement Transfer Network (OPTN) in the five boroughs of New York City from 1995–2003. In the first part of our study we examined zip codes for all PBC ($n = 99$) and PSC ($n = 73$) patients listed for liver transplant in New York City. Expected prevalence ratios, adjusted for age, gender and race were calculated. Median standardized prevalence ratios in zip codes containing/adjacent to a SFS ($n = 89$) were compared to the zip codes without SFS ($n = 85$). Median prevalence ratios for PBC were significantly higher in clusters associated with SFS compared with PSC (Table 2).

Table 2
Median std prevalence ratio of PBC significantly higher in clusters with SFS

	CLUSTERS WITHOUT SFS	CLUSTERS WITH SFS	P value
PBC	0.51	0.94	0.001
PSC	0.28	0.28	0.572

p: significance values for Mann-Whitney U test, 2-tailed Std prev ratio: observed/std expected prevalence.

In the second part of our study, we applied SaTScan technology (a validated statistical analysis software program looking for disease clusters) to detect specific clusters of PBC near SFS using our OPTN and MSSM PBC databases [10]. Again, an increased prevalence of PBC patients was identified in clusters around SFS. Furthermore, the clusters of PBC in our cohort (MSSM PBC database) correlated with the OPTN data [11]. The major contaminants in these superfund sites were halogenated aromatic hydrocarbons including polychlorethylene and benzene.

Since then many studies continue to show geographic clustering of PBC [8,9,11–18]. McNally et al showed space-time clustering in a population from Northeast England [19]. This phenomenon is interesting as it supports the role for a transient environmental agent in the disease pathogenesis. Space-time clustering is an epidemiologic term used to describe a large number of cases presenting in a small geographic area within a limited time period. When space time clustering is identified, a transient, possibly environmental agent becomes suspect. Using population based data from the northeast regions of England over a 16 year period (1987–2003), space time clustering was noted for PBC. The Knox test and K-function methods used in statistical determination of spatiotemporal patterns, were used in this study to analyze space-time clustering. Variations in population density were adjusted based on nearest neighbor thresholds. Individual space-time clusters were identified using Kulldorff's scan statistic. Using these methods, 1015 cases of highly significant space-time clustering were identified ($P < 0.001$). Cases diagnosed within 1–4 months of each other showed the highest degree of clustering. McNally's identification of space-time clustering using rigorous statistical tools in a stable population without migration is highly suggestive of transient environmental agent involvement in disease pathogenesis.

In the largest epidemiologic study involving PBC, Gershwin et al interviewed 1032 individuals with PBC and a randomly picked control group [20]. Using multivariate analysis, they were able to show multiple risk factors for developing PBC. These included: having a first degree relative with PBC, recurrent urinary tract infections, past smoking history, use of hormone replacement therapy, and the frequent use of nail polish. Of note, an increased frequency of urinary tract infections has previously been reported in PBC [21]. As will be described in the next section, this association may be one related to molecular mimicry.

5. Molecular data

5.1. Xenobiotics

Xenobiotics are small molecular weight foreign chemicals that can complex with the body's own proteins to alter structure and induce an immunogenic response. These small compounds are found ubiquitously in our environment in household detergents, cleaners, food preservatives, pesticides and pollutants among other things. They have been implicated in the pathogenesis of other autoimmune disease. For example, systemic lupus erythematosis can be induced by the drug hydralazine and scleroderma has been linked to silica exposure [22]. In a similar manner, the involvement of xenobiotics in the pathogenesis of PBC has also been studied. Most xenobiotics are processed by the liver.

The liver is the major detoxification organ of the body and the biliary tree is thereby exposed to high levels of xenobiotics during their clearance. Gershwin et al have extensively studied the immunoenicity of modified forms of PDC-E2, the major autoantigen recognized by anti-mitochondrial antibody. They propose loss of tolerance to PDC-E2, secondary to modification of the autoantigen by xenobiotic conjugation, as a mechanism for triggering autoantibody production (AMA) in PBC [23]. Several studies have implicated halogenated compounds as the mimotope that triggers this cascade of events [24].

In one study, rabbits immunized with 6-bromohexonate, a halogenated xenobiotic complex, all developed AMA that reacted with PDC-E2 and inhibited its enzymatic function [25]. Similar results were seen in guinea pig models [26]. Interestingly, in rabbit models, tolerance was recovered and a gradual reduction in autoantibody production was documented following repeated immunization with the xenobiotic [27]. Other examples of possible xenobiotics include halothane. Halothane is an antiquated anesthetic which oxidizes to a reactive intermediate that binds to cellular proteins leading to tri fluoro acetyls (TFAs). These TFAs not only cause an immunogenic response but also cross react with PDC-E2 [28].

2-octynoic acid has also been identified as having possible in vivo activity in modifying PDC-E2. This xenobiotic is widely used in perfumes, makeup and nail polishes possibly suggesting a linkage for the female predominance of the disease [29]. Additionally as mentioned earlier, an epidemiologic study suggested nail polish use as a risk factor for PBC. Despite the many identified immunogenic xenobiotics, identifying potential causative agents is not an easy task as exposures and molecular modifications likely take place long before a diagnosis of PBC is made. The triggering agent may no longer be present at the time of disease manifestation.

5.2. Microbial exposure

A number of microbes have been implicated in the pathogenesis of PBC. DNA of gram positive bacteria

and LTA, an antigenic bacterial cell wall component, have been detected in the bile of PBC patients. It is postulated that the peri- portal inflammation seen in PBC patients may cause increased ductal permeability. This may lead to leakage of bacterial antigens into the ductal system thereby provoking an immunogenic response [30]. The suggested mechanism of action for microbial exposure causing an immunogenic response is molecular mimicry. This refers to the hypothesis that antigens on bacteria and viruses may be similar enough to host antigens to elicit an autoimmune response by host T cells.

Escherichia coli have been suggested as a possible culprit. This is interesting because epidemiologic studies have shown an increased incidence of urinary tract infections in the PBC population [20,21,31]. As E. coli is the most common cause for these infections; this theory is an attractive one. The PDC-E2 auto-antigen is a highly preserved protein sequence in phylogeny and is found in many prokaryotes. Shimoda et al had shown that a peptide derived from E. coli was able to activate a host response against PDC-E2. These observations further suggest the possibility of molecular mimicry in the initiation of this disease [31]. Those that refute the theory have suggested that titers of antibodies against E. coli are often much less than titers against human complex. They are also often not seen in patients with early PBC but rather in those with advanced disease.

Another gram negative bacterium that has been implicated in PBC is the aerobic *Novosphingobium* aromaticivorans. This microbe is found in soil and water and is non-pathogenic in humans. Studies have shown a high degree of homology between the sequence of human PDC-E2 and lipoylated proteins for N. aromaticivorans. The reactivity against PDC-E2 is also 100 to 1000 fold greater than that seen with E. coli antigens. One study showed 100% reactivity with the bacterium in PBC patients compared to none in the control group [32]. Another study demonstrated that autoreactive AMA and T-cell mediated autoimmunity can be induced in the biliary ducts after immunization with this bacterium [33]. However, this was done in murine models. Interestingly, the bacteria also have a role in activating estrogens suggesting a role in the female predominance of PBC [34]. Furthermore, this bacterium is commonly found in soil and water, again linking with the aforementioned epidemiologic studies of PBC.

A role for viruses in the pathogenesis of PBC has also been explored. One group of investigators was able to isolate human beta retrovirus in the peri-hepatic lymph nodes of PBC patients. The retrovirus bore similarities to a murine mammary tumor and human retrovirus cloned from breast cancer tissue [35]. However this study could not be replicated by other groups. There have also been studies of Propionibacterium, Chlamydia and a case report of lactobacillus as causative factors of PBC; these findings have yet to be reproduced [36–38]. Identifying a single causative microbe in the pathogenesis of PBC is unlikely given the natural course of the disease. It is also probable that there are multiple agents capable of inciting the immune mediated response (Table 3).

Table 3
Proposed antigens in pathogenesis of PBC

Xenobiotics:	Benzene
	Polychlorethylene
	6-bromohexonate
	2-octynoic acid
	Halothane
	Other halogenated hydrocarbons
Microbes:	E. coli
	N. Aromaticivorans
	Human β retrovirus
	Chlamydia
	P. acnes
	Lactobacillus
	Mycobacterium

#5

5.3. *Current perspective and future direction*

Rather than solving the enigma of PBC, we have presented many of the individual pieces of this still unsolved puzzle. Much of our knowledge is based on observational and circumstantial evidence. While some of the pieces seem to form a cohesive theory others may be a "red herring". The current thought remains that the pathogenesis of PBC is both complex and multi-factorial in nature.

Like other autoimmune conditions, the two hit hypothesis has been suggested in which an already genetically susceptible individual faces a secondary insult triggering the cascade of autoimmune events. However, neither the genetic inheritance pattern nor the inciting triggers are yet clear. Though numerous studies have supported a role for environmental agents such as xenobiotics and microbes, further work is needed to elucidate this disease's development. The development of animal models will likely help us move towards putting the pieces of the puzzle together. Clearly, great strides remain to be made in the study of PBC and other autoimmune diseases.

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NIOSH Funded Projects

WTC Health Program Research Cooperative Agreements

Research Group

NIOSH has funded research projects and epidemiologic studies designed to help answer critical questions about physical and mental health conditions related to the September 2001 terrorist attacks. The areas of interest for these studies include: biomarkers of exposures or health outcomes; epidemiologic studies; exposure-response relationships; improvements in diagnosis and treatment; patterns of illness (age, gender, etc.); risk factors for disease; and other research studies on WTC-related health conditions or emerging conditions.

Below is a list of projects and studies currently underway. Included in the list is a description, the name of the principle investigator, and contact information for each project:

Agreements Awarded in FY 2013
Agreements Awarded in FY 2012
Agreements Awarded in FY 2011

Information on Research Funding Opportunities

At the current time, there are no research funding opportunities available for the WTC Health Program. We do anticipate that opportunities will be available during 2014. Please refer back to this page for additional information when the opportunity becomes available.

Cooperative Research Agreements Related to the World Trade Center Health Program (U01) (FOA Number PAR-12-126)

The maximum funding period for the FY 14 Announcement "Cooperative Research Agreements Related to the World Trade Center Health Program (U01)" is for up to two years. The table under the Research Objectives in Section I of the announcement **will be** amended to so that the **long-term project period will be up to two years.**

Agreements Awarded in FY 2013

- **Early Identification of World Trade Center Conditions in Adolescents** (See more / See less)
- **Mind Body Treatment for WTC Responders with Comorbid PTSD and Respiratory Illness** (See more / See less)
- **Post-9/11 Incidence of Systemic Autoimmune Diseases in the FDNY Cohort** (See more / See less)
- **Prostate Cancer Risk and Outcome in WTC Respondents** (See more / See less)
- **Trace Elements in Autopsy Tissues from World Trade Center Decedents** (See more / See less)
- **Uncontrolled Lower Respiratory Symptoms in the WTC Survivor Program** (See more / See less)

OVER →

Agreements Awarded in FY 2012

* MYRIS WEBBER, DR.PH

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SPOKE TO DR. WEBBER. HER AUTOIMMUNE STUDIES ONLY INCLUDE RHEUMATOLOGICAL CONDITIONS, NOT PBC.

Lab Order

Mitochondrial (M2) Antibody

Final, Reviewed
Diagnosis:
Ordered by MD on /2014 (Routine)
Collected on '2014 PM
Reported on '2014 PM
Reviewed by MD on '2014 AM

Result Note:
PERFORMED BY: DV LabCorp Denver
8490 Upland Drive
Englewood CO 801127115
8007953699
PERFORMED BY: DA LabCorp Dallas
7777 Forest Lane Suite C350
Dallas TX 752302544
9725986000
PERFORMED BY: BN LabCorp Burlington
1447 York Court
Burlington NC 272153361
8007624344

Test Name	Result	Units	Normal Range	Status
Mitochondrial (M2) Antibody	**(Abn: H)**	**Units**	**0.0-20.0**	**Final, Reviewed**

Note:

Negative	0.0 - 20.0
Equivocal	20.1 - 24.9
Positive	>24.9

Mitochondrial (M2) Antibodies are found in 90-96% of patients with primary biliary cirrhosis.

PENROSE HOSPITAL

#5

2222 N. NEVADA AVE.
COLORADO SPRINGS, CO, 80907

Name:			Specimen Number:
Medical Record Number:	Account Number:	Date of Birth:	Age:
Ordering Physician: MD 7321	Phone #: (719)635-	Gender: Female	Patient location: PHRCU
Collection date: '14 Received date: i4			

SURGICAL PATHOLOGY

SPECIMEN

A. Liver - 1. Liver bx x1

CLINICAL INFORMATION

The patient is a year-old female with elevated liver function tests. Her ALT was in 2013 and AST . ALT was in 2014. There is no history of significant alcohol use. The patient's body mass index is kg/m^2. Hepatitis serologies are reportedly negative. ANA has been negative. AMA is positive at (positive > 24.9). Iron studies were normal. Alpha$_1$-antitrypsin and ceruloplasmin levels were normal.

COPY TO: , MD; , MD

GROSS DESCRIPTION

Received in formalin labeled " liver biopsy x1" is a single cm portion of tan-red tissue, which is wrapped in lens paper and submitted in toto with a liver tag as (A1). '14

MICROSCOPIC DESCRIPTION

The sections of the liver biopsy are stained with H&E, PAS with and without diastase, iron, trichrome, and reticulin stains. Portions of approximately 12 portal tracts are present. The vast majority of the portal tracts are histologically unremarkable without acute, chronic, or granulomatous portal inflammation. Bile ducts are present. Step section 1 demonstrates a small collection of lymphocytes and macrophages in the vicinity of a portal tract. The block was ribboned completely through the entirety of the tissue with every third section stained with H&E. Step section 24 reveals a small collection of lymphocytes and macrophages within a portal tract which appears centered on a bile duct. This same step section also reveals an area of moderate lymphocytic cholangitis with small mature lymphocytes infiltrating bile duct epithelium with resultant bile duct epithelial cell nuclear disarray. There are scattered septal bile ducts within the biopsy which contain rare intraepithelial lymphocytes. There are no bile plugs or bile lakes. The hepatic acini demonstrate scattered, very small, non-necrotizing granulomas. The PAS stains show no fungal organisms within the granulomas, and an acid-fast stain contained a small portion of one granuloma which was negative for acid-fast bacilli. There are also a few areas of unicellular hepatocyte

Patient Name

Case Number

MD

2940 N Circle Dr

Colorado Springs, CO
80909

Copy For: [~ rpt sort key 1 nm]

necrosis characterized by small collections of lymphocytes and macrophages as well as a rare Councilman body. There is minimal steatosis involving less than 5% of hepatocytes. The iron stain is negative. The trichrome stain shows no fibrosis or cirrhosis. 14

FINAL DIAGNOSIS

LIVER, NEEDLE BIOPSY

- Favor non-suppurative destructive cholangitis (primary biliary cirrhosis).
- Grade 1 portal inflammation.
- Grade 1 lobular inflammation.
- Stage 0 fibrosis.
- Please see comment.

COMMENT:

The histologic findings within the liver biopsy are mild and extremely focal. Step section 24, however, demonstrates focal lymphohistiocytic inflammation centered on a bile duct as well as a second interlobular bile duct with a moderate degree of lymphocytic cholangitis. Well-formed granulomas are not identified within portal areas, but there are granulomas within the hepatic acini. The histologic and clinical laboratory findings are most consistent with non-suppurative destructive cholangitis (primary biliary cirrhosis). The histologic findings are not suggestive of chronic viral hepatitis, autoimmune hepatitis, steatohepatitis, or hemochromatosis. 14

CPT CODE(S):

MD

(Electronically signed by) (Final)
Verified: 14

* PLEASE NOTE - NO MICROBES ARE NOTED.

Patient Name

Case Number

MD

2940 N Circle Dr

Colorado Springs, CO
80909

Copy For: [~ rpt sort key 1 nm]

Associates in Gastroenterology, P.C.

AG

2940 N Circle Drive
Colorado Springs, CO 80909
Phone: 719-635-7321
Fax: 719-635-2510

'2014

RE:

To whom it may concern,

This letter is on behalf of She is under my care for Primary Biliary Cirrhosis. The exact cause of this disorder is difficult to determine; nowever, temporally the onset of liver test abnormalities has coincided with her toxic exposures during her volunteer work near "ground zero" after 9/11. It seems that this time of exposure could be the "trigger" for her condition. There is literature to support toxic exposures with the onset of Primary Biliary Cirrhosis.

Sincerely,

MD

From: @mountsinai.org
Subject: RE: 9/11 worker with PBC
Date: , 2014 at
To:

I hope this article helps
I have no data on patients

From:
Sent: , 2014
To:
Subject: 9/11 worker with PBC

Dr. ,

With your recent internet difficulties, I'm not sure if you received my previous email. Please contact me at .

I'd like any numerical data you may have on how many 9/11 workers Mt. Sinai is treating with autoimmune liver conditions and specifically PBC.

I am trying to add autoimmune liver conditions to the list of covered conditions for the World Trade Center Health Program.

Thank you in advance,

---------- Forwarded message ----------
From:
Date: 2014
Subject: Autoimmune PBC
To: " @mountsinai.org" < @mountsinai.org>

Hello

I am a yo 9/11 worker recently diagnosed by liver biopsy with autoimmune PBC. Do you have any medical evidence relating 9/11 workers to PBC, or more generally toxic exposure triggering a PBC response?

NIOSH and the WTC Health Program have certified several of my 9/11 conditions; however, I have to petition the CDC to add Autoimmune PBC to their list of covered conditions. Any related information, contacts, or studies especially involving 9/11 workers would be much appreciated.

I have the liver pathology report and blood work if needed.

Thank you for your time,

Sent from my iPhone

UMR

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