

Allergy and automobile pollution: experiments on humans

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SUMMARY

The increase in allergic airways disease in industrialized countries has been linked to particulates from fossil fuel combustion. We have employed human systems to investigate the effects of diesel exhaust particles (DEP) and the chemicals they contain upon allergic inflammation. We have shown that following nasal challenge of subjects DEP can enhance mucosal IgE production and induce an inflammatory response characterized by cell influx and increased production of chemokines and cytokines in the nasal mucosa. In combination with allergen, DEP can enhance local antigen specific IgE production, and drive *in vivo* isotype switch to IgE. These results can be duplicated by phenanthrene, an important polyaromatic hydrocarbon found in DEP. Low doses of allergen will synergise with DEP to initiate IL-4 secretion from CD117⁺ cells in the nasal mucosa thereby skewing the subsequent response toward a Th2 pattern. In addition, they can synergize with a neoantigen to drive primary sensitization. One potential mechanism for these effects is the enhancement of the role of macrophages as antigen presenting cells. Thus DEP can act as mucosal adjuvants at the cellular and molecular level and thus may cause both allergic sensitization and exacerbation.

KEY-WORDS: Diesel exhaust particles. – Allergy. – IgE regulation. – Mucosal immunity.

RÉSUMÉ

Allergie et pollution automobile: expérimentation chez l'homme. – L'augmentation des manifestations allergiques des voies respiratoires a été attribuée aux particules provenant de la combustion des matériaux fossiles. Nous avons recherché par études sur l'homme à préciser les effets des particules de combustion du diesel (DEP) et des substances chimiques qu'il contient sur l'inflammation allergique. Nous avons montré qu'à la suite de l'exposition nasale des sujets, le DEP peut augmenter la production muqueuse d'IgE et induire une réponse inflammatoire caractérisée par un afflux cellulaire et une production accrue de chemokines et de cytokines dans la muqueuse nasale. Associé à un allergène le DEP peut accroître la production antigénique locale d'IgE et entraîner *in vivo* une commutation isotypique de l'IgE. Ces résultats peuvent être reproduits par le phénanthrène, important hydrocarbure polyaromatique présent dans le DEP. La synergie de faibles doses d'allergène avec le DEP induit une sécrétion d'IL4 par des cellules CD117⁺ de la muqueuse nasale, déviant ainsi la réponse ultérieure vers le type Th2. En plus, elles peuvent s'associer à un nouvel antigène pour créer une sensibilisation primaire. Un mécanisme potentiel de ces effets est le renforcement du rôle des macrophages comme cellules présentatrices d'antigène. Ainsi, le DEP peut intervenir comme adjuvant muqueux aux niveaux cellulaire et moléculaire et causer à la fois la sensibilisation allergique et les poussées.

MOTS-CLÉS: Particules d'échappement du diesel. – Régulation des IgE. – Immunité des muqueuses.

DIESEL EXHAUST PARTICULATES AS A « MODEL POLLUTANT »

A link between industrialization and allergic disease has long been postulated. Indeed, only 50 years from the first reported case of allergy in 1819, Charles Blackley wrote that the « hay-fever epidemic » was associated with the movement of

people from the country into the cities [6]. The worldwide increase in atopy and allergic rhinitis has renewed speculation that urbanization is an important risk factor for these diseases [7]. In particular, both animal and epidemiological studies have implicated particulates from fossil fuel combustion [9, 11]. These combustion products consist of a variety of xenobiotic com-

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DIAZ-SANCHEZ D. – Allergy and automobile pollution: experiments on humans *Rev. fr. Allergol.*, 2000, 40 (1), 52-54

pounds among which polyaromatic hydrocarbons (PAH) and monofunctional inducers of oxidative stress (MIOS) abound. We have focused on diesel exhaust particulates (DEP) as a «model pollutant» because their occurrence has been well studied over time, they contain both PAH and MIOS and they make up over 40% of particles 10 microns or less in a city such as Los Angeles.

DEP AND ITS ASSOCIATED CHEMICALS EXACERBATE HUMORAL RESPONSES

We have employed human systems to investigate the effects of DEP and its constituent chemicals on allergic inflammation. These studies involved nasal provocation challenge by spraying 0.3 mg DEP into the nostrils of human test subjects. This dose is equivalent to a 1-3 day exposure to an outdoor environment in Los Angeles. A significant increase in total IgE production was evident in nasal lavages performed on these subjects within 24 hr of DEP exposure [3]. Maximal elevation of IgE occurred 4 days after challenge and had disappeared by day 7. In contrast, DEP did not alter levels of total nasal IgG, IgA, IgM or albumin, but did induce a small increase in IgG4 levels, an antibody often co-regulated with IgE. In cells recovered from the nasal lavage fluid, this rise in IgE protein was mirrored by a 25-fold increase in the levels of ϵ mRNA. Additionally, the number of IgE-secreting cells increased from less than 1 in 2,000,000 to greater than 1 in 100,000 whereas the number of IgA-secreting cells in nasal washes remained unchanged after DEP challenge. We next examined if DEP and allergen had synergistic effects. Nasal provocation challenge was performed with DEP, ragweed allergen *Amb a 1* or both simultaneously. These studies were performed in ragweed-sensitive subjects outside of the ragweed season. Following challenge with ragweed alone there was increased production of ragweed specific IgE but this was 16-fold higher when DEP was co-administered.

IgE synthesis by B cells is the end result of complex interactions between B cells and other cells that provide contact as well as soluble signals (e.g. T cells, macrophages, mast cells, epithelial cells etc.). We, therefore, tested whether DEP could alter the production of cytokines by cells residing in the nasal mucosa [5]. Prior to challenge, most atopic subjects expressed mRNA for interferon- γ (IFN- γ), IL-2, and IL-13, but failed to express IL-4, IL-5, IL-6 and IL-10 mRNA. However, after DEP challenge there was a significant increase in both the number of subjects in whom various cytokine

mRNAs could be detected and in the levels of mRNA for a widespread number of cytokines. Thus DEP markedly enhanced mucosal TH0-like cytokine production. In contrast in the presence of allergen, this cytokine production showed TH2-like deviation associated with increased IgE antibody production.

DEP consists of a carbon core and a complex mixture of organic compounds including a large number of PAH [1]. Extensive studies have characterized the nature of the PAHs present in diesel exhaust [15]. The major percentage of PAH found in DEP are relatively small polycyclic aromatic hydrocarbons containing up to four rings although larger compounds are present. The major family of PAHs are the phenanthrenes. Both the organic component of DEP and phenanthrene itself, enhanced IgE production by purified human B cells or from an IgE-secreting B cell line *in vitro* [12, 13]. The adjuvant potential of phenanthrene has also been confirmed *in vivo*. As with DEP, potentiation of the IgE response was observed after nasal challenge with 100 μ g phenanthrene (personal observation).

DEP INDUCES ALLERGIC SENSITIZATION

These studies demonstrated that DEP have a variety of effects on their own and can strongly enhance mucosal allergic inflammation and specific Ig responses in already sensitized individuals. *In vivo* studies in humans employing combined nasal challenge with DEP and allergen also demonstrated isotype switching to IgE *in vivo* [7]. This suggested that mucosal exposure to DEP would lead to sensitization, that is the production of IgE to a neoantigen. Therefore, we examined the ability of DEP exposure to lead to primary sensitization of humans by driving a *de novo* mucosal IgE response to a neoantigen, keyhole limpet hemocyanin (KLH) [3]. Nasal challenge of subjects to KLH alone resulted in production of anti-KLH IgG and IgA but not anti-KLH IgE. In contrast, when subjects were exposed to DEP prior to KLH, 60% subjects produced anti-KLH-specific IgE.

Sensitization to KLH when DEP was preadministered was characterized by the formation of a Th2-type cytokine pattern. Indeed, DEP can synergise with low doses of allergen which in themselves are insufficient to initiate a Th2 cytokine response. Within hours of DEP plus allergen exposure, IL-4 secretion occurs from CD117⁺ cells residing in the nasal mucosa (presumably mast

cells or basophils) [14]. Presumably, this early appearing IL-4 deviates the subsequent response toward a Th2 pattern by expanding the repertoire of IL-4-producing cells in the local microenvironment.

CELLULAR ACTIVATION BY DEP

In addition to their effects on antibody production, DEP can influence the cellular response. Challenge with DEP resulted in an increase in total and mononuclear cell counts in nasal lavage fluids and a significant enhancement of ECP levels at 6 to 24 hours after challenge. In addition, DEP induced production of certain members of the CC chemokines, a family of homologous chemotactic cytokines which are released as a response to injury, allergens, antigens and microorganisms which will cause cellular infiltration and are important mediators of the inflammatory response in the absence of allergen. Within hours of *in vivo* DEP exposure there is a burst of chemokine production from non-T cells.

One mechanism for the adjuvant effect of DEP is the enhancement of the role of macrophages as antigen presenting cells (APC). Others have shown that PAH present in DEP can enhance MHC-II gene expression in murine macrophages [10]. We have shown that CD80 (B7-1) protein

and message can be upregulated *in vivo* and *in vitro* by DEP or by PAH [12]. CD80 binds with high affinity to CD28, a major co-accessory receptor in T-cells which plays a role in antigen induced cytokine production. In nasal challenge studies, DEP can induce CD80 mRNA expression in nasal lavage. In a human macrophage cell line, THP-1, CD80 surface expression increases when exposed to a prototype quinone derivative, tert-butylhydroxyquinone (tBHQ). Additionally, PAHs can also affect macrophage-derived cytokines such as IL-1 and TNF- α that may play a role in allergic sensitization by inducing adhesion molecules, initiating cytokine cascades and recruiting pro-inflammatory cells.

CONCLUSION

Atopy and specifically allergic rhinitis has been increasing throughout the industrialized world. It is important to realize that many factors such as diet, lifestyles and changes to exposure of bacterial infection may all be involved. Epidemiological studies have indicated that combustion by products of automobile exhaust are also important contributors. Now, with experimental studies we can explore the potential mechanisms by which pollutants in the environment can interact with the immune system.

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