International Research Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **1(5),** 53-56, Sept. **(2012)** *I. Res. J. Biological Sci.*

Effects of Mold Exposure on Murine Splenic Leukocytes

Gorham K. and Hokeness K.
Department of Science and Technology, Bryant University, Smithfield, RI, USA

Available online at: www.isca.in
Received 7th August 2012, revised 13th August 2012, accepted 16th August 2012

Abstract

The relationship between exposure to mold spores and human disease is only beginning to be understood. While evidence exists of strong correlations between moldy environments and allergic and infectious diseases, the relationship between exposure to specific species and human immune responses to them is not fully understood. This paper seeks to clarify this relationship by analyzing the effects of exposing murine splenic leukocytes to volatile organic compounds (VOCs) produced by different toxic mold species. Here we report that the VOC 1-octen-3-ol can have deleterious toxic effects on the splenic leukocytes and can initiate cytokine production by them which ultimately can contribute to illness including the hypersensitivity diseases that have been observed in individuals exposed to indoor mold for long periods of time.

Keywords: Mold, volatile organic compounds, immune cells, cytokines.

Introduction

The World Health Organization recently put out a study that strongly correlates increased risk of respiratory symptoms and infections with damp and moldy environments[1]. Molds are a type of fungal species that are common in both indoor and outdoor environments. Molds reproduce via sporulation whereby spores are released into the environment and disperse. Under proper conditions they germinate and form new colonies on nutrient-rich substrates. Such substrates can include cellulose, starch, and lignin, which in indoor environments are found in drywall, fabric, food, and many other household items[2].

Several different human illnesses are thought to be caused by exposure to molds. Generally speaking, these illnesses can be categorized based on the type of immune response elicited by such exposure. Hypersensitivity diseases (such as allergies) involve an immune response to typically harmless microbes, while infectious diseases involve immune responses to actively colonizing pathogens. Additionally, certain organizations, such as the Occupational Health and Safety Administration, classify some molds as "irritants" – substances that invoke a temporary immune response that decreases as exposure decreases[3-6]. More specifically, toxins and volatile organic compounds (VOCs) produced by certain fungal species, particularly those associated with damp indoor environments, have been implicated in the condition known as "sick building syndrome" which is comprised of a long list of symptoms commonly exhibited by occupants and/or workers in some buildings, often those that have been exposed to water via flooding, fire (and thereby sprinkler systems), or some other mechanism.

Volatile organic compounds (VOCs) are organic compounds that can vaporize under normal conditions into the atmosphere, and are produced by several black mold species. Although literature on trichothecenes is relatively abundant, research on the effects of VOCs produced by molds is less prevalent. Many VOCs produced by molds are known toxins, and several previously ambiguous species have recently been shown to induce signs of neurotoxicity in invertebrates[7-11].

The work here is aimed at dissecting the effects of a known fungal volatile, 1-octen-3-ol on murine splenic leukocytes. Herein we show that this particular volatile is cytotoxic to leukocytes and can elicit chemokine production from these cells. The increased cell death and the resulting activation of the immune response can in turn lead to an altered immune status and contribute to the adverse health effects seen in those that have been exposed to mold volatiles.

Material and Methods

Isolation of Splenic Leukocytes: Splenic Leukocytes were isolated as follows. Spleens from 4 uninfected C57BL/6 mice (Jackson Laboratories) were harvested into 6-well plates containing 3ml of PBS/Serum. The spleens were then transferred to 0.3uM cell strainers and mechanically digested through the strainer into 3mL of PBS/Serum. The cellular mixture was centrifuged and washed two times with PBS/Serum. The red blood cells in the resulting cellular pellet were lysed using NH4Cl. The cells were then washed and centrifuged again. The total leukocytes isolated were counted and resuspended for separation.

Cell Sorting: Cells were resuspended at a concentration of 106/100ul in cold PBS/Serum. A PE-labeled F4/80 biomarker was used to sort for monocytes. Cells were incubated with PE-F4/80 and control IgG antibodies for 30 minutes on ice. Following cellular staining, the cells were washed twice with cold PBS/Serum. The stained cells were then sorted using a FACS/Aria (BD Biosciences). Purified monocytes were identified as being positive for F4/80. A total of 7.6X106 cells were isolated. Flow cytometry was performed at Brown University.

Trypan Blue Exclusion Assay: Cells were exposed to varying concentrations of 1-octen-3-ol (Sigma Chemicals) ranging from 0-0.1 % total volume for times indicated. Cells were then suspended at a 1:2 dilution in trypan blue dye. Live cells (clear) and dead cells (blue) were counted and the percentage of cell death was calculated based on the total number of cells. This assay was repeated 3 times and a representative graph is shown.

ELISA: One million cells were plated in 6-well plates and exposed to varying concentrations of 1-octen-3-ol ranging from 0-0.1% for times indicated. ELISA was performed to look at levels of secreted Murine Monocyte Chemoattractant Protein-1 (MCP-1) as indicated in the instructions (BD Biosciences). The assay was performed in technical replicate and a representative assay is shown.

Results and Discussion

1-octen-3-ol Decreases the Viability of Monocytes: 1-octen-3-ol is chemically classified as an alcohol and is a compound associated with the musty odor of mold. In invertebrate toxicity studies of thirteen VOCs produced by various mold species, 1-octen-3-ol displayed the most significant levels of toxic effects, killing 100% of *Drosophila melanogaster* subjects within a week of exposure[10]. For this reason, 1-octen-3-ol seems to be the most promising source of toxicity to cells exposed to mold and was chosen to investigate for this study. A monocyte cell population was isolated from the spleens of mice. To test the viability of monocytes after exposure to 1-octen-3-ol, Trypan Blue exclusion dye assay was used. Cells were exposed at concentrations of 0.01%, 0.05%, and 0.10% and incubated for 24 hours. Cell viability was tested at 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours. As expected, increasing VOC concentrations correlated with increased cell death, and longer incubation periods resulted in higher cell death percentages. Effects on cell viability were observed almost immediately (within one hour) and continued until most cells were dead after approximately 24 hours (figure-1).

1-octen-3-ol Induces MCP-1 Secretion by Monocytes: To further investigate the effects of 1-octen-3-ol on monocytes, chemokine production was analyzed. Monocyte chemoattractant protein-1 (MCP-1) is a chemokine that plays an important role in the recruitment of monocytes/macrophages to sites of injury and infection. In viral models, this recruitment occurs via stimulation of the CCR2 chemokine receptor, and increasing concentrations of MCP-1 secreted by immune cells corresponded with the progression of infection[12]. MCP-1 levels in the supernatant of cells exposed to 1-octen-2-ol using ELISA. One million cells were plated and exposed to increasing concentrations of the volatile ranging from 0-0.1% for varying exposure times. Results were congruent with viability result whereby increased volatile exposure corresponded with an increase in the levels of MCP-1 produced by the F4/80+ monoctyes. Significant levels of MCP-1 were observed within one hour, and production of the chemokine increased steadily over 24 hours. The increase in chemokine production is indicative of stimulation of the immune response by the fungal volatile 1-octen-3-ol figure-2.

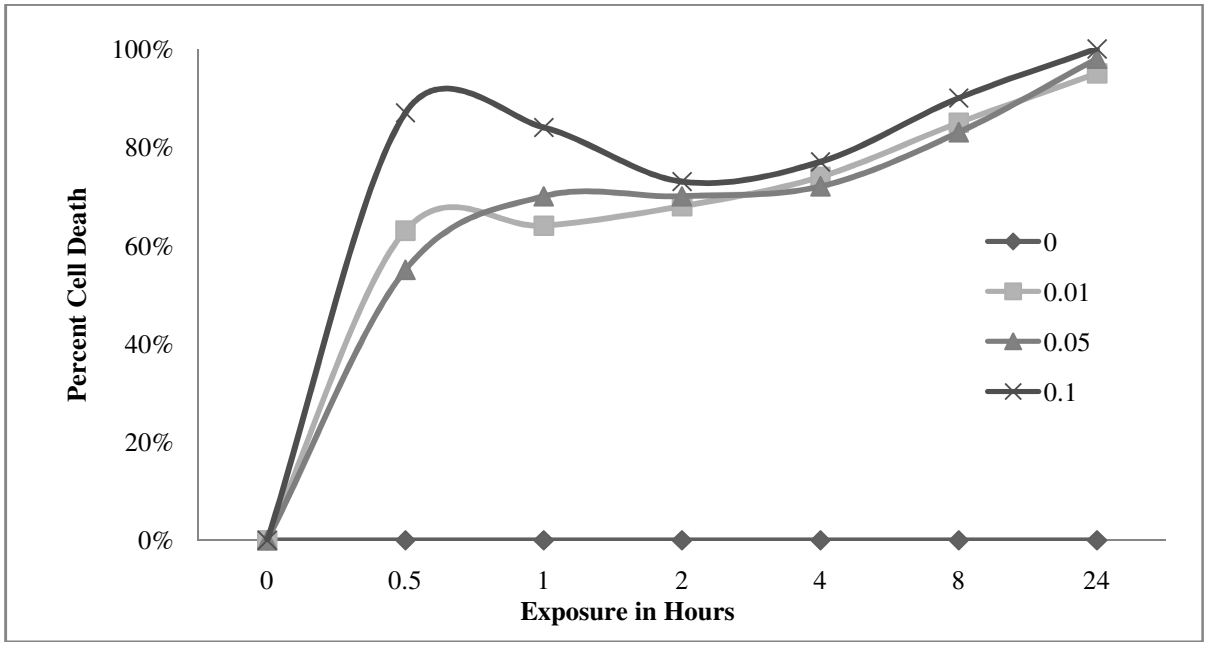

Figure-1
Toxicity of 1-octen-3-ol on murine splenic monocytes, Monocytes were exposed to 1-octen-3-ol for times ranging from 0.5-25 hours at concentrations of 0-0.1% total volume, Increased cell death is observed as exposure time and dose increases

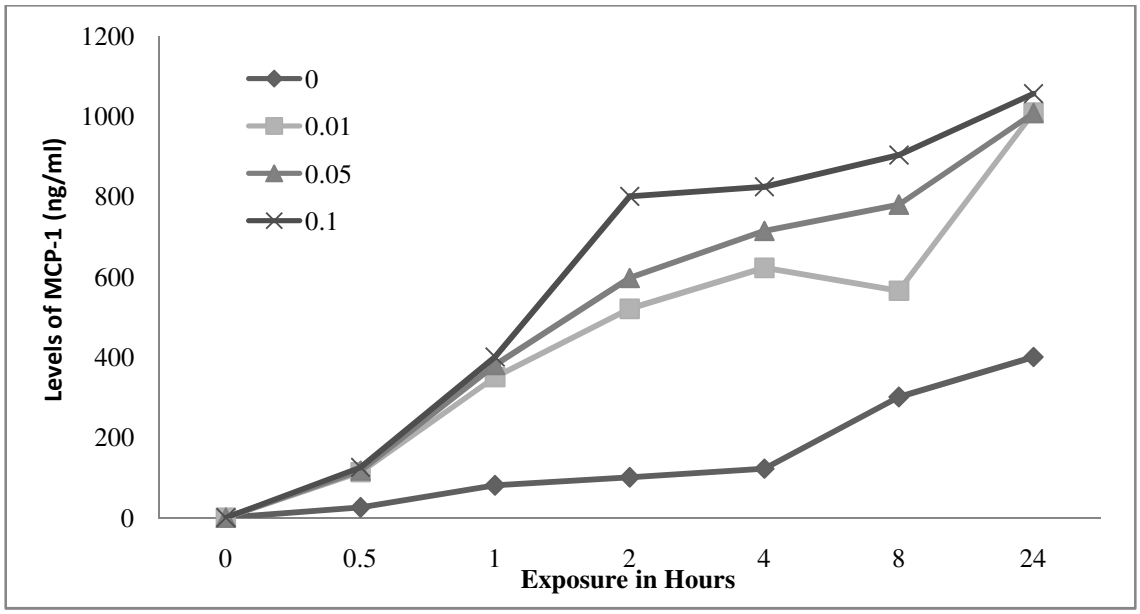

Figure-2

MCP-1 Production by Splenic Monocytes Exposed to 1-octen-3-ol. Monoctyes were incubated with varying concentrations of 1-octen-3-ol (0-0.1%) for increasing amounts of time (0-24 hours). MCP-1 was produced by the monocytes in a dose-dependent manner in response to VOC exposure

Conclusion

To date, the mechanisms by which molds can cause disease and the observed adverse health effects seen in those individuals exposed to moldy environments is poorly understood. The elucidation of these mechanisms is critical for the development of appropriate and effective treatment strategies. This study represents one of the first studies to examine the effects of the VOC 1-octen-3-ol on immune cells and their function. As shown, exposure of murine splenic monocytes to 1-octen-3-ol severely decreased the viability of murine monocytes. Significant decreases in viability were seen almost immediately, and continued steadily until the majority of cells were dead after 24 hours.

These results begin to offer some explanation for the immunosuppressive effects of mold exposure whereby decreasing monocyte populations can certainly negatively affect the body's ability to mount an effective immune response and could lead to altered immune states and the described poor health.

Similarly, an increase in MCP-1 production by monocytes was observed in cells exposed to 1-octen-3-ol. Since MCP-1 is involved with the recruitment of macrophages to sites of infection and injury, the overproduction of MCP-1 in response to exposure to the VOC represents a possible last-ditch effort by monocytes to call in other immune cells. This overproduction might also contribute to cell viability results described earlier as cytokines are often toxic to immune cells and tissues when produced in large enough doses. Increasing concentrations of MCP-1 may also be involved in the kinds of hypersensitive immune responses observed in cases of mold allergies as overproduction of cytokines and increased immune cell recruitment could lead to overstimulation of the immune response.

The work presented here begins to dissect the potential mechanisms whereby mold exposure can lead to altered health. It is consistent with previous studies in which mold exposure can affect immune function in a variety of cells[13-15] however it is the first to describe the effects of VOCs on immune cell activity. Currently, the field of biological sciences has a strong focus on antimicrobial discovery and treatment[16-20]. These studies also begin to provide a solid mechanistic link to mold exposure and health which is critical as to date the links have been casual at best. Such advances in knowledge will help shape policies on mold prevention and remediation and could thus lead to better health outcomes worldwide.

Acknowledgements

The project described was supported by grants from the National Center for Research Resources (5P20RR016457-11) and the National Institute for General Medical Science (8 P20 GM103430-11), components of the National Institutes of Health (NIH), and its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIGMS or the NIH. We would also like to thank the Salazar-Mather lab at Brown University for their help with the animal work.

References

1. World Health Organization, Guidelines for indoor air quality: dampness and mould, (eds: E. Heseltine and J. Rosen), Druckartner Moser, Germany **(2009)**

2. Bennett J.W. and Clich M., Mycotoxins, *Clinical Microbiology Review.*, **16,** 497-516 **(2003)**

3. Bush R.K., Portnoy J.M., Saxon A., Terr A.I. and Wood R.A., The medical effects of mold exposure, *Journal of Allergy and Clinical Immunology*, **117,** 326-333 **(2006)**

4. Andriessen J.W., Brunekreef B. and Roemer W., Home dampness and respiratory health status in European children, *Clinical and Experimental Allergy*, **28,** 1191-1200 **(1998)**

5. Greenberger P.A., Allergic bronchopulmonary aspergillosis, *Journal of Allergy and Clinical Immunology*, **110,** 685-692 **(2002)**

6. Luong A. and Marple B.F., Allergic fungal rhinosinusitis, *Current Allergy and Asthma Reports*, **4,** 465-470 **(2004)**

7. Hung F. and Clark R.F., Health effects of mycotoxins: a toxological overview, *Journal of Toxicology: Clinical Toxicology*, **42,** 217-34 **(2004)**

8. Kankkunen P., Rintahaka J., Aalto A., Leino M., Majuri M., Alenius H., Wolff H. and Matikainen S., Trichothecene mycotoxins activate inflammatory response in human macrophages, *Journal of Immunology*, **182,** 6418-6425 **(2009)**

9. Paananen A., Mikkola R., Sareneva T., Matikainen S., Andersson M., Julkunen I., Salkinoja-Salonen M.S. and Timonen T., Inhibition of Human NK Cell Function by Valinomycin, a Toxin from Streptomyces griseus in Indoor Air, *Infection and Immunity*, **68,** 165-169 **(2000)**

10. Inamdar A.A., Masurekar P. and Bennett J.W., Neurotoxicity of fungal volatile organic compounds in Drosophila Melanogaster, *Toxicol Sci*., **117(2),** 418-26 **(2010)**

11. Thompson W.L. and Wannemacher R.W. Jr., Structure-function relationship of 12, 13-epoxytrichothecene mycotoxins in cell culture: comparison of whole animal lethality, *Toxicon*, **24,** 985-994 **(1986)**

12. Salazar-Mather T.P., and Hokeness K.L., *Curr Top Microbiol Immunol*., **303,** 29-46 **(2006)**

13. Paananen A., Mikkola R., Sareneva T., Matikainen S., Andersson M., Julkunen I., Salkinoja-Salonen M.S. and Timonen T., Inhibition of human NK cell function by valinomycin, a toxin from Streptomyces griseus in indoor air, *Infect and Immun*., **68(1),** 165-9 **(2000)**

14. Bondy G.S. and Pestka J.J., Immunomodulation by fungal toxins*, J Toxicol Environ Health B Crit Rev.*, **3(2),** 109-43 **(2000)**

15. Methenitou G., Maravelias C., Athanaselis S., Dona A. and Koutselinis A., Immunomodulative effects of aflatoxins and selenium on human natural killer cells, *Vet Hum Toxicol,* **43(4),** 232-4 **(2001)**

16. Nakade Dhanray B., Antibiotic sensitivity of common bacterial pathogens against selected Quinolones, *ISCA J. Biological Sci.,* **1(1),** 77-79 **(2012)**

17. Shaziya Bi and Goyal P.K., Anthelmintic effect of Natural Plant (Carica papaya) extract against the Gastrointestinal nematode, Ancylostoma caninum in Mice, *ISCA J. Biological Sci.,* **1(1),** 2-6 **(2012)**

18. Masih Usha, Shrimali Ragini and Naqvi S.M.A., Antibacterial Activity of Acetone and Ethanol Extracts of Cinnamon (Cinnamomum zeylanicum) and Ajowan (Trachyspermum ammi) on four Food Spoilage Bacteria, *ISCA Res. J. Biological Sci.,* **1(4),** 7-11 **(2012)**

19. Illath Sujina and Subban Ravi, In-vitro Antimicrobial and Cytotoxic ativity of Methanolic extract of Osbeckia wynaadensis, *ISCA. Res. J. Biological Sci.,* **1(4),** 33-38 **(2012)**

20. Prajapati Ajaypal, Synthesis, Antimicrobial and Insecticidal Activity Studies of 5-Nitro N'-[Arylidenhydrazidomethyl Indole] 2-(Substituted Aryl)-3-(N'-Indolyl Acetamiddyl)-4-Oxothiazolidines**,** *Res. J. Recent Sci.,* **1,** 99-104 **(2012)**