Diesel exhaust compounds affect regulatory mechanisms of the immune system

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Diesel exhaust particles (DEP) have been shown to affect parameters of the immune system in many different assays, including mouse models and human exposure studies. This is to be expected, since the immune system is receptive to external influences, and responds in various ways. The modulation of immune parameters by air pollutants is a health problem, since the immune system is unable to mount meaningful responses towards pollutants, and the reactions induced are therefore at best useless and at worst detrimental. Examples for misregulation of the immune system are allergy, asthma, autoimmunity, and uncontrolled inflammation, where in various ways immune activation targets the wrong subjects, or immune suppression, where insufficient defenses are raised against infections.

Immune responses under exposure to pollutants could in principle be followed with mice, but these tests are expensive, time-consuming and technically difficult. Furthermore, screening of different engines, fuels etc. would have to be done in engineering facilities which are not equipped for animal experiments, while normal biological labs are clearly unable to run engine tests. A robust bioassay which does no require animals, is sensitive enough to give meaningful data, and can be performed in an engineering facility would be desirable.

We are working on the establishment of bioassays for measuring cytokines. Cytokines are immunoregulatory proteins which are used by the various immune cells to communicate with each other. By looking for cytokines it is possible to determine how active the immune system is and what kind of response is occurring. Out of the many different cytokines used by the immune system, we have selected a few key cytokines which will be measured.

Our strategy is to measure not the level of cytokine as such, but to determine how active the production of the cytokine is occurring. This can be measured in cell culture with human cell lines. Using genetic engineering methods, we have established cell lines which produce the enzyme Luciferase whenever one particular is cytokine is produced. This enzyme can induce the production of light (its normal place of work is in fireflies), which can readily be detected and quantitated. Once the appropriate cell lines have been produced, the method requires just basic cell culture equipment and can be performed after some training by any technician.

So far, we have produced cell lines for the cytokines IL-4 (involved in allergy and asthma), IL-8 (involved in cellular stress and inflammation) and IFN-γ (involved in antibacterial defense and autoimmunity). DEP were added at concentrations up to 125

μg/ml, which was not toxic for the cells as determined by MTT assay. By themselves, DEP did not influence the production of all three tested cytokines, but when additional stimuli were provided, costimulation with DEP reduced production of IL-4 and IFN-γ, and enhanced production of IL-8.

These cell lines, as well as other cell lines to be developed, will be exposed not only to collected DEP, but also on-line to freshly produced Diesel exhaust, including particles, volatile compounds and gas phase. The on-line exposure will be done at CERTAM facilities at Rouen. Parallel to the cell lines, mice will be exposed as well, to test whether the cell lines give responses in line to animal data. If such cell lines can be shown to give biologically meaningful data, they may present a suitable biosystem which is simple enough to be used in engineering contexts, but still gives useful answers to complex questions.

On-line exposures are in cooperation with Dr. Jean-Paul Morin (INSERM E9920) and Dr. Frederic Dionnet (CERTAM) at Rouen, France. This project is supported by the EU project MAAPHRI, QLK4-CT-2002-02357.

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Air pollution and immunity

- Environmental pollution has long been linked with allergy, but the epidemiological evidence is ambiguous
- Mouse models show a clear increase of allergic parameters when mice are exposed to DEP or organic extracts
- Polycyclic aromatic hydrocarbons (PAH) and transition metals affect the immune system, but the mechanisms are largely unknown

Los Angeles 1972, © both pictures EPA

Immune modulation

- **Allergy:** Hypersensitivity of the immune system against harmless agents
- **Autoimmunity:** Immune system attacks elements of the own body
- **Inflammation:** Immune activation, often by unspecific stimuli (cellular stress)
- **Immune suppression:** No defense against bacteria, viruses or parasites

Some problems with mice

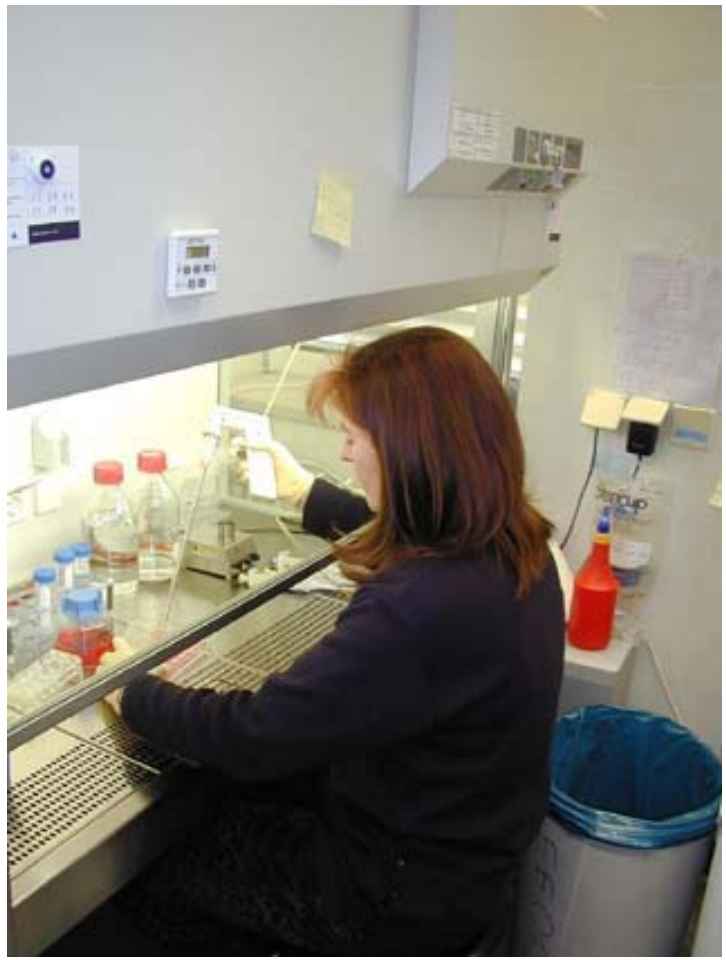
- The most frequently used mouse strain in pollutant studies is BALB/c. These mice are severe *atopics*, i.e. they develop allergies very easily
- Other diseases would require other mouse strains
- Monitoring at real-world particle concentrations would require a large number of animal experiments – not compatible with EU or US requirements

BALB/c mouse

Cell culture as an alternative

- + No animals needed
- + Cheaper
- + Faster
- + Right species
- + Easier to learn

- - A cell culture is not an organism
- - Problematic for multi-organ diseases
- - Some equipment needed



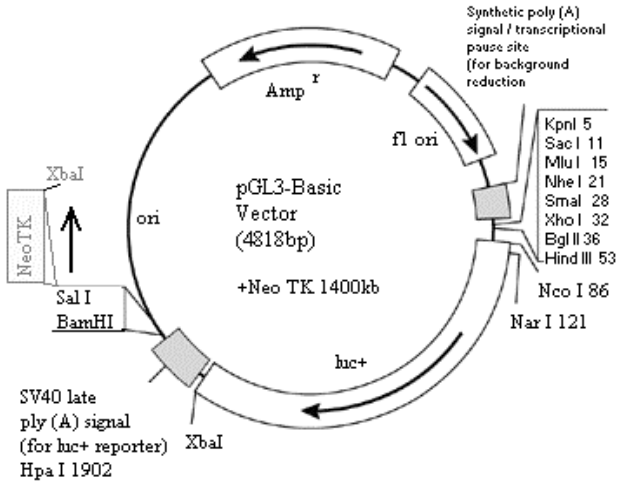
What should actually be measured?

- Immune cells communicate by secreting soluble proteins (Cytokines)
- The cytokines produced reflect the immune status
- Cytokines can be fairly well measured, but the assays are often not robust or sensitive enough

"Biochemistry", Jutta Duschl

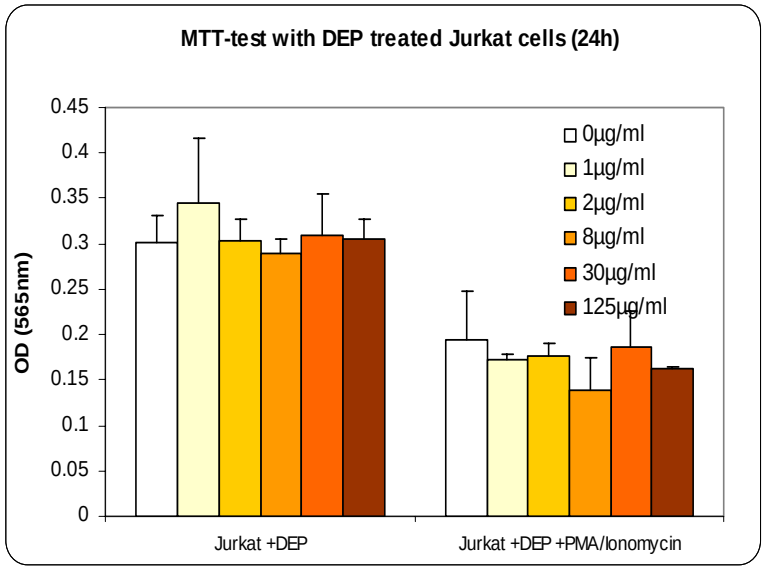
Our strategy: Stable cell lines

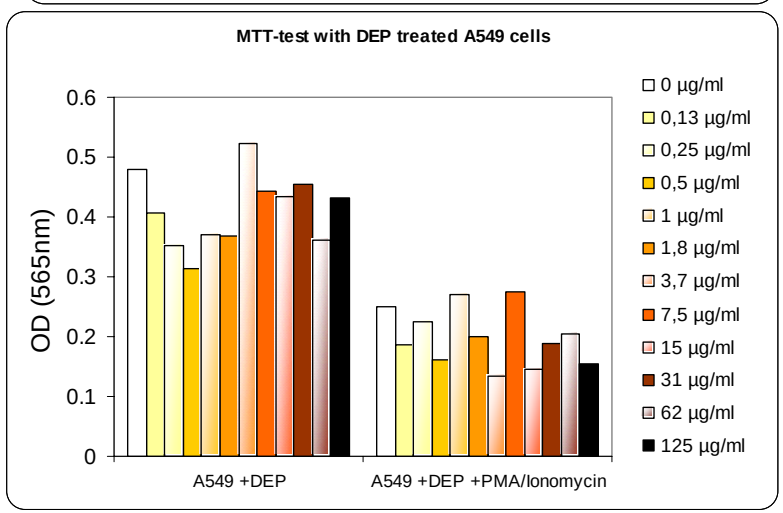
- Combine the regulatory region of a cytokine gene with a gene for the enzyme Luciferase from glow-worm
- Transport this DNA construct into immortal cell lines
- It is now possible to detect light emission when a particular cytokine is produced
- Integrate the DNA construct stably into the chromosomes (difficult step)
- Screen cells to identify suitable clones
- Maintain for 3 month:
  Stable reporter gene cell line



Toxicity

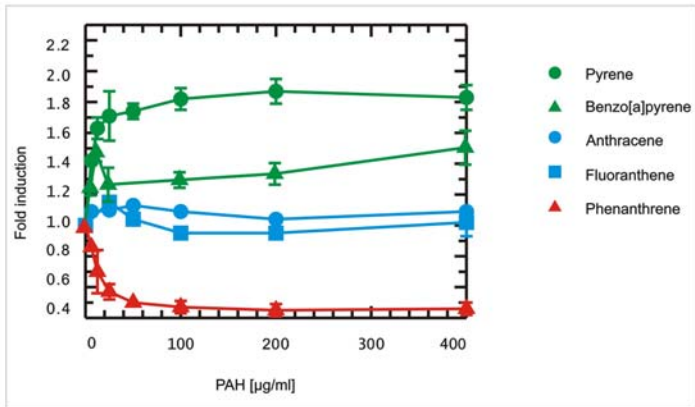
- DEP are very toxic: LC_{50} for human bronchial epithelial cells is 140 µg/ml
  (Matsuo et al., Biol. Pharm. Bull. 2003)
- DEP are quite harmless: Mice injected with 2 mg DEP show enhanced allergy but are otherwise healthy. (Mouse body volume 20 ml: 100 µg/ml)
  (Heo et al., Toxicology 2001)
- DEP in concentrations up to 125 µg/ml is not toxic for the cells we use (MTT assay)





Impact on allergy

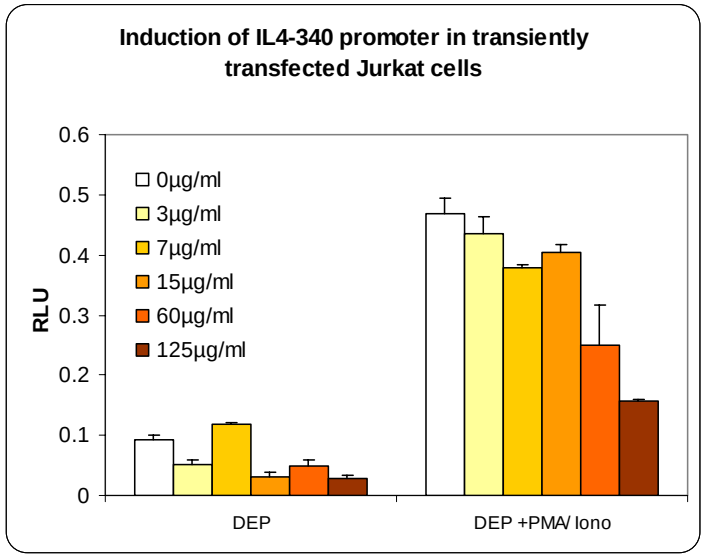
- PAH like Pyrene and Benzo[a]pyrene induce the pro-allergic cytokine IL-4 (Bömmel et al., J. Allergy Clin. Immunol. 2000)
- Anthracene, Pyrene and Phenanthrene as well as DEP increase IgE type antibodies which mediate allergy (Kanoh et al., J. Clin. Lab. Immunol. 1996, Suzuki et al., J. Clin. Lab. Immunol. 1996, Tsien et al., Toxicol. Appl. Pharmacol. 1997, Heo et al., Toxicology 2001)

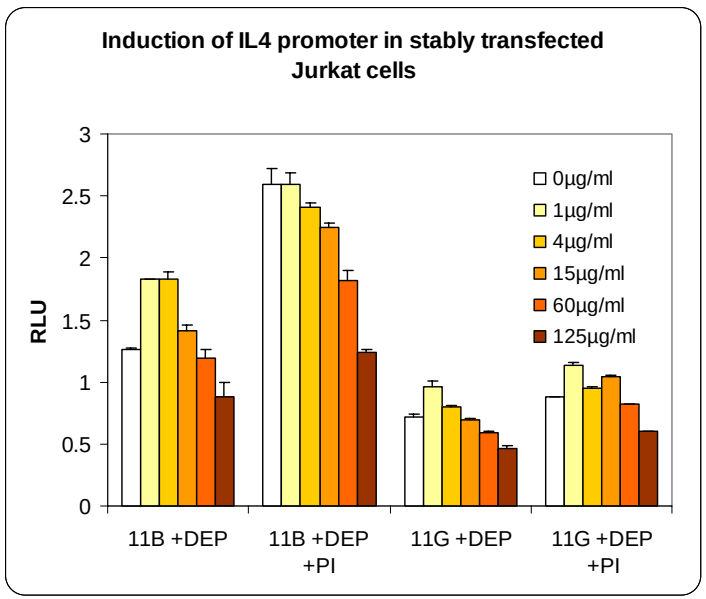


Bömmel et al., J. Allergy Clin. Immunol. 2000

Impact on allergy

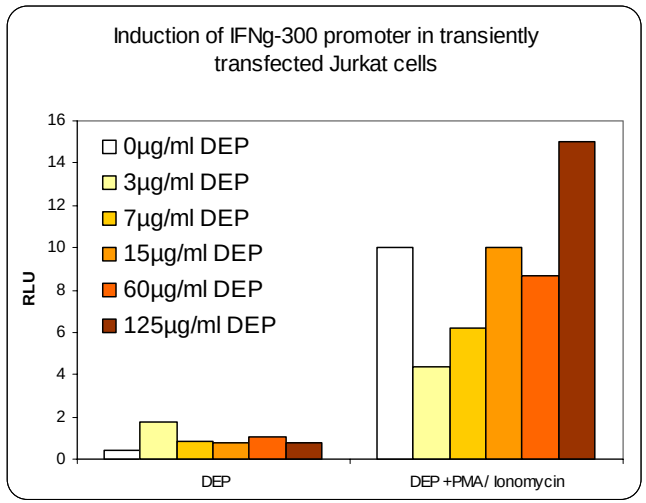
- DEP reduce production of the proallergic cytokine IL-4 in a human T-cell line
- Stable cells show the same effect
- Exeriment needed: Exposure of stable cell lines along with mice to real-life Diesel exhaust (to be performed at CERTAM within the EU-project MAAPHRI)

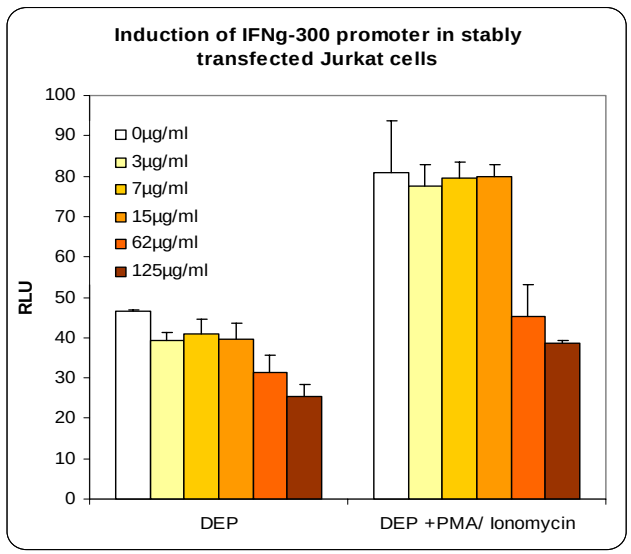




Impact on autoimmunity

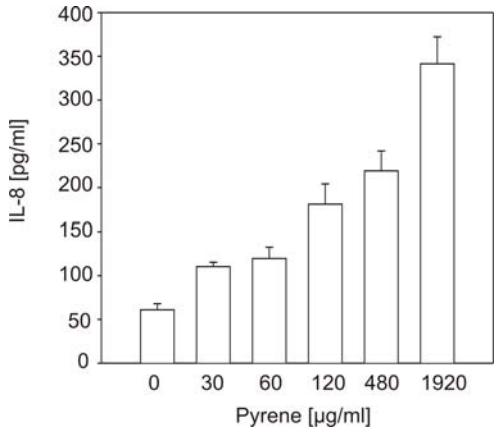
- Very little information available, but not unlikely given mechanisms of the immune system
- IFN-γ, a key regulator of antibacterial defense and antoimmunity is suppressed in transient and stable cells
- Exeriment needed: Exposure of stable cell lines along with mice to real-life Diesel exhaust
- IL-4 and IFN-γ are expected to behave different to each other



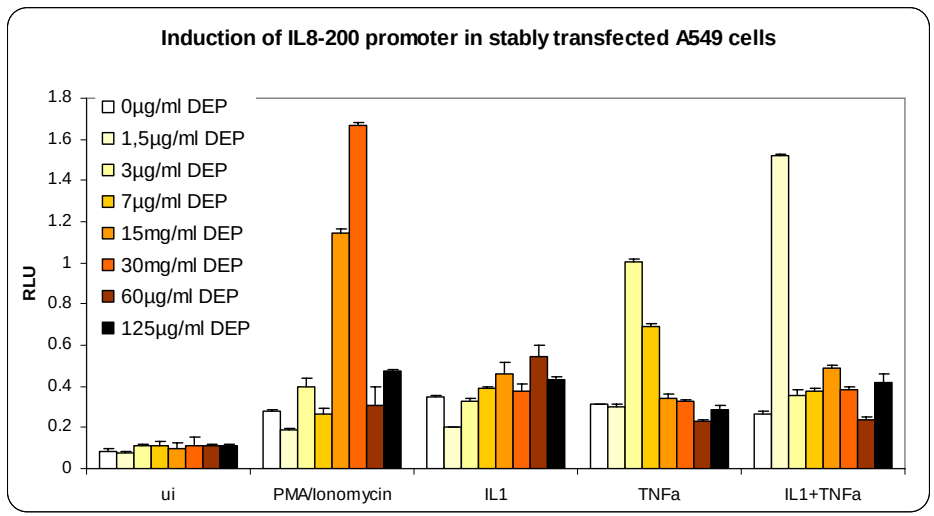


Impact on inflammation

- Pyrene induces production of IL-8, associated with cellular stress and local inflammation (Bömmel et al., Int. Immunopharmacol. 2003)
- So does DEP in stable cell lines, but depending on costimulus and concentration used
- Next cytokines studied: TNF-α (involved in inflammation and sepsis), IL-10 and TGF-ß (both immunosuppressive)



Bömmel et al., Int. Immunopharmacol. 2003



Summary

- Carbon core particles, associated PAH and transition metals, as well as gas components are all known to influence immunity
- Experimental problems both on the technical and the biological side make an assessment of the health impact difficult
- There is at present no reason to assume that different responses of the immune systems towards pollutants are all mediated by the same mechanism
- Stable cell lines may provide an assay which is relatively simple, stable and cheap

Bioassay partners

- Cooperators: Jean Paul Morin (Rouen Univ.), Frèderic Dionnet (CERTAM) along with the other MAAPHRI partners
- Cell lines: Jutta Horejs-Höck, Petra Luft, Maria Schmittner, Ulrike Tischner and Angela Schmidlechner (Salzburg), with Min Li-Weber (Heidelberg)
- Mouse studies: Angela Ehard and Annette Nelde (Salzburg), with Udo Herz (Marburg)
- Support of bioassay project: EU 5FP MAAPHRI

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