# Pollution and the immune response: atopic diseases – are we too dirty or too clean?

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## INTRODUCTION

The effect of pollutants on the immune system has filled tomes and is almost always controversial whether the topic is disinfectants in drinking water or particulates and lung cancer. Even whether outdoor and indoor air quality is improving or declining has been a source of considerable investigation, consultation and often litigation. Few areas, however, yield so much uncertainty and debate as the role of airborne pollutants in atopy.

Atopy is defined as an immunoglobulin E- (IgE) mediated allergic response to environmental antigens and can be manifested as rhinitis, asthma or atopic dermatitis. While there is some debate over whether there has been a real increase in atopy over the last decades, it is indisputable that there has been a dramatic change over the last 200 years. While isolated cases of anaphylaxis had been noted before, allergies were unheard of until the early 19th century. Only 54 years after the first description of hay fever in England in 1819, it was considered an epidemic and a clear association with urbanization had been reported.[1]

What aspect of urbanization is involved has been much debated. There has been much discussion amongst both experimental immunologists and epidemiologists over whether pollutants are responsible for increased allergic sensitization and/or severity.[2,3] In the last few years new studies have supported an alternative explanation, namely the decline in infectious diseases in industrialized countries.[4,5] Does this 'hygiene theory' toll the death knell for the 'pollution theory'? In this review I will argue that there is a compelling case to be made for both theories and that even this is not the entire picture.

## THE HYGIENE THEORY

Some studies have linked the rise of atopy with an increase in living standards and immunization programs in industrialised countries.[6,7] This has led to the theory that, while the normal response to childhood infections is the deviation of the immune system to a T helper (Th) 1-type cytokine response, the absence of these infections in 'Western' society has resulted in the predominance of a Th2-type response.[5,8,9] The Th2 cytokine environment is known to promote IgE production and atopic diseases.[10] In support of this theory, some epidemiological studies have shown an inverse association between the risk of atopy and sibling number,[11] birth order,[12] and nursery school attendance,[13] all factors that will increase the risk of infections in infancy. Perhaps the most cited evidence has come from a study looking at Japanese schoolchildren vaccinated against *Mycobacterium tuberculosis*.[14] There was an inverse relation between tuberculin responses and asthma, rhinitis and eczema. Additionally, serum IgE levels, both total and allergen-specific, were also lower in children with positive tuberculin responses.[14] While some studies looking at measles[15] or hepatitis A virus[16] infections have supported the conclusion that infections protect against atopy, others have shown an association between asthma or allergic disease and *M. tuberculosis* infection only in women but not in men[17] and yet others have failed to show similar associations between atopy and measles[18] or bacillus Calmette–Guérin (BCG) vaccination.[19] An alternative explanation has been proposed that mycobacteria do not lower the risk of atopy, but that atopics have an impaired ability to make a Th1 response to mycobacteria.[20] Yet, in experimental animal models, immunization with mycobacteria or other infectious agents will result in the promotion of a Th1 response and the inhibition of Th2 responses.[21,22]

As a cautionary note, it should be mentioned that, in the UK and the USA the greatest increase in atopy prevalence has come not in the last decades but in the years between 1830 and 1900. Deaths from infectious diseases have declined markedly in these countries only during the 20th century. By the turn of the century improvements in hygiene and immunizations regime were still limited and the life expectancy was around 47 years, only 20 years more than the Greco–Roman era, 2000 years earlier.[23] In 1900, 30·4% of all deaths in the USA occurred among children aged less than 5 years; in 1997, that percentage was only 1·4%.[24] There is broad agreement that the increased prevalence of atopy is due to the skewing of the immune system to a Th2 environment. While the 'hygiene theory' is an attractive explanation, other possibilities remain. Some have argued that the shift is due to diets,[25,26] others that it is because of the adjuvants used in immunizations,[27] others that high growth rates in successful pregnancies select for a Th2-type environment,[28] and, as we shall see, certain pollutants can interact with allergen to promote Th2 cytokines.

## POLLUTION AND EPIDEMIOLOGICAL STUDIES

The most cited argument against a role for pollutants in asthma and allergy onset is the comparative studies between the former

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nations of the now reunited Germany.[29,30] Despite increased particles and $SO_2$ levels in East Germany, allergic airway disease was significantly higher in West Germany. Additionally, prevalence of positive skin-prick tests (atopy) in an East German city (Leipzig) were half that of one in West Germany (Munich). Similar results have also been observed in comparisons between Sweden and Estonia.[31] Yet, these data are not as clear cut as it appears. Even within these studies, within each country, hay fever, atopy and asthma were all higher in industrialized polluted cities than in rural or non-industrial towns. This has led some to conclude that air pollution is an important risk factor but that some undefined feature of Western lifestyle may be even more so.[32] Indeed, since reunification, the prevalence of hay fever and atopy seems to have increased in the former socialist states[33] presumably because they are adopting a more 'Westernized' lifestyle. Subsequent studies have compared two polluted counties in East Germany with a neighbouring country without any source of industrial pollution, where lifestyles (including family size and infection rates) are similar in all three counties.[34] Again, children in the polluted counties had higher sensitization rates and specific IgE levels.

It is important to note that there seem to be distinctions between pollutants. Certain dusts and $SO_2$ associate strongly with infections and irritant responses, after controlling for as many lifestyle covariates as possible.[35] Conversely, parameters of allergic sensitization correlate significantly with combustion sources such as automobile exhaust. This agrees with epidemiological studies that have shown that in Japan, people living on busy roads lined with cedar trees have higher allergic cedar pollinosis than residents living on similar streets with much less traffic.[36] Similarly, allergic rhinitis is heavier in residential areas with heavy truck traffic[37] and some reports suggest that allergic symptoms correlate with the distance of residences to roads with heavy traffic.[38]

## DIESEL EXHAUST PARTICLES (DEP) AS MODEL POLLUTANT

The studies cited above have sparked renewed focus on the role of fossil-fuel combustion products on allergic inflammation. Because of their universal nature, indoor concentrations of these products are often equal to or exceed that found in ambient outdoor air. In particular, much of the work in the last decade has concerned diesel exhaust particles (DEP) and the xenobiotic compounds they contain. Consisting of an inert carbon core containing unburned fuel petrochemicals, DEP have been used as model pollutants to understand the mechanisms involved. Many of the chemicals they contain are also present in other pollutants.[39] For example, the prototypic polyaromatic hydrocarbon benzo(a)pyrene found in DEP,[40] is prominent in second-hand smoke, a risk factor for allergic sensitization identified in dozens of epidemiological studies.[41,42] In cities such as Los Angeles, 40% of the 10-μm particles (PM10) in the atmosphere are derived from diesel vehicle engines. This has become an area of increasing public health concern as diesel engines have come to dominate the trucking industry in recent decades; for example, 88% of all heavy-duty trucks (>26 000 lb.) now run on diesel fuel.[43] The rest of this review will use the example of DEP (as this is the best characterized and most studied pollutant of the last few years) to focus primarily on the changes that a pollutant can have on the immune response and especially allergic reactions.

## THE IMMUNOGLOBULIN RESPONSE

Much of the experimental work on pollution and atopy has centred on the ability to alter immunoglobulin production. In particular IgE regulation has been widely studied owing to the strong association of asthma with serum IgE levels and its central role in both the early and late phase of the mucosal response to allergens and the induction of allergic inflammation in humans. In 1986, Muranaka *et al*. published the first demonstration that DEP could have an adjuvant activity.[44] Instillation of ovalbumin (OA) or Japanese cedar pollen (JCP) mixed with DEP and injected intraperitoneally enhanced the antigen-specific IgE antibody response. Shortly after Takafuji *et al*. demonstrated an identical effect if an intranasal route of administration was used.[45] Since then, these studies have been validated many times and DEP shown to have similar effects if administered intratracheally or by aerosilization.[46–48]

Other studies have focused on IgG1. In mice and other animal models, IgG1 can also cause anaphylactic reactions and is sometimes the major reaginic antibody (e.g. in the guinea pig[49]) and may be important in the development of allergic airway inflammation.[50] There is no homologue to IgG1 in humans. Some studies using murine models have failed to show adjuvant activity for IgE but can show DEP can enhance IgG1 responses to allergen.[48,51] These differences probably result from differences in doses, route of administration, murine strains and exposure duration. The ability to modulate immunoglobulin production is not confined to DEP. Indeed the ability of both $SO_2$ and ozone to enhance allergen-induced anaphylactic responses in mice was reported 20 years ago.[52] Ozone can also increase the number of IgE-containing cells in the lung following allergen exposure.[53] Guinea pig exposure to $SO_2$ increases anti-OA IgG1 in serum and bronchoalveolar lavage (BAL) fluid. $NO_2$ exposure can augment local IgE (as well as IgA and IgG) to dust-mite antigen in rats.[54] Second-hand tobacco smoke, a pollutant which is often implicated as a risk factor for atopy, has many of the characteristics of DEP (e.g. a particulate nature) and shares many chemicals in common, has recently also been shown to increase IgE to OA in a mouse model.[55]

A major advantage of using DEP as a model pollutant has been the ease by which animal models have been transferred into human studies. These studies have categorically shown the potential of DEP to induce and exacerbate IgE responses *in vivo* in the human upper respiratory tract.[56] Human atopic volunteers were sprayed intranasally with DEP, with doses ranging up to 0·15 mg into each nostril.[57] Although this dose may seem high, in certain occupational or everyday settings (such as waiting at a bus stop) exposure to DEP can be much higher. DEP exposure resulted in a dose-response associated increase in total IgE production and ε mRNA in nasal lavage fluid.[57] Four days following DEP challenge, total IgE proteins levels found in nasal washes were up to 10 times greater than baseline values, while there was a 25-fold increase in the levels of ε mRNA in cells recovered from the nasal lavage fluid. By day 7, IgE levels returned to baseline. This response was isotype specific as levels of total nasal IgG, IgA, IgM were unchanged by DEP provocation. Cells recovered from nasal washes were

used to enumerate immunoglobulin-secreting cells. After DEP challenge the number of IgE-producing cells increased from less than 1 in 2000 000 to more than 1 in 1000 000 of total cells. However, the number of IgA-secreting cells remained unchanged after DEP challenge.[57] Thus DEP seems unlike other pollutants such as $NO_2$ or $SO_2$, which do not produce IgE in the absence of an allergen.[58]

Subsequent studies have investigated the ability of DEP to act as an adjuvant in humans *in vivo* to a co-administered antigen.[59] Ragweed-sensitive subjects were challenged with DEP, ragweed allergen (Amb a I) or both simultaneously. Nasal washes were performed at different days after challenge. At the peak of the response, ragweed-specific IgE was 16-fold higher following ragweed plus DEP challenge compared with ragweed alone. Once again, there was no change in any other immunoglobulin isotype except $IgG_4$, suggesting that DEP acts in an isotype-specific manner. Thus for both total and ragweed-specific responses, only IgE and $IgG_4$ levels were boosted by DEP. This may be because both $IgG_4$ and IgE production are under very similar, although not identical, controls.[60]

A popular explanation for the adjuvancy effect of DEP is the physical interaction between pollutants and pollen grains. It has been suggested that pollen from trees growing in cities are more allergenic.[61] Furthermore, purified grass pollen allergen molecules bind to DEP when incubated *in vitro*.[62] Thus it is possible that DEP may play a passive role, acting as a carrier that transports allergens into the airway. Although this may be the case in some instances, it is probably not the principle mechanism in most of the studies presented here. Enhanced IgE production in animals can occur when injected with DEP at sites remote from the respiratory tract. Carbon Black (elemental carbon) can also increase specific IgE production in mice and enhance the local inflammatory responses in cells of the draining lymph node, in mice challenged by OA injection into the foot pad.[63] However, the responses induced by Carbon Black are less than that seen with DEP.[63] In addition, the chemicals from DEP also can enhance IgE production; Pyrene, a polyaromatic hydrocarbon present in DEP, tobacco smoke and many other combustion products, can induce IgE production in mice injected intraperitoneally with OA.[64] The soluble organic fraction extracted from DEP also increased the IgE production of B cells co-stimulated with interleukin (IL) -4 and anti-CD40 mAb *in vitro*.[65] Phenanthrene, a principle polyaromatic hydrocarbon in DEP and also found in tobacco smoke, had similar effects.[65]

## THE TH1/TH2 RESPONSE

A more plausible explanation for the changes in IgE induced by pollutants is that they enhance on-going IgE production directly by acting on B cells, or indirectly by altering the local environment to one conducive for allergic inflammation, i.e. induce a Th2 cytokine mileau. A likely mechanism for this would be the induction of IL-4. In humans and mice IL-4 and IL-13 can initiate germline transcription and thereby in association with CD40 or CD58 ligation direct immunoglobulin class switching of B cells to IgE.[66–69] In addition, Th2 differentiation is critically dependent on the presence of IL-4.[70] Thus the induction of IL-4 by pollutants should result in increased IgE production and the initiation of a Th2 response.

Several pollutants have indeed been shown to enhance IL-4 production to allergen *in vitro* and in both animal and human *in vivo* models. Following intratracheal instillation of DEP with OA, IL-4 production in mediastinal lymph nodes was eight times greater than in mice injected with OA alone.[46] DEP plus JCP induced twofold higher levels than JCP alone in the same model. Similarly, the same group demonstrated that intranasal challenge with DEP and allergen induced increased IL-4 responses in cervical lymph node cells compared with allergen alone.[71] Subsequent studies demonstrated that DEP could have the same effects on IL-4 when used in a more physiologically relevant model that of administration through inhalation.[47] Similarly, spleen lymphocytes derived from mice treated with pyrene during house-dust-mite antigen sensitization secrete increased IL-4 upon antigen stimulation.[72] Human studies show that intranasal challenge of DEP alone can augment expression of many cytokines in a non-specific manner.[73] Therefore, levels of mRNA for interferon-γ (IFN-γ), IL-2, IL-3, IL-4, IL-5, IL-6 and IL-13 all increased in cells recovered from nasal washes. IL-4 protein levels, which are normally too low to be detected, could be detected in washes performed 18 hr after DEP challenge.[73]

In 1997 two studies (one in humans and one in mice) demonstrated the formation of an enhanced Th2 response following challenge with DEP and allergen.[51,59] In the first, ragweed-allergic subjects were challenged nasally with ragweed allergen, DEP, or both. Prior to challenge the only cytokines in nasal cells for which baseline levels of mRNA were detectable were IFN-γ, IL-2 and IL-13. Following challenge with ragweed alone, only low and inconsistent changes in cytokine expression occurred.[59] In contrast, challenge with both DEP and allergen resulted in significantly increased expression of IL-4, IL-6, IL-10 and IL-13, but decreased expression of the Th1 cytokine, IFN-γ. In mice intratracheally inoculated for 6 weeks, DEP with OA induced an eightfold increase in IL-5 protein in lung tissue and BAL, compared with OA-alone-treated mice.[51] Interleukin-5 is a hallmark Th2 cytokine, central to allergic asthma. While local levels of IL-2 and granulocyte–macrophage colony-stimulating factor (GM-CSF) also increased, it is significant that levels of IFN-γ were unchanged. These studies imply that the combination of DEP plus allergen selects against a Th1 profile and induces the formation a Th2-type cytokine response. In these studies, the induction of Th2 responses in the human and murine models were correlated with increased production of antigen-specific IgE and IgG1, respectively.

How DEP or pyrene induce a IL-4 production and the subsequent Th2 cytokine repertoire is still unknown. Several studies have implicated both direct mast cell and T-cell activation.[74,75] In atopic subjects, DEP can synergize with low doses of allergen, which in themselves are insufficient to produce measurable IL-4 protein.[59,76] In one study, IL-4 protein and $IL\text{-}4^+$ cells could be detected in nasal washes as early as 4 hr after challenge with DEP plus allergen.[74] At this time, the frequency of $CD3^+$ cells in the $IL\text{-}4^+$ cell population ranged from 0 to 12%, suggesting that T cells were not the main source of this early IL-4. In contrast, $CD117^+$ cells (primarily mast cells) constituted between 65 and 100% of $IL\text{-}4^+$ cells. No $IL\text{-}4^+$ $CD19/CD20^+$ (B cells) or $IL\text{-}4^+$ $CD56^+$ cells [natural killer (NK) cells] were detected at this time. As the allergic response progressed, the primary source of IL-4 changed. At the peak of IL-4 production, 18 hr after challenge, staining for intracellular IL-4 was observed predominantly (73–100%) in

$CD3^+$ cells. This study implies that mast cells produce the initial burst of IL-4, which deviates the subsequent response towards a Th2 pattern by expanding the repertoire of IL-4-producing cells in the local microenvironment. In support of this idea are studies that show that DEP can induce IL-4 and IL-6 production from purified bone marrow-derived mast cells *in vitro*.[77] Recently Bömmel *et al*. have shown that *in vitro* IL-4 production can be induced by pyrene.[75] Pyrene activated the IL-4 promoter in both T-cell lines and a human mast-cell line, but not in B-cell lines, suggesting that stimulation by pyrene of the IL-4 promoter is cell-type specific. Human IgE class-switching promoter is regulated by IL-4 by the STAT-6 pathway, which also regulates CD23 and major histocompatibility complex (MHC) II expression. Pyrene did not alter regulation of STAT-6 activation suggesting that it does not increase IL-4 expression by interfering with IL-4 signalling.

## ALLERGIC INFLAMMATION AND AIRWAY HYPERREACTIVITY

Animal models have demonstrated that the increased allergic antibody production and the generation of a Th2 response produced by interaction between allergen and pollutants may also result in enhanced effects on allergic asthma.[48,51,78–83] In a series of studies, Sagai and coworkers have shown that in mice repeatedly challenged intratracheally with DEP and OA, not only were IgG1 and IgE and Th2 cytokines higher than in OA alone inoculated mice but also there were clear signs of increased allergic inflammation.[48,51,80,82] Eosinophil infiltration of the submucosa of medium and small bronchioles was significantly enhanced, as were goblet cell numbers in the bronchi and bronchioles. Both these histopathologic changes correlated significantly with anti-OA IgG1 production. Additionally, cellular profile of BAL revealed that DEP augmented macrophage, eosinophil, neutrophil and lymphocyte numbers. Airway hyperresponsiveness was measured in these mice by respiratory resistance to acetylcholine and was found to increase with DEP dose. The same group extended their model to demonstrate that these results could be duplicated by diesel exhaust inhalation.[78,81] Mice were sensitized with alum plus OA *i.p.* and exposed to 3 $mg/m^3$ diesel exhaust for 6 weeks. Enhanced allergic airway inflammation and goblet cell hyperplasia was observed. The number of eosinophils per length of basement membrane of the bronchial epithelium was on average 4·4-times greater in mice receiving DEP plus OA than in those receiving OA alone, while goblet cell numbers were 6-fold higher. Similarly, BAL cell numbers and airway hyperresponsiveness were also augmented. Recently Ohta *et al*. have shown that in a DEP intranasal mouse model, antibodies raised against GM-CSF will abolish the DEP-evoked responses, suggesting that DEP may induce airway hyperresponsiveness by stimulating GM-CSF synthesis.[83]

Studies exploring effect of diesel exhaust on human lower airways have been restricted to normal healthy subjects. Rudell and co-workers have exposed non-smoking subjects for 1 hr to diluted diesel exhaust and performed BAL 24 hr after exposure.[84–87] Increased neutrophil, B-cell and alveolar macrophage recruitment in the airways was recorded along with increases in histamine and fibronectin. Additionally, phagocytosis by alveolar macrophage *in vitro* was decreased. Similar results have been observed following $NO_2$ or ozone exposure.[88,89] Bronchial biopsies performed after a 6-hr exposure to diesel exhaust have shown increases in neutrophils, mast cells and T cells, and up-regulation of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) both implicated in allergic asthma.[90] In human subjects, exposure to diesel exhaust induces increased concentrations of the antioxidant ascorbic acid in nasal cavity lining fluids.[91] Similarly, in the animal models, the effects of DEP could be reversed by introducing superoxide dismutase into the airway.[82] As asthmatic patients are thought to be subject to increased oxidative stress, these studies suggest that DEP and other pollutants are acting by induction of reactive oxygen species. (For a detailed review of the growing evidence to support this view please see ref. 56).

Exposure chamber studies to gaseous pollutants have been performed on asthmatic individuals. Enhanced airway responses to inhaled allergen has been reported following exposure to the combination of 400 p.p.b. $NO_2$ and 200 p.p.b. $SO_2$.[92] Subjects needed a reduced dose of dust mite allergen to induce a fall in forced expiratory volume in 1 second by 20% (PD20 FEV1), compared with prior exposure to air.

Several *in vitro* studies have demonstrated that both epithelial and mononuclear cells may release pro-inflammatory mediators, chemotactic cytokines and adhesion molecules, which may be involved in pollution-induced allergic airway inflammation observed *in vivo.* Devalia and co-workers have demonstrated that ozone, $SO_2$ and DEP could all cause release of sICAM-1 and pro-inflammatories such as GM-CSF and IL-8 from human normal bronchial epithelial cells *in vitro*.[2,93,94] Others have shown that DEP and benzo(a)pyrene can also have similar effects on transformed bronchial and basal-polyp-derived upper-airway epithelial cells.[95] Neither charcoal nor graphite had such stimulatory effects, demonstrating once again that the activity of DEP is not due to its particulate nature. The production of GM-CSF and IL-8 by airway epithelial cells can be regulated by the cytokines IL-1 and tumour necrosis factor-$\alpha$ (TNF-$\alpha$). Both DEP and DEP extracts can induce *in vitro* IL-1 and TNF-$\alpha$ production in rat pulmonary alveolar macrophages.[96] IL-8 is a member of the CXC family of chemotactic or chemokines and is mainly active on neutrophils. The CC chemokine family includes regulated on activation, normal, T-cell expressed, and secreted (RANTES), monocyte chemotactic protein (MCP), and eotaxin. This family is especially important in allergic inflammation as they are involved in chemotaxis of mononuclear cells, activation of eosinophils, mediator release from basophils and mast cells, and augmentation of T-cell proliferation and cytokine production. In addition, elevated expression of CC chemokines has been reported in allergic asthmatic patients and in nasal or bronchial secretions of allergic subjects following challenge with allergen.[97] Recently Fahy *et al.* have demonstrated that in peripheral blood mononuclear cells, the organic extracts from diesel caused an increase in both IL-8 and RANTES, but a decrease in MCP-1 production.[98] Furthermore, supernatants from cells stimulated with DEP extracts exhibited a significantly enhanced chemotactic activity for neutrophils and eosinophils.

It seems likely that while DEP synergizes with allergen to act on mast cells and lymphocytes to induce IL-4 and IgE production, it can act in the absence of allergen to induce

chemokine secretion from epithelial and mononuclear cells and thus promote inflammatory reactions. Whether these effects are all because of one active fraction, or whether different chemicals in DEP modulate different actions on different cells, is still unknown.

## SENSITIZATION

A common proposal to reconcile the experimental and epidemiological data is to suggest that pollutants may cause exacerbation of allergic disease, but can not increase the frequency of atopy, that is, induce the disease. However, at least in an experimental human model, the ability of the model pollutant DEP to cause allergic sensitization has been shown.[99] *In vivo* studies in humans employing combined nasal challenge with DEP and allergen, demonstrated isotype switching to IgE *in vivo*. Four days after challenge, clones of deleted switch circular DNA (Sε/Sμ), representing switching from μ to ε from nasal lavage cells, could be detected.[100] These results indicated that the combination of mucosal stimulation with DEP and ragweed allergen was capable of driving *in vivo* isotype switching to IgE in humans with ragweed allergy. These results were the first direct demonstration of *in vivo* IgE isotype switching in humans and suggested that mucosal exposure to DEP may lead to senzitisation.

In a subsequent study, the classic immunogenic neoantigen, keyhole limpet haemocyanin (KLH) was used to demonstrate the ability of DEP exposure to result in primary sensitization of humans by driving a *de novo* mucosal IgE response.[99] Atopic subjects were given an initial nasal immunization with 1 mg KLH followed by two bi-weekly nasal challenges with 100 μg of KLH. Challenge with KLH alone resulted in the formation of anti-KLH IgG and IgA antibodies in nasal washes; however, no anti-KLH IgE could be detected in any subjects. When the experiment was repeated on different atopic subjects, but DEP administered 24 hr prior to each KLH exposure, anti-KLH-specific IgE was detected in 60% of subjects studied. Furthermore, the response was characterized by the formation of a Th2-type cytokine pattern, with elevated protein levels of IL-4, but not IFN-γ, in nasal lavage fluids. In subjects receiving KLH alone, these levels were unchanged. It is unlikely that pollutants can convert a non-atopic into an atopic individual; however, these studies imply that DEP, and similar combustion pollutants, may cause those with the appropriate genetic predisposition to become sensitized to allergens to which they may not otherwise have become sensitized.

## CONCLUSION

Why the increased prevalence of atopy observed in industrialized countries in recent decades? While the 'hygiene theory' is both a plausible and attractive explanation it seems clear that it is not 'the' answer; indeed there is probably not one simple single answer. Epidemiological studies have certainly implicated lifestyles, including reduced exposure to infections, yet these studies also show a strong role for pollutants. Experimental animal and human models (both *in vitro* and *in vivo*) have shown the potential, and elucidated possible mechanisms by which pollutants may modulate the immune response to increase atopic severity and sensitization. As lifestyles become more and more similar throughout Europe, it is likely that pollution will become an ever more important risk factor for atopy.

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