

POLYAROMATIC HYDROCARBONS ADMINISTERED IN HUMANS BY DERMAL ROUTE INCREASE TOTAL IgE

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Inhalation of polyaromatic hydrocarbons (PAHs) extracted from diesel exhaust particles (DEP) enhances local (nasal) production of IgE in humans. The aim of the present research is to investigate whether in humans dermal exposure to PAHs which are not extracted from DEPs increases serum IgE and whether host factors modify the immunologic effect. In thirty-two patients with acute psoriatic lesions, a cream containing 3% of coal tar (which holds a variety of PAHs) was applied to the skin for 24 hours. Serum IgE were measured before (IgE₀) and four (IgE₄) and eight (IgE₈) days after application. Replicated means were compared by analysis of variance for repeated measures and by the Newman-Keuls' test. IgE₀, IgE₄ and IgE₈ were 151.19, 159.69 (a 6% excess) and 170.90 kU/L (a 13% excess) respectively; pairwise comparison showed IgE₈ was significantly higher than IgE₀ ($p < 0.05$). At multiple linear regression analysis, the percentage increase in serum IgE across observation days was the dependent variable against age, sex, cigarettes/day, urinary 1-pyrenol, atopy, skin area treated, and grams of cream. Of the independent variables, only age had a significant ($p < 0.028$) influence: the younger the age, the higher the IgE response to PAHs. We conclude that whatever the source and the route of entry (skin or respiratory tract), PAHs increase total serum IgE, mainly in younger age groups.

Numerous *in vitro* and *in vivo* studies have shown an increase in allergic antibody (IgE) production following exposure to polyaromatic hydrocarbons (PAHs) extracted from diesel exhaust particles (DEP) (1-3). *In vivo* studies on humans have, moreover, demonstrated that diesel exhaust particles can specifically enhance the local production of IgE in the upper respiratory mucosa (2).

Although most exposure to DEP-PAHs occurs through inhalation, in many cases dermal absorption is a significant component of total PAH exposure. Skin exposure to PAHs is common in various occupations. Moreover, young children may be exposed via dermal and oral routes as

they crawl on floor surfaces and place contaminated objects in their mouths (4). Recent data indicate that children have higher urinary levels of PAH metabolites than their mothers (5).

A cream containing coal tar (CT), which holds a variety of PAHs, is applied to the skin for at least 24 hours to treat acute lesions from psoriasis. Since psoriasis is not associated with altered total IgE (6), and since no studies have been undertaken in subjects exposed to PAHs through the skin, we measured serum IgE before and after four and eight days of treatment with CT for psoriasis in order to assess

1) the time response and the dose-response

Key words: polyaromatic hydrocarbons, total IgE, allergic diseases, psoriasis, coal tar cream, humans

relationships between PAHs administered through skin and production of total IgE antibodies; and 2) whether host factors, such as age, sex, and smoking modify the immunologic effect.

MATERIALS AND METHODS

Our series consisted of 32 patients (26 men, age: 48.7 ± 13.9 ; 6 women, age: 57.7 ± 16.6), who were consecutively admitted to the Section of Dermatology of the University of Padova from 01/02/2000 to 30/04/2000 for psoriasic lesions covering at least 30% of the skin surface area. The patients were treated for a single 24-hour day by applying a skin ointment that had the following composition (w/w): 33% distilled water, 24% stearyl alcohol, 24% vaseline, 0.015% nipasol, 0.9% sodium lauryl sulfate, 0.025% nipagin, 10% propylene glycol, 2% salicylic acid, 3% sulfur, and 3% coal tar (CT), which contains large quantities of PAHs. For each patient, the following information was collected: sex, age, occupational or environmental exposure to PAHs, cigarettes/day in smokers (who were not asked to interrupt their smoking-habit during treatment), and therapy at home (CT products: shampoos, soaps, lotions, etc.) or in hospital (CT cream applied in grams, skin area treated as a percentage of the body surface, topical steroids, phototherapy). The study was approved by the Ethics Committee of the School of Medicine, University of Padova. All patients gave their fully informed written consent. Patient anonymity was maintained.

A sample of peripheral blood (20-25 ml) was collected before, and on the fourth and the eighth days after the 24-hour skin treatment with CT. Serum was immediately obtained by centrifugation and the tubes were stored at 20°C until use. Twenty-four hour urine was collected immediately after treatment. Single micturitions, stored in polyethylene containers, were kept at -20°C in the dark until use. All measurements were carried out in one session, after collecting all samples.

Urinary 1-pyrenol was determined using a method described elsewhere (7). Briefly, urine was submitted for acid enzymatic digestion with β -glucuronidase/aryl sulfatase, followed by purification and enrichment on C18 resin (Sep-Pak C18). 1-Pyrenol was analysed by fluorescence high pressure liquid chromatography (HPLC). Individual values were expressed as μ moles of 1-pyrenol/mole of creatinine (μ moles/mole creatinine).

Serum IgE levels were measured using a fluoro-immunoassay method (UNICAP Total IgE- Pharmacia & Upjohn, Uppsala), and expressed as kU/L. Atopy, an evident source of high IgE levels, was assessed by the Phadiatop test, which uses a fluoro-immunoassay method (UNICAP Phadiatop-Pharmacia & Upjohn, Uppsala). The test determines several IgE antibodies in serum against a panel of respiratory antigens; if the finding is positive, the subject is classified as atopic (8,9).

We used one-way analysis of variance (ANOVA) for repeated measures (10) in comparing the IgE values measured in the same subjects before, and at the fourth and eighth days after treatment. The Newman-Keuls' test was then used to make pairwise comparisons of all the correlated means.

Serum IgE values were plotted against time (days 0, 4 and 8 from treatment) in each subject, the intercept (a) and the slope (b) being obtained by means of simple linear regression analysis. Then the ratio (b/a), expressing variations in IgE across the days of observation as a percentage of the baseline value, was used as a dependent variable in a stepwise multiple linear regression analysis, in which age, sex, cigarettes/day, urinary 1-pyrenol, atopy, skin area treated, and grams of CT cream were the independent variables. A statistically significant influence of one or more of the latter was assessed through the "t" test on the coefficients of regression.

RESULTS

Tab. I shows mean, standard error, and range for age, cigarettes/day, urinary 1-pyrenol, skin area treated, grams of CT cream, and IgE measured at days 0 (IgE_0), 4 (IgE_4) and 8 (IgE_8) following treatment in our subjects. In the total sample, IgE_0 , IgE_4 and IgE_8 were 151.19, 159.69 and 170.90 kU/L respectively; IgE_0 being 100%, IgE_4 and IgE_8 were 105.6% (an excess of 6%) and 113.0% (an excess of 13%), respectively. The values for 1-pyrenol greatly exceeded those found in non-smokers (99 percentile=0.67 μ moles/mole creatinine) (11), and the biological threshold limit of 2.3 μ moles/mole creatinine recommended for coke-oven workers (12). The wide range for most variables indicates that subjects are highly heterogeneous. The prevalence of atopy was 34.4%.

Tab. II shows that the F test result (analysis

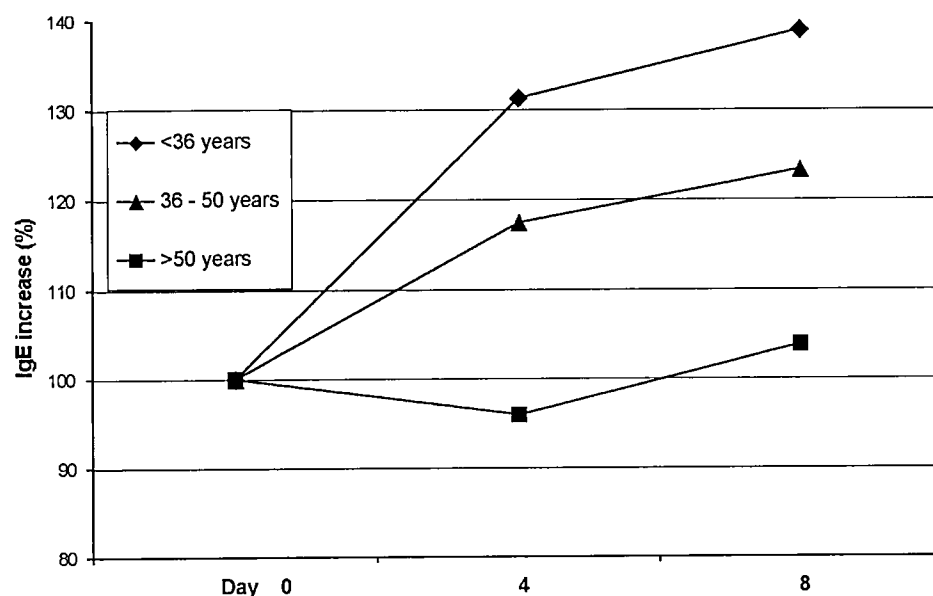


Fig. 1. Serum IgE at day 0 (baseline = 100%), 4 and 8 after skin treatment with coal tar: mean values in three age classes (<36, 36-50, >50 years).

Tab. I. Mean, standard error (SE) and range of the study variables in 32 psoriatic patients

Study variables	Mean	SE	Range
Age (years)	50.41	2.58	23 – 75
Cigarettes/day [§]	18.73	2.27	1 – 30
Urinary 1-pyrenol [°]	38.48	9.63	0.9 – 266.3
Skin area treated (%)	64.60	2.61	30 – 85
Coal tar cream (grams)	19.41	0.94	9 – 28
Total IgE before treatment	151.19	28.19	9.2 – 707.0
Total IgE on the day 4	159.69	28.64	8.9 – 673.0
Total IgE on the day 8	170.90	31.46	10.6 – 741.0

[§] in the 11 current smokers

[°]μmoles/mole creatinine

Tab. II. Analysis of variance for repeated measures, comparing total IgE values measured before and on days 4 and 8 following treatment with coal tar applied to the skin in 32 psoriatic patients.

Sources of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F
Between subjects	2532970.4	31		
Whitin subjects	56822.3	64		
Between days	6241.0	2	3120.5	3.82*
Residual	50581.3	62	815.8	

* 0.01 < p < 0.05

of variance for repeated measures) indicates statistically significant ($p < 0.05$) differences among the three replicated means. In the pairwise comparison, only the difference between IgE_8 and IgE_0 exceeded the critical value of the Newman-Keuls' test ($p < 0.05$).

As it can be seen in Fig. 1, the highest percent increase in serum IgE (more than 40% above baseline) occurred at day 8 after CT cream application in subjects younger than 36 years, although absolute IgE_0 values among age groups were not significantly different.

At stepwise multiple linear regression analysis, of the independent variables only age had a significant influence on the percentage increase in IgE (the b/a ratio) across the days of observation (regression coefficient: - 0.12; standard error: 0.05; "t" test: -2.34; $p < 0.028$). The negative sign of the coefficient indicates that with increasing age there is a decreased IgE production after exposure to CT cream containing PAHs. IgE changes were not correlated to grams of CT cream used, skin area treated, 1-pyrenol excretion, sex, cigarettes/day, and atopy.

DISCUSSION

Smokers have higher serum IgE levels than non-smokers, probably because of PAHs in cigarette smoke (13). In our study, however, smoking habits (and atopic status) could not have confounded the time trend of IgE, because each subject was his/her own control.

On searching Current Contents (1995 to 2001) and Embase (1993-2001), no paper was found associating IgE changes with any cream component other than PAHs: stearyl alcohol, vaseline, nipasol, nipagin, propylene glycol, salicylic acid, sulfur, and sodium lauryl sulfate, a known skin irritant (14) that induces neither IgE- nor T cell-mediated hypersensitivity responses (15).

Moreover, according to Weigl (6), the clinical features of acute psoriasis (high cortisol, reduced norepinephrine, markedly low IgM, IgG, and IgE in serum) are close to those of an immune deficiency syndrome. Lastly, the IgE in plasma from psoriatic patients are not altered by phototherapy (16).

In view of the above, the serum IgE changes

observed in our patients after application of CT cream to the skin may therefore be attributed to the PAH content of the cream. To our knowledge, ours is the only study to evaluate the skin route of exposure in examining the effect of PAHs on the level of the total IgE. Our findings are in agreement with those reported in experimental and epidemiologic studies (17), confirming that PAHs, whatever the route of entry (skin or respiratory tract), entail a risk of high IgE levels.

According to Diaz Sanchez (18), in nasal provocation studies the local level of IgE peaks at day 4 after exposure to PAHs, and returns to baseline values at day 8. However, according to Manetz (15), the peak time point is day 10 for the IgE+B220+ population in lymph nodes draining the dermal exposure site. This "local lymph node assay" is used to investigate the IgE- or T cell-mediated hypersensitivity responses to substances applied to the skin. In establishing the chronology of observation in our study, we had to choose between 4 and 10; day 8, determined by considerations as to feasibility, was probably too early, and this may have resulted in an underestimation of the IgE response after skin treatment with PAHs.

When transformed B cell line were exposed in vitro to phenanthrene (19) or when nasal washes were performed in humans after exposure to DEP (20), IgE were about three or, respectively, more than four-fold the initial value. The lower IgE increase in our study may be due to the fact that dermal route of entry may be less efficient than inhalation because in the airways DEP-PAHs interact with common environmental allergens in enhancing the production of IgE (21).

Chemical species of pollutants can play a role in underestimating the IgE increase. Phenanthrene constitutes 52% of DEP according to Barfknecht (22), and 31% of the CT cream administered to psoriasis patients (23). Moreover, diesel and gasoline-powered engines generate nitrated polycyclic aromatic hydrocarbons in their exhausts, rather than PAHs (24).

Therefore, although small, the time variations in total IgE are meaningful because they were observed in a context of immunosuppression and, probably, some days before the peak time point.

While in subjects submitted to nasal challenge studies the age range was narrow (20, 21), our patients were from 23 to 75 years of age. This enabled us to identify the modifying effect of age: the younger the age the higher the IgE response to PAHs. To our knowledge, this observation has never been made in the literature, and may explain the recent increase of allergic airway disease mainly in the early decades of life (17).

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REFERENCES

1. Takafuji S., S. Suzuki, K. Koizumi, K. Tadokoro, H. Ohashi, M. Muranaka, et al. 1989. Enhancing effect of suspended particulate matter on the IgE antibody production in mice. *Int. Arch. Allergy Appl. Immunol.* 90:1.
2. Diaz-Sanchez D., A.R. Dotson, H. Takenaka and A. Saxon. 1994. Diesel exhaust particles induce local IgE production in vivo and alter the pattern of IgE messenger RNA isoforms. *J. Clin. Invest.* 94:1417.
3. Takenaka H., K. Zhang, D. Diaz-Sanchez, A. Tsien and A. Saxon. 1995. Enhanced human IgE production results from exposure to the aromatic hydrocarbons from diesel exhaust: Direct effects on B-cell IgE production. *J. Allergy Clin. Immunol.* 95:103.
4. Talaska G., P. Underwood, A. Maier, J. Lewtas, N. Rothman and M. Jaeger. 1996. Polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs and related environmental compounds: Biological markers of exposure and effects. *Environ. Health. Perspect.* 104;(S) 5:901.
5. Kang D.H., A. Correa-Villasenor, P.N. Breyse and P.T. Strickland. 1995. Correlation of urinary 1-hydroxypyrene glucuronide concentration between mothers and their children. *Proc. Annu. Meet. Am. Assoc. Cancer Res.* 36(S):107.
6. Weigl B.A. 1998. Immunoregulatory mechanisms and stress hormones in psoriasis (part 1). *Int. J. Dermatol.* 37:350.
7. Jongeneelen F.J., R.B.M. Azion and P.T. Henderson. 1987. Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine. *J. Chrom.* 431:227.
8. Dekker F.W., J.D. Mulder-Dzn, J.A. Kramps, A.A. Kaptein, J.P. Vandenbroucke and J.H. Dijkman. 1990. The Phadiatop in vitro test for allergy in general practice: is it useful? *Fam Pract.* 7:144.
9. Matricardi P.M., R. Nisini, J.G. Pizzolo and R. D'Amelio. 1990. The use of Phadiatop in mass-screening programmes of inhalant allergies: advantages and limitations. *Clin. Exp. Allergy.* 20:151.
10. Winer B.J. 1971. Statistical principles in experimental design, 2nd ed. McGraw-Hill Book Inc. New York, p. 1.
11. Pavanello S., A. Genova, V. Foa and E. Clonfero. 2000. Assessment of occupational exposure to aromatic polycyclic hydrocarbons determining urinary levels of 1-pyrenol. *Med. Lav.* 91:192.
12. Jongeneelen F.J. 1992. Biological exposure limit for occupational exposure to coal tar pitch volatiles at cokeovens. *Int. Arch. Occup. Environ. Health.* 63:511.
13. Jensen E.J., B. Pedersen, E. Schmidt and R. Dahl. 1992. Serum IgE in nonatopic smokers, nonsmokers, and recent exsmokers: relation to lung function, airway symptoms, and atopic predisposition. *J. Allergy Clin. Immunol.* 90:224.
14. Basketter D.A., J. Miettinen and A. Lahti. 1998. Acute irritant reactivity to sodium lauryl sulfate in atopics and non-atopics. *Contact Dermatitis.* 38:253.
15. Manetz T.S., D.A. Pettit and B.J. Meade. 2001. The determination of draining lymph node cell cytokine mRNA levels in BALB/c mice following dermal sodium lauryl sulfate, dinitrofluorobenzene, and toluene diisocyanate exposure. *Toxicol. Appl. Pharmacol.* 171:174.
16. Jones C.D., M. Guckian, A.A. el Ghor, N.K. Gibbs and M. Norval. 1996. Effects of phototherapy on the production of cytokines by peripheral blood mononuclear cells and on systemic antibody responses in patients with psoriasis. *Photodermatol. Photoimmunol. Photomed.* 12:204.
17. Peterson B. and A. Saxon. 1996. Global increases in allergic respiratory disease: the possible role of diesel exhaust particles. *Ann. Allergy Asthma Immunol.* 77:263.
18. Diaz-Sanchez D. 1997. The role of diesel exhaust particles and their associated polyaromatic hydrocarbons in the induction of allergic airway disease. *Allergy* 52(S38):52.
19. Tsien A., D. Diaz-Sanchez, J. Ma and A. Saxon. 1997. The organic component of diesel exhaust particles and phenanthrene, a major polyaromatic hydrocarbon

- constituent, enhances IgE production by IgE-secreting EBV-transformed human B cells in vitro. *Toxicol. Appl. Pharmacol.* 142:256.
20. **Diaz-Sanchez D., A. Tsien, A. Casillas, A.R. Dotson and A. Saxon.** 1996. Enhanced nasal cytokine production in human beings after in vivo challenge with diesel exhaust particles. *J. Allergy. Clin. Immunol.* 98:114.
21. **Diaz-Sanchez D., A. Tsien, J. Fleming and A. Saxon.** 1997. Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. *J. Immunol.* 158:2406.
22. **Barfknecht T.R., R.A. Hites, E.L. Cavaliers and W.G. Thilly.** 1982. Human cell mutagenicity of polycyclic aromatic hydrocarbon components of diesel emissions. *Dev. Toxicol. Environ. Sci.* 102:77.
23. **Clonfero E., M. Zordan, D. Cottica, P. Venier, L. Pozzoli, E.L. Cardin, et al.** 1986. Mutagenic activity and polycyclic aromatic hydrocarbon levels in urine of humans exposed to therapeutical coal tar. *Carcinogenesis* 7:819.
24. **Bauer S.L. and P.C. Howard.** 1991. Kinetics and cofactor requirements for the nitroreductive metabolism of 1-nitropyrene and 3-nitrofluoranthene by rabbit liver aldehyde oxidase. *Carcinogenesis* 12:1545.