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The worldwide allergy and asthma epidemic

The incidence of allergic diseases like hay fever, house dust allergy, animal hair allergy and allergic asthma has increased significantly in recent decades. This increase is associated with a "Western life style", since it is most prominent in areas like Western Europe, North America, Japan and Australia [1]. In many Third World countries, asthma and allergies are predominant in cities and nearly absent in the countryside, but for industrialized countries there is often no clear difference between urban and rural areas [2]. In some countries, like Australia, the incidence of asthma is actually higher in rural areas [3]. It is still not quite clear what features of "Western" societies make them more susceptible to allergies.

It has been speculated that air pollution may be one of the new factors which promote allergic diseases. Studies in Japan have shown that children living close to major roads had a higher level of allergic diseases than other children [4, 5]. These findings have focused the interest of allergy researchers on car derived pollutants, in particular on Diesel exhaust particles (DEP). As mentioned above, the epidemiological evidence linking air pollution and allergy is equivocal, but in the wake of the Japanese studies, DEP were studied in mouse models of allergy.

Studies on mice and humans link allergic diseases to air pollutants

The common white laboratory mouse readily develops allergic diseases upon exposure to suitable allergens. When mice were exposed at the same time to DEP, a more severe form of the disease developed and less allergen was needed to induce symptoms [4, 6-8]. The same result was obtained when organic extracts containing the polyaromatic hydrocarbons (PAH)

associated with DEP were used. DEP or PAH by themselves did not induce allergic symptoms, but they promoted the development af allergies against common allergens like plant pollen or house dust. Since allergens and particulate pollutants are nearly continuously present in our environment, it is conceivable that this combination promotes allergy in humans as well.

It has indeed been shown that allergic human volunteers which were exposed to DEP during a challenge with allergens developed more severe symptoms [9]. It has even been possible to induce allergy against a new allergen by co-exposure with DEP in humans [10]. The DEP doses used in these experiments are described as corresponding to exposure "from standing at a bus stop when a bus starts up or being in a freeway tunnel for 10 min" [9]. These data provide direct evidence for an allergy-promoting effect of particulate air pollutants, presumably mediated by PAH associated with these particles.

Interleukin-4, a key regulator of allergy

The experimental evidence suggests that PAH promote all allergies, not just some specific ones. This has led us to speculate that PAH may somehow influence Interleukin-4 (IL-4). IL-4 is a regulatory protein which is secreted by cells from the immune system. Its natural function is to induce allergic responses. If IL-4 is produced at the beginning of an immune response, an allergic response will be developed towards the stimulus which has prompted the immune reaction. This central role of IL-4 has made it a favourite target for the development of new anti-allergic drugs [11, 12].

The purpose of the allergic system is of course not the fight against harmless plant pollen or cat hair, but a defense against large parasites, like worms [13]. However, if this defense system is activated in the absence of a parasite, it may instead turn against a harmless substance. Allergies can be described as situations where our natural anti-parasite defense is turned on when it should not. Since IL-4 production is a key step in this event, anything affecting IL-4 will change the risk to develop an allergic response.

PAH and IL-4

We have tested the effects of several PAH on IL-4 production in cell based assays. Cells in cell culture can be engineered to produce an enzyme whenever they produce IL-4. An enzyme has the advantage that it catalyzes chemical reactions which lead to easily detectable signals like color development or light emission. Such a system allowed us to screen a number of individual compounds and focus on positive substances for further tests.

Some but not all PAH induced a higher production of IL-4 in the cells [14]. Surprisingly, most PAH affected IL-4 production very little or not at all. PAH which induced a good signal in this screening test included Pyrene, Benzo(a)pyrene and some derivatives of these substances. PAH which were active in the screening were also found to stimulate production of IL-4 in normal, unmodified blood cells derived from healthy blood donors; this IL-4 is measured with immunological tests in the culture medium surrounding the cells [14].

The allergy-promoting effect is independent from the carcinogenic effect of some PAH: Benzo(a)pyrene is well-known as a potent cancer-inducing substance, but Pyrene is completely harmless in that respect [15]. However, since Pyrene is an even better inducer of IL-4 than Benzo(a)pyrene, it is probably a more efficient promoter of allergy development.

Small variations in the structure of the PAH can have a strong influence on the proallergic effects (Kusnetzow, Bömmel and Duschl, unpublished). At present, it is not understood which structural motifs are required to turn a PAH into an IL-4 inducer. We are now trying to identify the cellular receptor which interacts with PAH. These ongoing studies should allow us to outline the molecular mechanisms by which PAH modulate IL-4 production.

PAH as modulators of the immune system

We have observed that PAH can not only stimulate production of IL-4, but also of other immunoregulatory proteins. These other factors regulate different branches of the immune system, and their production out of turn may also lead to inappropriate immune activation. For example, Pyrene promotes production of Interferon-γ (Bömmel and Duschl, unpublished), which organizes the defense against some types of bacteria, including those responsible for tuberculosis and leprosy [16]. However, production of this protein in the wrong context promotes autoimmune reactions, which lead to tissue destruction in diseases like multiple sclerosis, insulin-dependent diabetes, or rheumatoid arthritis. The general effect of PAH seems to be an activation of the immune system, which may increase the chance for inappropriate immune activation and lead to disease.

PAH and its immune effects: What can be tested?

The biological test systems used in this study are suitable to test *e.g.* different particle fractions or particles derived from different sources for their potential to modulate the human immune system. It is for example not really clear how DEP would perform compared to particles derived from other sources of air pollutants, or to what extent engine or fuel types

influence the biological effects. Cell based assays allow to estimate immunomodulatory properties and they can give clues as to what disease may be associated with a particular compound. Positive substances can then be analyzed in mouse studies, which offer very good models for human disease, including a dependence on IL-4 for induction of allergy [17]. These bioassays should also lead us to an understanding why some particle-associated PAH influence the immune system, and why most others fail to do so.

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