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13. ABSTRACT *(Maximum 200 words)*
We have continued our pulmonary research for JP-8 toxicity over the past three years as well as continued our collaboration with other AFOSR-funded investigators by serving as the primary site for JP-8 jet fuel exposures. This research produced 13 published manuscripts over the past three years along with numerous abstracts. Additionally, we have commercialized our substance P research sponsored by the U.S. Air Force Office of Scientific Research with the formation of immuneRegen Biosciences, Inc., a public company that is listed on the NASDAQ stock market. The major scientific findings from this past three years of research in our laboratory are the establishment of a minimum dose of JP-8 jet fuel (50 mg/mg3) that demonstrates any evidence of pathological injury in the lung terminal bronchioles, co-cultures of pulmonary alveolar macrophages and alveolar type II epithelial cells caused significant differences in cytokine production, demonstration of vast differences in a young mouse lung - vs- old mouse lung in response to JP-8 fuel exposure, lung proteomic studies demonstrating significantly reduced alpha- 1-antitrypsin in mice after a moderate exposure of JP-8 jet fuel of 200 mg/m3 for one hour/day for seven days, and prior JP-8 jet fuel exposure before subsequent infection with the Hong Kong influenza virus significantly increases lung injury compared to Hong Kong virus-only controls.

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FINAL TECHNICAL REPORT FOR THE PERIOD OF MAY 15, 2000 TO November 15, 2003

Chronic Effects of JP-8 Jet Fuel Exposure on the Pulmonary System

Air Force Office of Scientific Research Grant Number F496200010119

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Collaborations with other AFOSR-Funded Scientists-

(1) Dr. David Harris. We exposed numerous mice to JP-8 jet fuel for his immune system studies.

(2) Dr. Frank Witzmann. We are working closely with Dr. Witzmann to characterize various organ systems' proteomic response to JP-8 jet fuel.

(3) Dr. Vijayalaxmi. We are exposing mice to low levels of JP-8 jet fuel for DNA micronuclei studies.

(4) Dr. Mark Smulson. We are working with Dr. Smulson to characterize various organ system's genomic response to JP-8 jet fuel.

(5) Dr. Larry Fechter. We are exposing rats to various levels of JP-8 jet fuel exposure for hearing studies.

(6) Dr. Jeff Fisher. We are conducting pharmacokinetic studies for both aerosol and vapor JP-8 jet fuel exposure scenarios.

Manuscripts Published from 2000-2003 with Key Findings-

(1) Robledo RF, Young RS, Lantz RC, Witten ML: Short-term pulmonary response to inhaled JP-8 jet fuel aerosol in mice. TOXICOLOGIC PATHOLOGY, 2000, 28:656.

We found that JP-8 jet fuel exposures as low as 50 mg/m^3 caused pathological evidence of lung injury at the lung terminal bronchioles.

(2) Kornguth S, McGuire S, Wright L, Bostad E, Nelson S, Daggett D, Witten M, Siegel F: Increased immunoreactivity of glutathione-s-transferase in retina of Swiss-Webster mice following inhalation of JP-8+100 aerosols. ARCHIVES OF TOXICOLOGY, 2000, 74:276.

Kornguth et al. found evidence that oxygen radical production in the retina, as evidenced by increased glutathione-s-transferase levels, increased after JP-8 jet fuel exposure.

(3) Harris DT, Sakiestewa D, Titone D, Robledo RF, Young RS, Witten M: Effects of JP-8 jet fuel exposure on cell-mediated immunity. TOXICOLOGY & INDUSTRIAL HEALTH, 2000, 16:78.

Harris et al. demonstrated that even a single dose of JP-8 jet fuel exposure could alter immune cell-mediated immunity.

(4) Witzmann FA, Bauer MD, Fieno AM, Fultz CD, Grant RA, Keough TW, Kornguth SE, Lacey MP, Siegel FL, Sun Y, Wright LS, Young RS, Witten ML: Proteomic analysis of simulated occupational jet fuel exposure in the kidney. ELECTROPHORESIS, 2000, 21:976.

Witzmann et al. profiled protein changes in the rat kidney after JP-8 jet fuel exposures. My laboratory conducted the JP-8 jet fuel exposures for Dr. Witzmann.

(5) Wang S, Witten ML: Neurokinin-1 (NK_1) receptor activation changes eicosanoid generation of acute respiratory distress syndrome. PROCEEDINGS OF THE 2000 TACHYKININS INTERNATIONAL CONFERENCE, La Grande Motte, France.

A substance P analog, Sar9, Met (O2)11-substance P treatment in our acute diesel fuel smoke exposure model caused decreases in PGE2 and PGI2 in the lungs of rabbits, possibly demonstrating a protective effect for the substance P analog. The Sar9, Met (O2)11-substance P was administered by intravenous methods rather than aerosol delivery. This finding is important since the U.S. Food & Drug Administration wants us to develop a direct injection method for administration of Sar9, Met (O2)11-substance P.

(6) Baldwin CM, Houston FP, Podgornik MN, Young RS, Witten ML: Effects of aerosol-vapor JP-8 jet fuel on the functional observational battery, and learning and memory in the rat. ARCHIVES OF ENVIRONMENTAL HEALTH, 2001, 56:216.

Baldwin et al. demonstrated that JP-8 jet fuel exposure of three weeks in duration caused changes in the functional observational battery tests, especially excitability levels, in adult male rats.

(7) Harris DT, Sakiestewa D, Titone D, Robledo RF, Young RS, Witten M: Substance P as prophylaxis for JP-8 jet fuel-induced immunotoxicity. TOXICOLOGY & INDUSTRIAL HEALTH, 2001, 16:253.

Harris et al. demonstrated that Sar9, Met (O2)11-substance P treatment attenuated JP-8 jet fuel-induced immune system injury in mice.

(8) Wang S, Young RS, Sun NN, Witten ML: In vitro cytokine release from alveolar type II epithelial cells following JP-8 jet fuel culture. TOXICOLOGY, 2002, 173:211.

We demonstrated that co-cultures of pulmonary alveolar macrophages and alveolar type II epithelial cells with JP-8 jet fuel exposure caused significant differences in lung cytokine production. We speculate that these data demonstrate "cross-talk" between pulmonary alveolar macrophages and alveolar type II epithelial cells. This "cell-to-cell" communication may be crucial for the lungs' defense against the damaging effects of JP-8 jet fuel exposure.

(9) Wang S, Young RS, Witten ML: Age-related differences in the pulmonary inflammatory responses to JP-8 jet fuel aerosol inhalation. TOXICOLOGY & INDUSTRIAL HEALTH, 2002, 17:23.

We demonstrated vast differences in young mice's lungs' (6 weeks old) response to JP-8 jet fuel exposure –vs- one year old mice's lungs' response (equivalent to middle-aged humans). This research has vast implications for Air Force personnel who are exposed to continual JP-8 jet fuel exposure throughout their military careers.

(10) Hays AM, Lantz RC, Witten M: Correlation between in vivo and in vitro pulmonary responses to jet propulsion fuel-8 using precision-cut slices and a dynamic organ culture system. TOXICOLOGIC PATHOLOGY, 2003, 31:200.

We have developed an in vitro lung cell culture system to investigate toxic effects of JP-8 jet fuel exposure and possibly utilize the two-photon confocal microscope system in a "real-time, in-line" JP-8 jet fuel exposure scenario in lung slices.

(11) Harris DT, Sakiestewa D, Witten ML: JP-8 jet fuel exposure results in immediate immunotoxicity, which is cumulative over time. TOXICOLOGY & INDUSTRIAL HEALTH, 2003, 18:77.

Harris et al. demonstrated that JP-8 jet fuel exposure has an immediate effect on immune system cells and that repeated JP-8 jet fuel exposures have a cumulative effect on immune system parameters.

(12) Drake M, Hyde J, Witzmann F, Witten ML: Lung proteomic profiles at two different JP-8 exposure levels. TOXICOLOGY, 2003, 191:199.

We investigated changes in lung proteins at low levels of JP-8 jet fuel exposure, 200 mg/m^3 for one hour/day for seven days. We found that this exposure level in mice significantly reduced alpha-1-antitrypsin levels in the lungs. These findings may have very important implications for Air Force personnel since deficiency of alpha-1-antitrypsin in humans is known to contribute to the development of emphysema.

(13) Wong S, Hyde JD, Rowland S, Sun NN, Witten ML: Hong Kong respiratory virus-induced lung injury is potentiated by prior JP-8 jet fuel exposure. TOXICOLOGY (in press).

We have developed a JP-8 jet fuel-exposed influenza respiratory virus, A/Hong Kong/8/68 mouse-adapted virus, model that demonstrates that JP-8 jet fuel exposure prior to respiratory virus infection potentiates the severity of respiratory illness from the influenza virus. The mice were administered 10 microliters of the Hong Kong virus in their nasal passages. On Day +7 after viral inoculation, the JP-8 jet fuel + Hong Kong virus mice demonstrated obvious signs of illness by lethargy, dehydration, decreased

body weight, and Acute Respiratory Distress Syndrome. Recently, we administered our substance P analog, Sar9, Met (O2)11-substance P, after JP-8 jet fuel exposure followed by inoculation with the Hong Kong virus. We demonstrated that our substance P analog significantly decreased lung leukotriene B4 levels by three-fold as well as brought lung inflammatory cell levels to normal values. Additionally, on a pathological basis, treatment with our substance P analog preserved lung airway cilia as well as the airway epithelial cells had normal mitochondria levels. Furthermore, we did not observe any Hong Kong virus virons in the mice treated with our substance P analog, possibly indicating that pulmonary alveolar macrophages were activated to phagocytize the Hong Kong virus virons. These findings have important implications for Severe Acute Respiratory Syndrome (SARS) and Avian Flu outbreaks in Asia as well as Air Force personnel transferred to foreign areas for combat operations where they potentially could encounter SARS, Avian Flu, or other new types of respiratory viruses.

Scientific Personnel Supported by AFOSR Grant-

(1) Mark L. Witten, Ph.D. 75% effort
(2) Simon Wong, M.D. 25% effort
(3) Nina Sun, M.S. 25% effort
(4) Juanita Hyde, M.S. 50% effort

Inventions/Patents/Discoveries

(1) Provisional patent by the University of Arizona concerning the development of a diagnostic test for acute leukemia patients to predict success of chemotherapy treatment.

(2) Provisional patents for SARS, hair replacement treatment, acute radiation syndrome, and respiratory viruses for our substance P analog, Sar9, Met (O2)11-substance P drug compound.

Transitions/Technology Transfers-

We have formed ImmuneRegen Biosciences, Inc. and ImmuneRegen Biosciences-Asia with ImmuneRegen Biosciences, Inc. being a public company as of July 2, 2003. ImmuneRegen Biosciences-Asia was formed in early March 2004 in Singapore. The substance P patents developed under sponsorship of the Air Force Office of Scientific Research were transferred to ImmuneRegen Biosciences, Inc. and the company is pursuing a four-pronged strategy to bring the Sar9, Met (O2)11-substance P drug to market as quickly as possible for the benefit of mankind and for possible use in the War on Terrorism. The drug development process is for the following four applications-

(1) Acute Respiratory Distress Syndrome including the SARS virus. We have signed a consulting agreement with Ever Progressing Systems of Singapore to begin clinical trials in Singapore for Acute Respiratory Distress Syndrome within the next six months.

(2) Attenuation of Cigarette-Smoke Induced Lung Injury. We have signed a consulting agreement with Ever Progressing Systems of Singapore to begin clinical trials in Singapore within the next 6-9 months.

(3) Acute Radiation Syndrome. We have demonstrated a 50% survival rate in mice administered a lethal dose of gamma radiation. We met with the U.S. Food & Drug Administration on March 12, 2004 in Rockville, Maryland. The Division of Counter-Terrorism gave us a 10 page summary of our drug development at this point in time. Two key points were raised by the U.S. FDA and they are the following-

(a) They want us to develop a direct injection method for the administration of Sar9, Met (O2)11-substance P for possible use by the U.S. military against acute radiation exposure.

(b) They want us to develop a rapid-response team to administer the Sar9, Met (O2)11-substance P drug anywhere in the world in response to terrorist attacks with "dirty radioactive bombs".

(4) Hair Replacement Treatment. The gamma radiation-exposed mice also were observed to retain their hair. This is a potential treatment for humans undergoing chemotherapy or radiation treatments for cancer.

The contact for ImmuneRegen Biosciences, Inc. is the following-

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