Gordon Research Conferences

Meeting Details

Phagocytes

Gordon Research Conference

The Behaviour and Function of Phagocytes in Health and Disease

Dates

May 31 - June 5, 2015

Location

Waterville Valley
Waterville Valley, NH

Organizers

Chair:
Paul Kubes

Vice Chair:
Dianne Cox

Meeting Description

The 2015 Phagocytes Gordon Conference will bring together scientists from around the world to discuss the most recent advances in the area of myeloid cells during homeostasis and during disease. Expert invited speakers will present their latest unpublished research related to the many facets of phagocyte biology. There will be 4 major topics across 4 days starting with phagocytosis, the formation of the phagosome and regulation of these events culminating with a talk on negative regulation of Fc receptors. The second day will discuss the very dynamic placticity of monocytes and macrophage ending with focus on specialized macrophage including a talk on the newly identified GATA6 lineage of macrophage. The third day will focus on new ways of killing of pathogens and dying of immune cells ending with talks about the release and life span of neutrophils using humans as the experimental model. The program will end with some exciting new imaging of innate immunity ranging from visualizing the heart, kidney, lung and other organs and the war between pathogens and myeloid cells. Speakers will also highlight areas of translation to humans. The program is designed to encourage vigorous discussion and the free exchange of ideas. Thus, each scientific session will be chaired by a prominent researcher who will highlight recent progress and knowledge gaps related to each topic, and guide subsequent discussion. As participation of junior scientists is a key goal of the meeting, the conference will also include two interactive sessions for presentation of ~90 posters, and additional speakers will be chosen from among the submitted abstracts. The collegial atmosphere of this conference is exemplified by the shared meals, and the substantial time provided for informal gatherings in the afternoons and evenings to further promote interactions between attendees at all levels. The overall environment is therefore designed to provide multiple opportunities for learning and for the development of exciting collaborations.

To facilitate the participation of students and postdoctoral fellows, the conference will be preceded by a Gordon Research Seminar (GRS). The **Phagocytes GRS** is planned by and for trainees, and provides an opportunity for our future leaders to present their research via seminars and posters in a supportive and interactive environment. The GRS has been well received and adds substantial value to the Gordon Research Conference experience for these junior investigators.

Related Meeting

Multi-walled carbon nanotubes activate macrophages and NF-κB signaling to elicit inflammatory and fibrotic responses in mouse lungs

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Abstract

Macrophages play multiple roles in the pulmonary response to deposition of foreign bodies, such as particles, fibers, and microbes, in the lungs. As the major first line of defenders, macrophages phagocytize and kill in the case of microorganisms, or transport for clearance in the case of particles and fibers. Macrophages also secrete numerous soluble mediators to stimulate inflammatory and fibrotic responses to facilitate clearance and tissue repair. Under pathologic conditions, the responses become exacerbated and pulmonary fibrosis takes place, causing excessive deposition of collagen fibers and destruction of lung structures. Carbon nanotubes (CNT) are new nanomaterials made of one-atom-thick carbon nanotubes in either a single layer (SWCNT) or concentric multiple layers (MWCNT). CNT have been developed for a wide range of industrial and commercial applications. However, their small size and fiber-like shape make them respirable and potentially pathogenic, causing adverse effects in the lungs. Understanding the health effects of exposure to CNT has become increasingly important in nano safety. We found that MWCNT administered to mice by pharyngeal aspiration elicited significant inflammatory and fibrotic pathology in the lungs. The early phase response was characterized by prominent inflammatory infiltration, elevated levels of proinflammatory cytokines in the BAL fluid and lung parenchyma, and increased deposition of collagen fibers in the interstitial space. Macrophages were a dominant cell type during inflammatory infiltration in the early phase response and for the formation of granulomas and chronic fibrosis. In cultured macrophages, MWCNT directly stimulated secretion of proinflammatory and profibrotic cytokines and growth factors, and activation of the NF-κB pathway. Our study provided direct evidence for a critical role of lung macrophages in the development of pulmonary fibrosis following exposure to carbon nanotubes.

Results

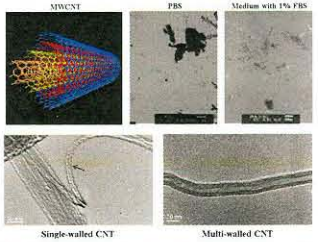

Figure 1. Illustration, dispersion, and scanning electron microscopy of CNT.

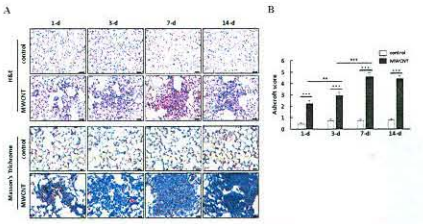

Figure 2. Time course analysis of fibrotic response to MWCNT in mouse lungs. (A) H&E and Mason's Trichrome stainings. (B) Ashcroft score of fibrosis. Mice were dosed at 40 μg/mouse. MWCNT were shown as black deposits.

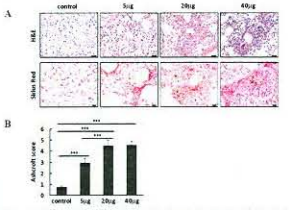

Figure 3. Dose response analysis of fibrotic response to MWCNT in mouse lungs. (A) H&E and Sirius Red stainings. (B) Ashcroft score of fibrosis. Fibrosis was analyzed at 7 days post-exposure.

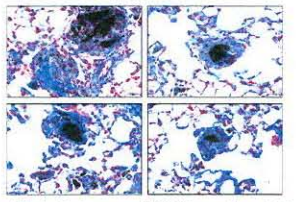

Figure 4. Chronic fibrotic response to MWCNT in mouse lungs at 28 days and 56 days post-exposure (40 μg/mouse). Mason's Trichrome staining. MWCNT were shown as black deposits.

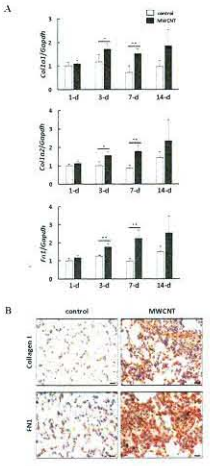

Figure 5. Expression of fibrotic marker genes. (A) mRNA expression by real-time PCR. (B) Immunohistochemistry staining of Collagen 1 and FN1. 40 μg/mouse, 7 days.

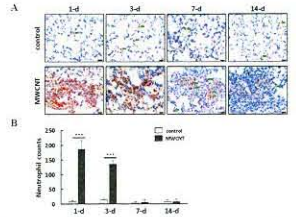

Figure 6. Rapid infiltration of neutrophils. (A) Immunohistochemistry staining of neutrophils with anti-Ly-6G and anti-Ly-6C. (B) Quantification.

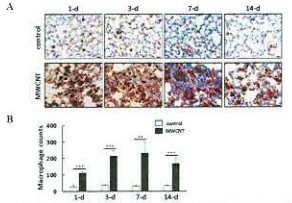

Figure 7. Rapid infiltration of macrophages. (A) Immunohistochemistry staining of macrophages with anti-Mac-2 antibody. (B) Quantification.

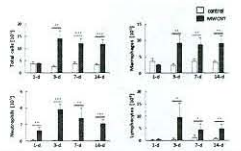

Figure 8. BAL cells were analyzed from mice dosed with MWCNT at 40 μg/mouse.

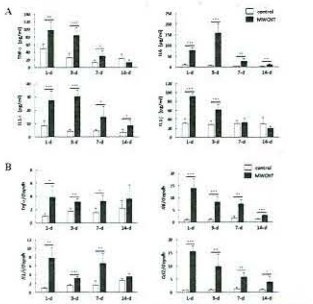

Figure 9. (A) Cytokine protein levels in BAL fluid from mice treated with DM or MWCNT (40 μg/mouse) by ELISA. (B) mRNA expression in the lungs by real-time PCR.

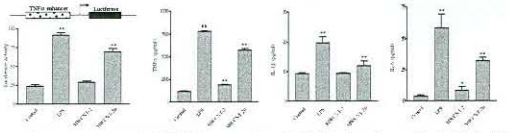

Figure 10. Induction of cytokines by MWCNT in macrophages. RAW264.7 cells stably transfected with the TNFα promotoer/luciferase reporter were treated with MWCNT at 0, 2, and 20 μg/ml for 16 hr. LPS at 1 μg/ml was used as a positive control. Expression of luciferase or cytokines were measured from cell-free culture media.

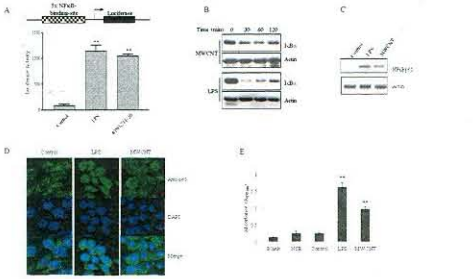

Figure 11. MWCNT activate NF-κB in macrophages. RAW264.7 cells stably transfected with a luciferase expression construct under the control of 5X NF-κB binding sites were treated with MWCNT or LPS. (A) Induction of luciferase expression. (B) Induction of IκBα degradation. (C) Nuclear enrichment of NF-κBp65 protein. (D) Immunofluorescent imaging of NF-κBp65 nuclear localization by MWCNT. (E) Binding of NF-κB to DNA quantified by ELISA.

Methods

Male C57BL/6J mice (8-10 weeks old) from Jackson Laboratories were treated with 50μl of vehicle control (DM) or MWCNT solution by pharyngeal aspiration.

H&E, Mason's Trichrome, and Picro-Sirius Red stainings were performed using standard procedures.

mRNA isolation and qRT-PCR were carried out using QIAGEN kits.

Immunohistochemistry of formalin-fixed paraffin-embedded lung tissue sections was performed with ImmPRESS Polymer Detection kits (Vector Laboratories).

ELISA analysis was performed using kits obtained from R&D System.

The significance of the differences between groups was calculated using standard statistical methods.

Conclusions

MWCNT potently induced rapid pulmonary fibrosis upon a single exposure at low doses in C57BL/6J mice, which progressed to granuloma formation and chronic fibrosis.

MWCNT exposure elicited a dramatic inflammatory response in the early phase, which appeared to resolve in the chronic phase.

Macrophages were major effector and regulator cells recruited during the acute phase to mediate the acute inflammatory response, and transformed to epithelioid cells to promote granuloma formation in the chronic phase, in the lungs exposed to MWCNT.

MWCNT activated macrophages *in vitro* to secret proinflammatory and profibrotic cytokines, which in part is mediated through NF-κB-controlled gene transcription.