Polycyclic aromatic hydrocarbon-induced oxidative stress and lipid peroxidation in relation to immunological alteration

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ABSTRACT

Objectives We evaluated the association between exposure to polycyclic aromatic hydrocarbons (PAHs) and immunological measurements using blood samples from coke oven workers exposed to high and low PAH levels.

Methods A population-based cross-sectional study was conducted from 2008–2009 with coke oven workers and steel-rolling workers as the exposed and control groups, respectively. Questionnaires on basic demographic information were administered. Personal breathing zone and urine samples were collected to quantify personal PAH intake and biological response doses. Immunological and cytokine parameters in serum were analysed. Urinary malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) were analysed to determine oxidative stress induced by PAHs in relation to altered humoural immunological status.

Results Mean levels of serum immunoglobulin A (IgA) and TNF-α were significantly increased in coke oven workers compared to steel-rolling workers who had no or minimal PAH exposure ($p=0.0033$ and $p<0.0001$, respectively). There were no significant differences in mean levels of IL-4 and IL-10 between coke oven workers and steel-rolling workers. Moderate activation of lipid peroxidation and oxidative damage as determined by plasma MDA and 8-OHdG levels were detected simultaneously with significant alterations in IgA and IgE levels. Multiple regression analyses demonstrated that PAHs with high molecular weights >252 (dibenzo(a,h) anthracene, benzo(a)pyrene, benzo(a)anthracene and/or indeno(1,2,3-cd)pyrene) correlated with IgA and IgE levels.

Conclusions This study showed that coke oven workers with chronic exposure to PAHs may develop immunological alteration. Oxidative stress and lipid peroxidation induced by PAHs may partly explain the alteration in immunological parameters.

What this paper adds

- This study supports and supplements existing evidence that polycyclic aromatic hydrocarbons (PAHs) alter humoural parameters of the immune system.
- Oxidative stress-induced lipid peroxidation may play a moderate role in the alteration of humoural immunological parameters.
- Awareness of PAHs and their association with autoimmunity could prompt or alter monitoring, testing and treatment regimens for exposed individuals or populations.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), a group of toxic and lipophilic chemicals, have become a widespread environmental contaminant due to their prevalence in petroleum, coal, soot, cutting oil, tobacco and charbroiled meat. Several studies have consistently shown that they can alter specific or non-specific immunity in animal and human cells.[1–3] However, epidemiological studies have reported inconsistent results regarding alteration of immunological profiles induced by PAH exposure. For example, Szczeklik *et al* observed that coke oven workers exposed to PAHs had markedly suppressed serum IgA and IgG,[4] and slightly increased serum IgE levels as compared with workers with no PAH exposure.[4 5] Karakaya *et al* have found that serum IgG levels were significantly higher in PAH-exposed male road-paving workers than in non-exposed workers.[6] Currently, only sporadic information about immunological changes after chronic occupational exposure to PAHs is available from existing human epidemiological databases. However, limited studies that investigated the relationship between metabolites resulting from PAH exposure and immunological status may be useful for examining the mechanism of toxicity of PAHs to the human immune system.

PAHs can cause toxicity after they are metabolically activated to become electrophilic intermediates.[7 8] These reactive intermediates are then capable of covalent binding to DNA or engaging in redox cycling. In turn, this leads to over-production of reactive oxygen species (ROS), which causes oxidative stress. For example, levels of urinary 8-hydroxydeoxyguanosine (8-OHdG), a biomarker of oxidative damage, have been found to be higher in coke oven workers compared with non-exposed workers.[9 10] The levels of 8-OHdG significantly correlated with urinary 1-hydroxypyrene (1-OHP), a reliable biomarker for biological response doses of PAH exposure.[9] Studies have suggested that oxidative stress-induced lipid peroxidation may play a role in toxic responses to PAH in immunological systems. Animals had a 1.5- to 2-fold increase in lipid peroxidation products in blood plasma after 6 months of PAH inhalation as compared with controls.[11] The increased lipid peroxidation products were linked to modification

of leucocyte membranes and reduced activity of lymphocyte enzymes.[11]

This study was designed to (1) assess humoural immunological alterations in serum samples from coke oven workers exposed to PAHs, and (2) examine oxidative biomarkers, such as plasma malondialdehyde (MDA) and 8-OHdG, for PAH exposure in relation to immunological alterations to explore underlying mechanisms of PAH-induced immunotoxicity. We collected serum samples, administered a questionnaire and took personal breathing zone samples from coke oven workers as the exposed group and from steel-rolling workers with no or minimal exposure to PAHs as the control group. Total PAH levels and urinary 1-OHP levels were quantified to determine intake doses and biological response doses of PAHs. Oxidative status was evaluated by measuring levels of urinary MDA and 8-OHdG. A variety of immune parameters were assayed, including immunoglobulins (IgG, IgE and IgA) and cytokines (TNF-α, IL-4 and IL-10). Finally, statistical analyses were conducted to examine the association between exposure to PAHs and alteration of immunological parameters.

MATERIALS AND METHODS

Study design and participants

We conducted a cross-sectional study to assess the relationship between exposure to PAHs and immunological response in coke oven workers. Recruitment took place in the largest steel plant in Taiwan where 1000 workers were employed. We chose this plant because (1) the coking process in the plant had remained the same for over a decade and (2) the steel company offers annual health check-ups for coke oven workers at a clinic in the plant. This allowed us to collect specimens from workers during their annual physical examinations in order to minimise interference with normal work schedules. Features of this study design included efficient control for confounding factors, accurate exposure ascertainment, and sufficient power to detect exposure-related changes in immunological indicators.

Topside coke oven workers, side coke oven workers and steel-rolling workers were recruited to serve as high exposure, low exposure and control groups, respectively. Topside coke oven workers were defined as staying in the topside-oven area for more than 6 h per day. Side coke oven workers were defined as staying in the side-oven area more than 4 h per day without any visits to the topside area. The steel-rolling workers had minimal PAH exposure, since the rolling steel plant is approximately 1 km away from the coke-oven plant. The steel-rolling workers were blue collar workers with similar educational and social backgrounds and geographical location as the coke oven workers. The selection criteria included: (1) at least 1 year of employment at the plant and 3 months in the same work location; (2) age between 25 and 50 years; and (3) no medical history of allergy, asthma or allergic rhinitis. Workers with skin infections or fever during the sampling period were excluded from the study.

All participants signed consent forms prior to data and specimen collection. Questionnaires were administered and urine and blood samples collected during annual health check-ups in the company's clinic. A structured questionnaire was filled out for each worker in a face-to-face interview to determine socioeconomic background, health and medical history, employment history and habits. After subjects completed the questionnaire, blood and urine specimens were collected. Subjects were instructed not to eat for at least 6 h before blood sampling. Blood was centrifuged and serum was stored in a freezer at −70°C. Serum IgA, IgE and IgG were measured within 1 month. Urine specimens were used to analyse MDA, 8-OHdG and 1-OHP levels. The experimental protocol was approved by the Institutional Review Board of Kaohsiung Medical University.

Exposure assessment

The coking process of the coke-oven plant had not particularly changed over the past decade. On-going air monitoring in this plant indicated that total respiratory particulate PAH levels surrounding the coke-oven area ranged from 9700 to 1400 ng/m^3 depending on distance from the coke ovens. These readings were at least 200 times higher than in non-exposed area, including the rolling steel plant (6.7 ng/m^3).[12] To assess the PAH intake levels of the workers, we quantified PAH levels in workers' personal breathing zones. In addition, urinary 1-OHP levels were measured to determine the PAH biological response dose. The personal breathing zone air samples were collected using battery operated personal air sampling pumps (model 224PCXR7; SKC, Müllheim, Germany) with an average flow rate of 2.01 l/min.[13] The levels of the following 16 PAHs recommended by the US Environmental Protection Agency were quantified: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, fluoranthene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene and dibenzo(a,h)anthracene. PAHs were analysed using a gas chromatograph/quadruple mass spectrometer (GC/MS) with an automatic sampler system.[14 15] The lowest detection limits of the 16 PAHs ranged from 7.86 ng for naphthalene to 0.4 ng for dibenzo(a,h)anthracene. Measurements below the detection limit in each air sample were set at half the detection limit.

Urinary 1-OHP was analysed using an HPLC with a fluorescent detector.[14 15] Normalised levels of urinary 1-OHP were expressed as ng/g creatinine as measured by the Jaffe reaction.[14 16] The detection limit was found to be approximately 0.1 mg/l based on seven repeated analyses of 1-OHP at 15.0 mg/l. The variation in the coefficients of repeated analyses for urinary 1-OHP was less than 10%.

Determination of serum immunoglobulins and cytokines

Serum IgA and IgG levels were determined by the nephlometric method,[17] and serum IgE levels were determined by isotype-specific enzyme-linked immunosorbent assays.[18] Serum TNF-α, IL-4 and IL-10 levels were ascertained by a immunometric assay (Diagnostic Products Corporation, Los Angeles, California, USA). The range of the assay for TNF was 1.7−1000 pg/ml, so the non-detection level was set at 2.0 pg/ml. The range of the assays for IL-4 and IL-10 levels with eight measurements was 0−16 pg/ml (0, 0.25, 0.5, 1, 2, 4, 8 and 16 pg/ml) and 0−50 pg/ml (0, 0.78, 1.56, 3.12, 6.25, 12.5, 25 and 50.0 pg/ml), with R^2 at 0.9996 for IL-4 and 0.9979 for IL-10, respectively.

Urinary MDA and 8-OHdG

Urinary MDA levels were measured with an HPLC system (model 980-PU; JASCO, Tokyo, Japan) using a C18 column and an ultraviolet-visible detector at 532 nm (JASCO UV-975).[19 20] The mobile phase was comprised of a methanol/potassium phosphate (9:11) buffer and the flow rate was 1.2 ml/min. The samples were analysed for MDA based on the thiobarbituric acid (TBA) reaction, with HPLC separation of the MDA (TBA) 2 adduct, using tetraethoxypropane as a standard. A detection limit of 0.06 mg/l was obtained from seven repeated analyses of deionised water, and the coefficient of variation in repeated analyses was less than 10%. The urinary MDA levels for each individual were corrected according to urine creatinine values.

8-OHdG levels were measured using the LC/MS/MS method.[9] A 500 μl sample of urine was loaded into a Sep-Pak C18 cartridge preconditioned with 1 ml methanol and 1 ml of distilled water. To purify the crude urine sample, the fraction containing 8-OHdG was eluted with 1 ml of 40% (v/v) methanol, collected and dried under vacuum for 2 h, and dissolved in 500 μl of 80% acetonitrile containing 0.1% formic acid. Then, 20 μl of the sample solution was injected into the HPLC/MS/MS instrument. The HPLC system consisted of a PE 200 autosampler and two PE 200 micropumps (Perkin Elmer, Norwalk, Connecticut, USA), and a polyamine-II endcapped HPLC column (150×2.0 mm, 5 μm, YMC) with an identical guard column. The mobile phase was 80% acetonitrile with 0.1% formic acid, delivered at a flow rate of 300 μl/min. The eluent of the HPLC system was connected to a triple-quadrupole mass spectrometer (API 3000; Applied Biosystems, Foster City, California, USA) equipped with a turbo-ion-spray source. Electrospray ionisation was performed in the positive mode. For all samples, the $[M+H]^+$ ion was selected by the first mass filter. After collisional activation, the $[M+H-116]^+$ ions, corresponding to $BH2^+$, were selected by the last mass filter. Nitrogen was used as the nebulising, curtain, heater (6 l/min) and collision gas. The turbo-ion-spray probe temperature was set at 300°C.

Statistical analysis

To reduce the influence of outliers and normalise residual distribution, we used the natural logarithm of PAHs, and immunoglobulin and cytokine levels. The Student t test and Bonferroni statistics were used to compare PAH levels and immunoglobulin and cytokine levels in the three subject groups. The general linear regression method was used to evaluate any significant difference in immunological parameters, cytokine levels and PAH levels before and after adjusting for covariates. Pearson's correlation coefficient was calculated to assess association between PAH levels and serum immunoglobulin and cytokine levels, and was useful to select variables for the regression models. For example, if more than one PAH species correlated with IgE, IgA and IgG, multiple regression models were then used to determine the relationships between various PAHs and IgE, IgA and IgG. This occurred after adjusting for potential confounding variables, such as age, smoking status, alcohol consumption and vitamin consumption. A p value of <0.05 was considered significant.

RESULTS

Table 1 summarises the demographic data of the topside coke oven workers, side coke oven workers and steel-rolling workers. We found no significant differences among the three groups regarding age, alcohol consumption, betel chewing, asthma or allergic history. However, the topside coke oven workers had less cigarette smoking than the side coke oven workers and steel-rolling workers (p=0.04). Also, both topside coke oven workers and side coke oven workers had significantly higher vitamin intake than the steel-rolling workers (p<0.001).

Table 2 shows the mean levels of serum immunoglobulins and cytokines. The highest mean levels of serum IgE and TNF-α were in the topside coke oven workers, followed by the side coke oven workers and steel-rolling workers. Both topside and side coke oven workers had significantly higher mean levels of IgE and TNF-α than steel-rolling workers (p=0.0033 and p<0.0001, respectively). Changes in mean levels of IgE and TNF exhibited a dose–response pattern in coke oven workers exposed to PAHs. In contrast, coke oven workers had significantly lower mean values of IgA than steel-rolling workers (p=0.008), whereas mean levels of serum IgG were not significantly different among the three groups (p=0.1896).

Table 1 Demographic data of exposed and non-exposed workers

	High exposed group (n=66)	Low exposed group (n=162)	Non-exposed group (n=168)	p Value
Age (years)	42.1±8.4	41.8±8.6	43.2±6.8	0.52
BMI (kg/m^2)	24.7±5.3	25.2±8.9	26.4±12.8	0.31
Cigarette smoking				0.04*
Yes	37 (56.0)	62 (38.0)	57 (34.0)	
No	29 (44.0)	100 (62.0)	111 (66.0)	
Alcohol consumption				0.36
Yes	10 (15.8)	(16.2)	22 (19.8)	
No	56 (84.2)	(83.8)	80 (80.2)	
Betel chewing				0.19
Yes	16 (9.2)	14 (8.3)	13 (2.0)	
No	60 (90.8)	148 (91.7)	155 (98.0)	
Regular intake of vitamins				<0.12
Yes	26 (39.2)	49 (30.1)	26 (15.6)	
No	40 (60.8)	113 (69.9)	142 (84.4)	

Values for age and body mass index (BMI) are means±SD; other values are number (%). No log transformation on the data was needed.
*p<0.05.

The total PAH levels and 1-OHP levels in topside coke oven workers were significantly higher than in side coke oven workers (table 3). The mean values of 14 of the 16 targeted PAHs (acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene) were significantly different between the topside coke oven workers and side coke oven workers. The levels of benzo(a)pyrene were strongly and positively correlated with the levels of total PAHs and 1-OHP levels (r=0.97, p<0.001; r=0.98, p<0.001, respectively). 1-OHP levels correlated with IgE levels (r=0.89, p<0.01) and were weakly correlated with IgA and IgG levels (r=0.37, p=0.06; r=0.25, p=0.09, respectively).

Table 4 shows the mean levels of two oxidative stress parameters, MDA and 8-OHdG. Topside coke oven workers had the highest MDA levels, followed by side coke oven workers and steel-rolling workers. However, there was no significant difference among the three groups (p=0.396). Mean levels of 8-OHdG in the topside coke oven workers were higher than in the side coke oven and rolling-steel workers. However, there was no significant difference among the three groups (p=0.092). The MDA and 8-OHdG levels moderately correlated with large increases in the levels of IgE and TNF-α (r=0.67).

When the correlation between PAHs and immunological parameters was examined, body mass index (BMI) and asthma

Table 2 Mean values for serum immunological parameters in the workers

	High exposed group (n=66)	Low exposed group (n=162)	Non-exposed group (n=168)	p Value
IgA (mg/dl)	255.77±117.71	259.99±99.96	314.08±123.23	0.0080*
IgE (mg/dl)	231.52±99.96	150.83±286.82	97.59±251.27	0.0033*
IgG (mg/dl)	1255.78±255.96	1292.15±245.77	1300.48±264.70	0.1896
TNF-α (pg/ml)	11.94±12.63	6.48±7.27	2.04±0.34	<0.0001*
IL-4 (pg/ml)	0.0005±0.003	0.0013±0.016	0±0	0.5011
IL-10 (pg/ml)	0.960±1.96	0.677±1.86	0.561±1.635	0.3068

Values are means±SD. Serum IgA, IgE and IgG levels were $\log_{10}$ transformed to achieve normality of distribution. Comparisons of the serum immunological responses among the exposed and control groups used log transformed data.
*p<0.05.

Table 3 Mean concentrations of total polycyclic aromatic hydrocarbons (PAHs) and 16 selected PAH species in coke oven workers

PAHs (ng/m^3)	Top-oven (n=16)	Side-oven (n=24)	p Value
Naphthalene	40.83±26.53	110±35.60	0.4375
Acenaphthylene	5.56±12.38	0±0	0.0328*
Acenaphthene	0.69±2.12	0±2.12	0.2019
Fluorene	5.90±6.80	0.69±4.03	0.0326*
Phenanthrene	134.46±178.24	2.10±17.91	0.0045*
Anthracene	43.29±64.86	5.54±6.81	0.0070*
Fluoranthene	778.66±757.82	44.84±52.61	0.0466*
Pyrene	629.86±379.09	58.94±39.45	0.0423*
Benzo(a)anthracene	1363.13±3133.07	41.96±71.38	0.0471*
Chrysene	987.55±3971.83	50.80±63.53	0.0176*
Benzo(b)fluoranthene	2045.31±667.00	152.61±191.11	0.0243*
Benzo(k)fluoranthene	714.16±267.00	57.74±52.79	0.0149*
Benzo(a)pyrene	1603.06±539.47	78.21±100.78	0.0403*
Indeno(1,2,3-cd)pyrene	1110.07±2216.85	76.99±102.92	0.0274*
Dibenzo(a,h)anthracene	276.77±628.38	17.49±24.04	0.0491*
Benzo(g,h,i)pyrene	690.98±1209.49	62.47±80.69	0.0148*
Total PAHs	10430.28±1034.70	760.38±80.69	0.013*
1-OHP	68.24±75.02	32.83±51.25	0.0001*

*p<0.05.
Values are means±SD.

allergy history were not included in the regression models since they did not significantly predict serum IgA, IgE, IgG or TNF-α levels and did not affect the significance of exposure status (high and low versus no exposure). As only five readings of fluorene levels were recorded in the personal breathing zone samples, fluorene was excluded from the regression models due to lack of statistical power. After controlling for covariates, including cigarette smoking, alcohol consumption and vitamin intake, the stepwise selection approach included dibenzo(a,h)anthracene, benzo(a)pyrene and benzo(a)anthracene in the best fit regression model in relation to IgA levels. These PAH species explained the variations in IgA levels in the exposed and non-exposed groups. Indeno(1,2,3-cd)pyrene was included in the best fit regression model in relation to IgE levels (table 5).

DISCUSSION

Our research shows that PAHs play an important role in stimulating IgE production, which is consistent with findings from other epidemiological studies.[2 5 21] Studies on diesel exhaust particles (DEPs) found that high PAH content induced significantly increased IgE production through enhanced allergic inflammation in humans.[21 22] We observed an inverse association between levels of PAHs and IgA, which is primarily responsible for protecting mucosal surfaces (eg, the respiratory and gastrointestinal tracts). So far, limited studies have yielded inconsistent findings. Szczeklik *et al* found marked depression in mean IgA levels in coke oven workers who had been chronically exposed to PAHs.[4] In contrast, Diaz-Sanchez demonstrated that DEPs with high PAH content had no effect on IgA levels in the upper respiratory tract of human subjects with a short exposure of 0.30 mg of DEPs into their nostrils.[23] Our results appear to agree with findings related to chronic rather than acute exposure to PAHs. The decrease in IgA production suggests that PAHs may compromise protection of mucosal surfaces in the respiratory tract. This issue area warrants future study to confirm such an effect.

Table 4 Mean urinary concentrations of multiple oxidative biomarkers in human subjects

	High exposed group	Low exposed group	Control group	p Value
MDA	2.08±1.74	1.58±1.35	1.45±1.06	0.0923
8-OHdG	12.98±12.32	10.09±7.92	10.61±7.77	0.115

Values are means±SD (μmol/mol creatinine); MDA, malondialdehyde; 1-OHP, 1-hydroxypyrene.

The well-characterised three levels of PAH exposure were suitable for examining whether the PAHs affected IgE levels in a dose–response fashion. After controlling for possible confounders, such as smoking and alcohol consumption, the greatest decrease in IgE levels was observed in the topside coke oven workers exposed to the highest PAHs levels, followed by those exposed to low PAH levels, and then controls. Such dose–response data are valuable for risk assessment to quantify the likelihood of immunological effects in individuals or populations exposed to PAHs.

Multiple regression analysis revealed that PAH species with heavier molecular weights in the range of 252–278, such as dibenzo(a,h)anthracene, benzo(a)pyrene, benzo(a)anthracene and indeno(1,2,3-cd)pyrene, correlated with marked increases in IgE levels and decreases in IgA levels. PAH species with molecular weights above 252 have been found to have higher redox activity than those with low molecular weights.[12 13] Higher redox activity can lead to ROS formation, causing oxidative stress to biological systems.[12 13] PAHs are highly hydrophilic and can directly enter cells, where they are metabolically activated by cytoplasmic enzymes (CYP450 family) to become electrophilic intermediates, such as semiquiones and quinones.[8 24]

Table 5 Multiple liner regression analysis on polycyclic aromatic hydrocarbon species as predictors for IgA and IgE concentrations

	IgA (mg/dl)			IgE (mg/dl)		
	R^2	Estimate (SE)	p Value	R^2	Estimate (SE)	p Value
Crude association						
Dibenzo(a,h)anthracene	0.0008	0.097	0.268		–	–
Benzo(a)pyrene	0.020	0.354	0.020	–	–	–
Indeno(1,2,3-cd)pyrene	0009	0.247	0.042	–	–	–
Benzo(a)anthracene	0.0007	0.206	0.058	–	–	–
Indeno(1,2,3-cd)pyrene						
Adjusted association†	–	–	–	0.1702	0.098	0.013*
Benzo(a)pyrene	0.030	0.508	0.0332	–	–	–
Indeno(1,2,3-cd)pyrene	0.122	0.413	0.0533	0.4202	0.1133	0.0054*
Benzo(a)anthracene	0.027	0.273	0.1128	–	–	–

*We adjusted for age, smoking and alcohol consumption and selected PAH species into the best fit models using stepwise selection. All data were $\log_{10}$ transformed.
†p<0.05 ($\log_{10}$ transformed).

These redox-reactive intermediates can undergo redox cycling and generate large amounts of radical species that upset the oxidative balance of homeostasis. Thus, it is likely that heavier PAH species increased redox activity and subsequently induced generation of ROS, which altered IgE and IgA levels in workers exposed to PAHs.

Moderate activation of lipid peroxidation was detected simultaneously with significant changes in humoural immunity shown by a decrease in IgA levels and an increase in IgE levels. Also, moderately increased 8-OHdG levels were found in topside coke oven workers. The data suggested that production of ROS results in lipid peroxidation, which was detected both systemically and locally, may effect the immune response of cells. Cellular and animal experiments have shown that a decrease in humoural immunity may be caused by activated lipid peroxidation resulting in modification of leucocyte membranes and consequent alteration of B cell and/or T cell maturation or function.[25 26] PAHs appeared to directly impact on B cells leading to increased IgE production and allergic inflammation.[27]

A general observation is that PAHs are cytotoxic at high doses, causing lymphoid organ atrophy, while lower doses of PAHs frequently result in alteration of immunological responses without cytotoxicity. In an in vitro study, Diaz-Sanchez *et al* showed that PAHs can exert an effect on total IgE synthesis by stimulating increased RNA transcription and translation.[28] This finding was in accordance with the ability of PAHs to alter transcription of a number of genes via the cytoplasmic Ah receptor complex.[29 30] There is good evidence that the Ah receptor complex is involved in the biological effects of PAHs in lymphocytes and B cells.[8 30] Studies have demonstrated that activation of mature human B cells up-regulates Ah receptor expression, suggesting that human B cells are direct PAH targets.[30 31]

Another model suggested that the toxic effects of PAHs on immune cell response may result from interference with or stimulation of cytokine secretion or cytokine receptor expression. Our study showed that increasing IgE accompanied increased production of TNF-α, a potent modulator of immune and inflammatory response. The mean level of TNF-α in the topside coke oven workers was 11.94 pg/ml, which was significantly higher than 2.04 pg/ml in the non-exposed group ($p<0.0001$). The data suggest that this cytokine is predominantly produced by activated macrophages. Although our study did not generate data on the origin of the macrophages, it is, nevertheless, fair to speculate that macrophages in the lungs may be a primary source of the increase in IgE levels. Also, direct peroxidation of cellular membranes likely occurs in the lung cells of affected individuals. We further examined those workers who regularly took at least one vitamin pill per week and observed that they had lower IgE and TNF-α levels. The antioxidant effects vitamins provide can directly and/or indirectly protect the host against the damaging influence of cytokines and oxidants. Antioxidants exerted a beneficial effect, which supports our findings on the involvement of oxidative stress induced by PAHs in immunotoxicity. IL-4 has been known as a type-2 cytokine, which is produced by type-2 T cells and a canonical cytokine for immunoglobulin class switching.[16] In this study, mean levels of IL-4 in exposed groups were the same as in controls, although IgE levels were significantly increased in exposed subjects. Perhaps PAHs activate cell division of pre-existing B cells already committed to producing IgE, instead of causing class switching.

CONCLUSION

This study supports and supplements existing evidence that PAHs can alter the humoural parameters of the immune system. Certain PAH species, particularly those with high molecular weights, alter PAH-induced immunological parameters. Oxidative stress-induced lipid peroxidation may play a moderate role in the alteration of humoural immunological parameters. The effect of PAHs on the immune system may stem directly from cytotoxicity of cells in the immune system. Due to widespread PAH contamination, the effect of PAHs on humoural immunity is a significant occupational and environmental risk for human health. Therefore, awareness of PAHs and their association with autoimmunity could prompt or alter monitoring, testing and treatment regimens for exposed individuals or populations.

To gain a better understanding of the mechanisms of PAHs' immunological effects, we suggest future studies should examine oxidative stress in depth as regards the effect of PAHs on humoural immunity. Also, further research should clarify the effects of PAHs on lymphocytic cells and their subsets, mitogen stimulation of lymphocytes and cellular signalling pathways. Finally, study of the role of polymorphisms in Ah receptor expression would be valuable to determine the risk of PAH-induced immunotoxicity in humans.[23] Such results could prove helpful to determine why people respond with different sensitivities to these agents.

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Competing interests None.

Ethics approval This study was conducted with the approval of Kaohsiung Medical University.

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