

Fig. 2. Prevalence of selected diseases in the North Bohemian region: relative values in relation to Czech Republic = 100 %. The first row represents children under 5 years of age, the second those from 5 up to 15 years and the third one older populations. 1 – pulmonary diseases, 2 – allergic affections, 3 – endocrine diseases, 4 – mental diseases, 5 – skin diseases, 6 – diseases of the upper airways, 7 – diseases related to social conditions.

Partial knowledge on the influence of noxious substances in the indoor atmosphere clearly indicates the necessity of monitoring constituents affecting the general health status and immune indices of exposed population (16). Sociological investigations show that humans spend at least 80 % of the time indoors. It is important to stress here the influence of social, psychic and aesthetic factors which are perceived with much greater intensity indoors than outdoors. It is therefore necessary to recognize the reflection of these influences on immunity and health not only from the point of view of their action on the central nervous system (1, 16).

Similarly as indoor spaces of our homes, social facilities and means of transport are characterized by certain and for them typical features, indoor working spaces are characterized by specific noxious substances which may cause a specific occupational risk of impairment of the immune system and subsequently of health. It is important to remember that the impact of this factor is limited by time and often by a series of other risk factors typical for a given locality. While model experiments made so far demonstrate clearly impairment of immune mechanisms due to the action of various xenobiotics, investigation of similar effects on man under conditions of the usual load associated with a broad spectrum of interfering factors is a far more complicated problem. Many xenobiotics followed up at present are not only components of the environment (water, atmosphere) but also of food chains, indoor contaminants, dwellings and transport environment. Furthermore, there are various physical and other factors (4). Different findings may therefore be obtained in occupationally exposed groups in different areas with a different environment etc. The complexity of immunotoxicofogical research in this sphere is, moreover potentiated greatly by possible effects of various noxious substances present e.g. in tobacco smoke etc. (16–18).

The subject of studies of preventive immunology with possible application both in individual and mass diagnosis need not be the above facts. The possibility and frequently clinical manifestations preceding recognition of health impairment and clinical condition may facilitate application of various compensatory measures as well as monitoring of their desired effects. This does not imply only possible elimination of exposure to noxious substances threatening a given population but also application of positive nutritional intervention, changes in the established life-style and habits. We cannot but agree with the credo of L. B. Lave: „The public requires a healthy and clean environment, a safe working atmosphere and wholesome food. For humans who have succeeded in photographing Earth from cosmic space and launching first man to the Moon these requirements should not be an insolvable problem. The public is right to be impatient over the lack of solution in regard to the above requirements (9).

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THE EFECT OF CIGARETTES SMOKING ON THE INDEXES OF IMMUNITY AND ACUTE PHASE REACTION IN SUBJECTS WITH OCCUPATIONAL EXPOSURE TO ORGANIC SOLVENTS

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SUMMARY

The study was carried out in 156 men, including 49 nonsmokers and 47 smokers who had never been exposed to chemicals, 19 nonsmokers exposed to organic solvents, and 41 smokers exposed to organic solvents. The results of toxicological analysis of air in the working place carried out in the range depending on the type of solvents used in the process of lacquering of steel cans and on the data obtained from the producer showed that the solvents contained benzene, toluene, xylene and their derivatives partly hydrogenated, paraffin hydrocarbons, oleins, naphthenes (components of painter's naphtha), monohydric and polyhydric alcohols (butanol, cyclohexanol, butyloglycol), esters (ethylglycol acetate, butyl acetate) and ketones (methyl isobutyl ketone, cyclohexanone). Measured benzene concentrations varied from 0 to 370 mg x m^{-3} (0 to 116 ppm), with arithmetic mean annual averages of about 100 mg x m^{-3} (31 ppm) in the late 1960's and less than 50 mg x m^{-3} (16 ppm) in the 1970's. In the 1980's values for the TWA were 0–38 mg x m^{-3} (0–12 ppm) with arithmetic mean averages of about 19 mg x m^{-3} (6 ppm) and for the level of benzene 0–351 mg x m^{-3} (0–110 ppm), with arithmetic mean annual averages of about 48 mg x m^{-3} (15 ppm). Phenol concentration in the urine of the workers in groups was 7.9 ± 3.5; 10.0 ± 5.8; 16.8 ± 6.2 and 18.4 ± 9.7 mg x l^{-1} respectively. Hippuric acid concentration in the urine of the workers in groups was 496 ± 326, 538 ± 341, 982 ± 420 and 1107 ± 507 mg x l^{-1} respectively.

The parameters of immunity and proteins acute phase reaction were determined, measuring the count of T, B, and „non-T, non-B" circulating lymphocytes, the concentration of immunoglobulins, lysozyme, C3c, C4, α_1-acid glycoprotein, haptoglobin and ceruloplasmin in serum. The results of the presented study suggest the role of cigarette smoking as a co-factor in the immunological changes brought out by occupational exposure to organic solvents. This phenomenon is reflected in the changes of IgA, IgD, IgG, IgM and lysozyme in the serum, and number of circulating T cells.

Key words: tobacco smoke, solvents, lymphocytes, antibodies, complement

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The assessment of the effect of a given chemical compounds on the immune system during occupational exposure is difficult, since only exceptionally the personnel of industrial plants is exposed to only one chemical substance. Most frequently, the workers are exposed to several chemical compounds at the same time, and tobacco smoke is an additional factor changing the immune status of many workers. Generally speaking, tobacco smoke exerts a depressing effect on the function and counts of T and B cells (1–4). Organic solvents impair also the functions of T and B cells, inhibit enzyme activity and cause destabilization of lysosomes in peripheral blood lymphocytes (2, 5–11). The acute effect of cigarette smoking induces bronchoconstriction (12). It seems likely that the activation of complement can induce the release of leukotrienes, and that these leukotrienes cause bronchoconstriction (13). Activated complements promote phagocytosis, acute chemotactic attraction of phagocytic cells, and generate anaphylatoxin. Chronic smoking is considered one of the predisposing factors for developing chronic airway obstruction.

In view of these data it seemed worthwhile to carry out a study of certain immunity system indexes and acute phase reaction in workers occupationally exposed to organic solvents, with analysis of the effect of tobacco smoking on them.

MATERIAL AND METHODS

The men who volunteered for study were divided into four groups: group I included 49 workers aged 17 to 50 years (mean age 31 years) doing moderately heavy physical work from 1 to 32 years, who never smoked cigarettes and had never been exposed to chemicals or harmful physical factors in their occupational work; group II – 47 workers aged 24 to 42 years (mean age 36 years) doing moderately heavy physical work from 3 to 24 years, who smoked 15 to 25 Polish mouthpiece cigarettes of various sorts per day (mainly of the „Klubowe" brand) since at least 10 years and had never been exposed to chemicals or harmful physical factors in their occupations; group III – 19 men aged 18 to 47 years (mean 30 years) exposed to organic solvents in their work in the production of steel cans (they had been working there since from 5 to

108 months, average – 62 months) in a three-shift work with working days 7–8 hours. They never smoked. In group IV 41 men aged 22 to 56 years (mean age 41 years) had been exposed to organic solvents for from 8 to 182 months (mean 71 months) in the same industrial plant; they gave a history of smoking various sorts of Polish cigarettes, mainly of the „Klubowe" brand for from 5 to 39 years (mean 21 years). The social-economic and nutritional conditions were similar in all the investigated subjects. They lived in partly industrialized region.

The chemical analysis performed at the Technical Research Centre in Finland demonstrated that the smoke from one cigarette of the „Klubowe" brand contained 21,5 mg tar substances, 1,14 mg nicotine, 11,7 mg carbon monoxide, and 261 μg hydrogen cyanide (14). The tobacco of one cigarette mentioned above contains also 2,7 μg of cadmium.

All these subjects were subjected to medical examination and to basic haematological and biochemical tests for determination of their present health state.

Concentrations of benzene in plant in various work areas had been measured from 1968 to 1982 by different groups, using various measured devices. In the 1980's gaschromatographic determination of benzene collected in the charcoal tubes were carried out according to the NIOSH method (15). In the 1990's five main work areas were identified. The exposure data were classified according to the shift time weighted average (TWA) and the level of benzene (time unspecified).

Exposure to benzene was assessed by the phenol test colorimetrically using 4-aminoantipyrin, the exposure to toluene was assessed by the hippurate test by the Burkiewicz-Zielinska method (16). The levels of phenol and hippuric acid were determined in urine collected between 6 and 8 hours of work.

For the immunological tests blood samples were taken from an antecubital vein before breakfast.

The concentrations of immunoglobulins A, G, M, complement components C3c and C4, α_1-acid glycoprotein, ceruloplasmin and haptoglobin were determined by radial immunodiffusion using NOR-Partigen plates and standard sera produced by Behring. For IgD determination I.C-Partigen was used, according to producer's instruction.

Serum lysozyme activity was determined by the Smolelis-Hartsell method modified by Harrison and Barne which is a turbidimetric method in which a suspension of *Micrococcus lysodeicticus* is used (17).

Lymphocytes were isolated by centrifugation of heparinized venous blood in a Ficoll 400 – Uropolinum density gradient for 30 minutes at room temperature, at 400 g. The cell suspension obtained from the interphase contained on avarage 91 % lymphocytes, 2 % neutrophils and 7 % monocytes. The viability of lymphocytes was tested with 1 % trypan blue solution at room temperature. It ranged from 97 % to 99 %.

The determination of T lymphocytes count was based on their ability to nonimmunologic binding with sheep red blood cells (SRBC) evaluated by the E rosette test. The E_a rosette test was done for the determination of direct rosettes (the preparation was made without earlier incubation) and the E_{18h} rosette test (the preparation was made after incubation for 18 hours). B cells were identified by the receptor for the C3 component on their surface which bound immune complexes made up of SRBC, anti-SRBC and complement in the EAC-rosette test. These tests were done according to the generally accepted principles observing the recommendations of the WHO Report (10). The methodological details have been described elsewhere (9, 10).

The count of the „non-T, non-B" lymphocytes was calculated as the difference between the total number of lymphocytes and the number of lymphocytes with surface markers.

The results were subjected to statistical analysis by Student's „t" test or Cochrane-Cox „C" test.

RESULTS

All the studied subjects were regarded as healthy. In none of them clinical sings of chronic bronchitis were found. The results of toxicological analysis of air in the working place carried out in the range depending on the type of solvents used in the process of lacquering of steel cans and on the data obtained from the producer showed that the solvents contained benzene, toluene, xylene and their derivatives partly hydrogenated, paraffin hydrocarbons, oleins, naphthenes (components of painter's naphtha), monohydric and polyhydric alcohols (butanol, cyclohexanol, butylglycol), esters (ethylglycol acetate, butyl acetate) and ketones (methyl isobutyl ketone, cyclohexanone). Measured benzene concentrations varied from 0 to 370 mg x m^{-3} (0 to 116 ppm) with arithmetic annual averages of about 100 mg x m^{-3} (31 ppm) in the late 1960's and less than 50 mg x m^{-3} (16 ppm) in the 1970's. In the 1980's values for the TWA were 0–38 mg x m^{-3} (0–12 ppm) with arithmetic mean averages of about 19 mg x m^{-3} (6 ppm) and for the level of benzene 0–351 mg x m^{-3} (0–110 ppm) with arithmetic mean annual averages of about 48 mg x m^{-3} (15 ppm). For the bulk of the exposure period, average concentrations were less than 64 mg x m^{-3} (20 ppm) on an annual basis. Detailed results of toxicological analysis have been published elsewhere (7, 9, 10). The solvent of the lacquer „Kreolak" was examined chromatographically since it was relatively frequently used in the production. The lacquer contains xylene 3 %, butanol 12 %, cyclohexanol 35 %, ethylglycol acetate 25 % and butyloglycol 25 %. Painter's naphtha contains about 12 % of aromatic hydrocarbons.

Phenol concentration in the urine of the workers in groups I, II, III and IV was 7.9 ± 3.5; 10.0 ± 5.8; 16.8 ± 6.2 and 18.4 ± 9.7 mg x l^{-1} respectively. Hippuric acid concentration in the urine of the workers in groups I, II, III and IV was 496 ± 326, 538 ± 341, 982 ± 420 and 1107 ± 507 mg x l^{-1} respectively. These results demonstrate increased concentrations of phenol and hippuric acid in the urine of the workers in groups III and IV confirming thus the absorption of small amounts of benzene and toluene. In relation to group I the concentrations of IgA were lower in groups II and IV (Table 1). In group IV this concentration was low also in relation to group II and III, while in group III IgA concentration was higher than in groups II and IV.

IgD concentration was low only in group IV workers.

In relation to group I a low level of IgG was observed in smokers in group II and in group IV workers. In the latter group IgG level was also lower than in groups II and III. In group III the concentration of this immunoglobulin was higher than in groups II and IV.

The concetration of IgM was lower in group II in relation to group I, and in group IV workers in relation to groups I and III.

Lysozyme concentration was decreased in group II and group IV in relation to group III and group I (Table 2). The absolute lymphocyte count was reduced only in group IV as compared to group I (Table 3). The population of T cells was decreased in groups II and III, and even lower it was in group IV. The count of „non-T, non-B" lymphocytes was raised in group II in relation to group I. On the other hand, the count of these cells was lower in groups III and IV than in smokers in group II. No changes were noted in the count of B cells and the concentrations of C3c, C4, α_1-acid glycoprotein, haptoglobin and ceruloplasmin.

Table 1. The blood serum immunoglobulin concentration in relation to the interaction between the cigarette smoking and the exposition to organic solvents (g x l^{-1})

Group		IgA	IgD	IgG	IgM
Nonsmokers n = 49 (I)	$\bar{x}$	2.33	0.23	11.92	1.77
	SD	0.78	0.17	2.92	0.71
Smokers smoking more than 10 years n = 47 (II)	$\bar{x}$	1.74^{x}	0.20	9.03^{x}	1.50^{x}
	SD	0.49	0.15	1.36	0.54
Not smoking workers n = 19 (III)	$\bar{x}$	$2.17^{\varnothing}$	0.24	$11.55^{\varnothing}$	1.71
	SD	0.54	0.18	2.16	0.42
		xxx ØØØ	xxx ØØØ	xxx ØØØ	xxx ØØØ
Smoking workers n = 41 (IV)	$\bar{x}$	1.51°	$0.12^{\circ\circ}$	8.11°	1.32
	SD	0.46	0.07	2.40	0.30

n - number of subjects
Statistical significance:
I : II, I : III, I : IV
x – p < 0.05
xxx – p < 0.001

II : III, III : IV
Ø – p < 0.05
ØØØ – p < 0.001

II : IV
° – p < 0.05
°° – p < 0.01

Table 2. The lysozyme (IU/ml), C3c, C4 α_1-acid glycoprotein, haptoglobin, and ceruloplasmin blood serum concentration (mg/dl) in relation to the interaction between the cigarette smoking and the exposition to organic solvents

Group		Lysozyme	C3c	C4	a_1-acid glycoprotein	Haptoglobin	Ceruloplasmin
Nonsmokers n = 49 (I)	$\bar{x}$	312	94.2	34.6	92.4	134	26.7
	SD	103	16.1	9.2	18.3	41	4.9
Smokers smoking more than 10 years n = 47 (II)	$\bar{x}$	498^{x}	90.3	33.8	105.3	145	25.8
	SD	61	17.8	12.4	27.6	62	6.7
Not smoking workers n = 19 (III)	$\bar{x}$	$268^{\varnothing\varnothing\varnothing}$	87.3	38.1	104.7	142	29.0
	SD		12.4	16.2	20.2	57	5.2
Smoking workers n = 41 (IV)	$\bar{x}$	$215^{\circ\circ}$ xxx	92.7	32.9	108.1	130	25.3
	SD	59	18.0	10.3	30.2	49	7.5

n – number of subjects
Statistical significance:
I : II, I : III, I : IV
x – p < 0.05
xxx – p < 0.001

II : III, III : IV
°° – p < 0.01
ØØØ – p < 0.001

The concentrations of IgA, IgD, IgG, IgM lysozyme and the T cell number in smokers exposed were lower than in not smoking workers (Fig 1).

DISCUSSION

The results of the presented study suggest the role of cigarette smoking as a co-factor in the immunological changes brought out by occupational exposure to organic solvents. Lysozyme is the enzyme hydrolysing the 1–4 glycoside bonds between N-acetylglucosamine and N-acetylmuramic acid. This type of bonds is found in mucopeptides of the cell wall of Gram-positive and Gram-negative bacteria. The hydrolytic effect of lysozyme damages the bacterial cell wall facilitating destroying of bacteria by other defensive mechanisms in the organism. The concentrations of IgA, IgD, IgG, IgM, lysozyme and the T cell number in smokers exposed to solvents were lower than in nonsmokers working under conditions of similar occupational exposure. Emphasis should be placed on the reduction of the number of mature T cells determined by the E_{18h} test and the T cells recognized by the E_a test which identifies young cells and activated cells. In smokers not exposed to organic solvents the reduced number of T cells was associated with a simultaneous rise in the population of „non-B, non-T" lymphocytes which may suggest rather a damage to the surface receptors of T cells. No such observation was noted in smokers exposed to solvents in whom T cell number reduction had no effect on the count of „non-B, non-T" lymphocytes, but was associated with a reduction in the total lymphocyte count. On the other hand, exposure to organic solvents alone had only a slight effect on T cells. The mechanism of the synergic action of tobacco smoke and organic solvents on an immunity system is still poorly known. These factors alone damage the genetic material impairing the effector functions and changing the composition of circulating lymphocytes (2, 5, 18, 19). Causing destabilization of lysosomal membranes they destroy the lysosomal apparatus which

Table 3. T, B and „non-T, non-B" circulating lymphocytes (x 10^9/l) in relation to the interaction between the cigarette smoking and the exposition to organic solvents

Group		Lymphocytes	T lymphocytes		B lymphocytes	„Non-T, non-B" lymphocytes
			$test_E$	$test_{E\,EAC}$		
Nonsmokers	x	2.39	1.29	1.68	0.54	0.17
n = 49 (I)	SD	0.82	0.39	0.42	0.20	0.09
Smokers smoking more than 10 years	x	2.27	1.06[x]	1.44[x]	0.54	0.30[x]
n = 47 (II)	SD	0.67	0.37	0.49	0.16	0.11
Not smoking workers	x	2.20	1.10	1.43[x]	0.57	0.20[o]
n = 19 (III)	SD	0.74	0.43	0.38	0.11	0.08
			xxx oo	xxx o		
Smoking workers	x	2.01[x]	0.84[+]	1.21[+]	0.60	0.20[o]
n = 41 (IV)	SD	0.62	0.29	0.35	0.13	0.07

n – number of subjects
Statistical significance:
I : II, I : III, I : IV
x – $p < 0.05$
xxx – $p < 0.001$

II : III, II : IV
o – $p < 0.05$
oo – $p < 0.01$

III : IV
+ – $p < 0.05$

is of essential importance for a normal development of lymphocyte activation (11, 19).

The synergism of tobacco smoke and other chemical substances in their effect on the concentration of serum immunoglobulins was reported also in subjects exposed to asbestos (20) and radioactive radiation emitted by uranium-containing rocks (21), while it was not observed in subjects exposed to vinyl chloride (22). The clinical importance of this interaction has been the subject of only few studies. Tobacco smoke impairs the induction of Ig-antibodies to antigens present in water vapor in subjects working in the production of microprocessors (23). Tobacco smoking by farmers is also a factor inhibiting the production of IgG antibodies to the antigens of *Aspargillus umbrosus* (24). It may be supposed that the immunosuppressive effect of tobacco smoke and organic solvents combined impairs in a higher degree the immunity against infections and neoplasmas than the action of either of these factors alone. This view is confirmed by epidemiological observations showing that tobacco smoke has a synergistic action with certain chemical carcinogenes, e.g. in smokers exposed to arsenic in occupational work the incidence of lung cancer was higher than in nonsmokers (25). A similar observation was made in the population of workers exposed to asbestos (26).

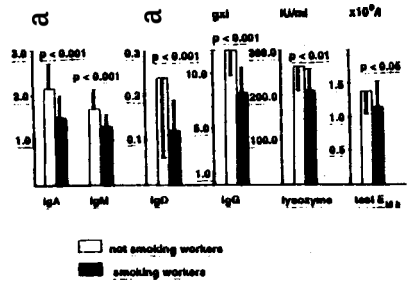

Fig. 1. Statistically significant differences of immunologic indices between smokers exposed to solvents and not smoking workers.

The acute phase accompanies inflammations and its intensity is proportional to the degree of tissue damage and manifests itself as a rise in the serum levels of certain proteins, among them: C-reactive protein, ceruloplasmin, mucoprotein, α_1-acid glycoprotein, haptoglobin and fibrinogen. The present study failed to demonstrate changes in the C3c, C4 and the acute phase proteins levels. It should be emphasized that only few of large group of heterogenic plasma proteins were examined. The cigarette smoking induces an increase in peripheral venous blood leukotrienes, and complement C3a and C5a concentration (13). The intensity of smoking in that study was five cigarettes in 20 min, which was interpreted as the maximal tolerated dose. Levels of stimulation did not occur to this extent in my study, where smoking one-three cigarettes per hour took place.

CONCLUSIONS

The assessment of the immunotoxic effect of organic solvents during occupational exposure should take into consideration the possibility of a synergistic action with tobacco smoke.

The concentrations of IgA, IgD, IgG, IgM, lysozyme and number of circulating T cells which are lower in smokers exposed to solvents than in nonsmokers working under similar conditions corroborate this phenomenon.

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