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Research Article

Beyond Single-Agent Toxicology: Two-Hit Models as a Framework for Understanding Environmentally Driven Immune Dysregulation

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Abstract

Persistent organic pollutants (POPs) continue to pose significant public health challenges despite their regulatory restrictions worldwide. Due to their environmental persistence, bioaccumulation, and biomagnification, POPs continue to expose both humans and animals to low doses through contaminated food, water, and the environment. Emerging evidence suggests that environmentally driven diseases arise from the interaction of multiple exposures over time rather than from single toxicants alone. Traditionally, toxicological studies of pesticides have focused on well-recognized end points such as neurotoxicity, carcinogenicity, and reproductive toxicity etc. In contrast, the long-term effects of these chemicals on the immune system, particularly in non-target organs, remain relatively unexplored. Recent studies indicate that long exposure to environmental contaminates can alter immune signaling, leading to immune dysregulation and altered responses to subsequent infectious or inflammatory insults. Supporting this concept, recent findings from a two-hit murine model demonstrate that dietary γ-hexachlorocyclohexane (γ-HCH; lindane) exposure reshapes pulmonary immune responsiveness to a later endotoxin challenge. The study provides evidence that subchronic chemical exposure can alter innate immune signaling and immune dysfunction rather than simply cause direct toxicity. It also highlight the need to move beyond traditional single-agent toxicology and adopt experimental model that better reflect real-world exposure scenarios in environmental health research.

Keywords: Environmental immunotoxicology; Pesticide; γ-hexachlorocyclohexane; Innate immunity; Mouse model; Pulmonary inflammation

Commentary

For decades, environmental health research has traditionally focused on the direct toxic effects of individual chemicals on specific organs, including nervous system, kidney, reproductive organs etc. Accordingly, regulatory frameworks classify chemicals primarily based on endpoints such as neurotoxicity, reproductive toxicity or carcinogenicity. While these endpoints remain essential, an equally important yet comparatively neglected aspect is the ability of environmental contaminants to modify immune function. Usually, single agent exposure system does not fully reflect the complexity of the real world, where people constantly encounter multiple chemical, biological, and physical stressors. Chronic health issues usually originate from prolonged simultaneous or sequential exposure to multiple environmental contaminants [1,2]. This concept is particularly relevant for persistent organic pollutants (POPs), which continue to circulate in ecosystems despite restrictions on their use [3]. Owing to their lipophilic nature and

resistance to degradation, POPs accumulate in soil, water, wildlife, livestock, and ultimately in the human food chain. Consequently, dietary exposure has become the primary route of exposure to many organochlorine pesticides, including γ-hexachlorocyclohexane (γ-HCH; lindane) [4]. To better understand the health risk associated with these chemicals, environmental toxicology must move beyond single-agent models toward frameworks that account for multiple, sequential, or concurrent environmental insults. Within this context, the study by Tewari et al. [5] makes a significant contribution to immunotoxicology by demonstrating that subchronic dietary exposure to γ-hexachlorocyclohexane (γ-HCH) modifies pulmonary immune responsiveness to a subsequent bacterial challenge (LPS).

The key strength of this study is its realistic, sequential two-hit experimental model. It tested a prolonged low-dose dietary exposure to γ-HCH, followed by an acute endotoxin exposure. This approach more accurately mimics environmental conditions for agricultural workers, livestock handlers, and rural residents, who routinely encounter both pesticides and infectious agents [2]. This dualistic exposure is highly relevant in agrarian countries like India, where a large portion of the population and workforce lives in rural areas. Consequently, understanding how one exposure modulates responsiveness to another is increasingly critical for risk assessment and disease prediction.

These findings suggest that prolonged low dose γ-HCH exposure triggers a state of immune priming rather than simple toxicity. This is defined by elevated levels of inflammatory mediators, increased TLR4 expression, enhanced neutrophil recruitment, and structural changes in pulmonary tissues [4]. Notably, the subsequent LPS challenge did not uniformly amplify inflammatory responses than those observed in LPS-only groups. Instead, it produced a mixed effect, with many immune responses altered, dysregulated, or partially attenuated. These results point to an activated yet functionally compromised immune state, in which activated inflammatory signaling may coexist with impaired immune competence. It shows that combined exposures do not always result in additive effects; rather, they may produce unexpected patterns of immune dysregulation that traditional experimental designs could overlook. Multi-hit models therefore provide a more realistic approach for studying the non-linear interactions that occur under real-life exposure conditions.

The study also provides valuable mechanistic insights by focusing on TLR4-mediated innate immune signaling. Toll-like receptors (TLRs) are pattern-recognition receptors that detect both pathogen-associated molecular patterns (PAMPs) and endogenous danger-associated molecular patterns (DAMPs). Environmental pollutants capable of inducing oxidative stress can promote DAMP release from injured tissues, thereby activating TLR-mediated signaling even in the absence of infection [2,6]. In the γ-HCH model, increased expression of TLR4 was accompanied by higher production of pro-inflammatory cytokines (IL-1β and TNF-α) and greater recruitment of neutrophils and macrophages in lungs. These findings suggest that TLR4-dependent innate immune pathways may play an important role in pollutant-induced immune reprogramming [7]. Nevertheless, the current evidence remains largely associative, and direct mechanistic links have yet to be established.

Another important consideration is the distinction between immune activation and protective immunity. Elevated cytokine production or inflammatory cell recruitment are frequently interpreted as evidence of stronger immune response. However, an exaggerated or poorly regulated inflammatory response does not necessarily improve host defense. Instead, it may cause tissue damage while failing to enhance pathogen clearance. The γ-HCH two-hit model clearly illustrates this complexity, demonstrating increased inflammatory signaling without evidence of proportionally improved protective responses following endotoxin exposure. Future studies using receptor antagonists, TLR4-deficient animal models, transcriptomic analyses, and pathway-specific interventions will be necessary to establish causal relationships and identify the exact molecular mechanism involved.

Another important aspect of the study is the association of systemic immune crosstalk following oral exposure to environmental toxicant. The observation that dietary γ-HCH influences pulmonary homeostasis indicates systemic immune communication beyond local toxicity. One possible mechanism is the gut-lung axis, where changes in gut microflora, microbial metabolites, and intestinal barrier function shape immune responses to in the lungs [8,9]. Future studies integrating microbiome analyses, metabolomics, and systems could provide a more comprehensive understanding of the pathways connecting environmental contaminants to pulmonary immunity.

The study further emphasizes the need to progress beyond descriptive inflammatory markers and pursue functional assessments of immune competence. Although changes in cytokine profiles, histopathology, and receptor expression yield insightful information, subsequent studies must assess whether pollutant-induced immune changes affect host defense, including responses to infections, vaccination, and lung function. Such functional assessments will clarify whether the observed molecular changes translate into meaningful biological outcomes. Another limitation that deserves attention is the sex-specific responses. Since only male mice were used in this study, it remains unclear whether females would respond similarly. Including both sexes in future research will be key to developing a complete understanding of pollutant-induced immune dysfunction.

More broadly, the study is consistent with the emerging concept of the exposome, which recognizes that health outcomes are determined by cumulative exposures over time rather than individual agents [10]. The two-hit model described here provides a practical framework for translating exposome theory into mechanistic research. Applying this approach to other pollutants, environmental stressors, and infectious agents may improve our knowledge of environmentally driven disease susceptibility.

Conclusion

In conclusion, Tewari et al. [5] demonstrate compelling

evidence that subchronic γ-HCH exposure can modify pulmonary immune responses to a secondary inflammatory challenge. By advancing past traditional single-agent toxicology and adopting a biologically relevant two-hit framework, the study gives us a much clearer picture of how stubborn environmental pollutants actually hamper our immune systems in the real world. These findings add to the expanding field of environmental immunotoxicology and provide a foundation for future research into the complex relationships among environmental exposures, immune function, and disease risk.

Acknowledgement

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Conflict of Interest

No Conflict of interest.

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