Phenotype analysis of lymphocytes of workers with chronic benzene poisoning

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Abstract

Lifetime exposure to benzene is associated to a variety of blood disorders, and except for the risk of cancer, almost nothing is known concerning health impairment in individuals who are no longer exposed. In Brazil, this exposure is one of the serious problems in workplaces, and many workers have been laid off their jobs due to this intoxication, particularly in the State of Bahia, the largest producer of benzene in Latin America, which is the area of this study. From a larger study to describe health effects and genetic polymorphisms among workers with chronic benzene poisoning (CBP), this previous specific investigation analyzes the association between CBP and the pattern of sub-populations of lymphocytes. The study was performed with a CBP group ($n = 24$) and a control group with other occupational diseases ($n = 24$); both were selected at the Workers Health Study Center in the State of Bahia, Brazil. Clinical and epidemiologic variables were collected from medical records and from a detailed questionnaire. The average age was similar in the two groups (51.1 and 50.7, respectively). Analyzing the mean proportions of the sub-populations of lymphocytes, statistically significant differences were found for T cytotoxic cells (TCD8) (27.9; 19.4; $p = 0.002$) and T helper memory cell (CD4CD45RO) (31.2; 37.0; $p = 0.015$), respectively, for the CBP group and control group. These results should be viewed with caution because of the small sample size, but they strengthen a previous impression that workers exposed to benzene have their immune system impaired, even in the long term, which may contribute to some disorders and carcinogenesis process. These workers must be strictly followed up in a medical surveillance program. Although this problem has been known for a long time, this is the first attempt to study these specific effects in Brazil.
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1. Introduction

In Brazil, exposure to benzene has been seen as one of the serious problems noted in work places [1]. The largest benzene production in Latin America, around 400,000 t/year, occurs in the State of Bahia, more precisely in the Petrochemical Complex of Camaçari. At the beginning of the 1990s, many workers were laid off work in this region due to the presence of hematological alterations, most notably the reduction in the number of leucocytes/neutrophils in peripheral blood.

Although there are many forms of benzene exposure, such as industrial emissions through gasoline vapors, motor

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exhaust fumes, cigarette smoke and water source contamination, occupational exposures are considered to be of a higher level [2]. Evidence of the noxious effect of benzene dates back to the end of the 20th century [3,4] and its carcinogenesis on bone marrow is known [5–13]. In addition, this substance is involved in a variety of blood abnormalities of which there are many reports [14–17]. Accidentally, some studies with the object of analyzing the relation between exposure to benzene and leukemia have also shown an increase in the risk of lymphoid neoplasias among workers exposed to benzene [18]. In a review of 18 epidemiological studies about the association between exposure to solvents and non-Hodgkin's lymphoma (NHL) found that out of the nine studies that characterized exposure more precisely, only one was categorical in demonstrating the non-existence of an association [19], which may mean an important indication of the existence of this association. It was concluded that considering the limitations of each study and the leucemogenic action of benzene, it is reasonable to classify it as one of the probable agents involved in the etiology of NHL [20]. However, in spite of this recognized carcinogenic action on the lympho-hematopoietic system (classification in Group 1 of the *International Agency for Research on Cancer* IARC, the patterns of exposed populations (workers and the community in general) becoming ill is still being discussed, and except for the risk of cancer, little is known with regard to immunotoxicity [21].

The effects of immunotoxicity induced by benzene are probably a reflection of the toxicity to the bone marrow [22], inducing depression and altering both the immune system mediated by cells and the humoral with decrease in the lympho-proliferative response of T and B cells and inhibition of the activity of T cytotoxic cells [23]. The increase in the susceptibility to infections as a result of a depression of the bone marrow may be the main cause of death related to chronic exposure to benzene [24].

The majority of data available in literature about CBP and immunotoxicity was obtained from studies in animals [15,25–35]. Data in humans are scarce and involves groups of workers during the exposure [36–39]. Except for the risk of cancer, little is known with respect to immunotoxicity [21] and the alterations to health among individuals in whom occupational exposure has ceased.

The purpose of this study is to describe and analyze the phenotypical profile of lymphocytes, comparing the leucogram values of two groups (individuals with and without CBP). This study is a part of a broader research, in progress, about laboratory assessment of immunocompetence, of genotoxic alterations and genetic polymorphism.

In spite of the various forms of toxicological assessment, the evaluation of the immunological response is an important parameter that could provide information about the immunotoxic mechanisms that may be responsible for a series of reactions leading to pathogeneses. The findings in this study will help to describe later evens in individuals with a history of CBP.

2. Material and method

2.1. Population and the study area

Within the scope of a wider project "Hematological, Genotoxic, Immunological Alterations, Genetic Susceptibility and Occupational Exposure to Benzene" in workers registered in the Work Disease Outpatients ("Ambulatório de Doenças do Trabalho (ADT)") of the Worker Health Study Center ("Centro de Estudos da Saúde do Trabalhador (CESAT)") in the period from 1988 to 1999, a hematologist selected 24 patients with a most probable diagnosis of chronic intoxication by benzene based on the following criteria: (1) occupational exposure to benzene; (2) number of leucocytes lower than 4000 and neutrophils lower than 2000; (3) decreasing trend in the number of these cells, as from the beginning of exposure. The controls were selected from the same file and from where cases came from (CESAT) diagnosed with other occupational diseases, at the same time as the cases. The patients mainly originate from the Metropolitan Region of Salvador, and work or worked for companies, generally in the industrial sector of this region.

2.2. Study design

This study is a part of a larger case control study. From the definition of the two groups, those who have and those who do not have CBP, a phenotype analysis was carried out of the lymphocytes and comparisons were established between the groups.

2.3. Data collection

2.3.1. Questionnaire

The individuals were asked to answer a questionnaire, structured specifically for the assessment of exposure, personal data, determination of life-style factors like smoking and alcohol and use of medications, among others.

2.3.2. Obtainment of peripheral blood samples

Blood was collected at fasting by venous puncture in the antecubital region in a vacuum collection tube (Vacutainer®) with tri-potassium EDTA.

2.3.3. Leucogram

For analyzing the blood sample, a hematological self-analyzer Adivia 120-Bayer was used. Complete leucograms were made and the total numbers of leucocytes, lymphocytes and segmented neutrophils were analyzed. For all the reagents used to acquire and analyze the samples, the manufacturer's recommendations and guidance were followed.

Morphological study of the samples in blood smears was assessed by a hematologist of the group.

2.3.4. Preparation and analysis of peripheral blood lymphocyte populations and sub-populations by flow cytometry

EDTA was placed in a test tube and $2\,\mu L$ of the following monoclonal antibodies conjugated with fluorochromes, in double and triple combinations were added: (1) CD3PE/CD4FITC (for lymphocytes $TCD4^+$); (2) CD3FITC/CD8PE (for lymphocytes TCD8); (3) CD19FITC/CD56PE (for lymphocytes B and NK cells, respectively); (4) CD4FITC/CD8PE/CD25CY (for activated T lymphocytes); (5) CD4FITC/CD8PE/CD45ROCY (for memory T lymphocytes). The monoclonal antibodies were obtained from Caltag Laboratories, Bayshore Blvd., Burlingame, CA, USA).

The incubation of the tubes was standardized for 30 min with the above-mentioned antibodies. The red cells were lysed with lysing solution (Beckman-Coulter). After being lysed, each sample was centrifuged twice, at 1000 rpm for 5 min. The cells were re-suspended in $300\,\mu L$ of PBS.

The acquisition and analysis of immuno-marked cells was standardized for 10,000 events per sample in FACSort BD (US–BD Biosciences Clontech). Percentages were obtained of each lymphocyte sub-population and the absolute numbers of the lymphocyte sub-populations was also calculated.

3. Ethical considerations

The project was submitted to and approved by the Ethical Commission on Research of the "Maternidade Climério de Oliveira (UFBa)" and all the participants in the study were informed about the research objectives and asked to formalize participation by signing a Free and Informed Consent Term ("Termo de Consentimento Livre e Esclarecido (TCLE)" following the current norms of the Ministry of Health for studies with human beings. The patients received a cost allowance to reimburse expenses with travel and meals.

The individual results were passed on to patients with an indication about the diagnostic procedures and/or therapies required. Individuals that presented periodontal disease were duly treated at the "Hospital Roberto Santos" by the Periodontics service. At the end of the study, copies of the results were sent and a medical bulletin attached, reporting the alterations and referring the patient to the Hematology Outpatient Department of the UFBA Faculty of Medicine.

4. Results

4.1. Study population

The long time interval between the last CESAT consultation and the beginning of the study made it difficult to locate some individuals due to the lack of updated addresses, causing losses. Fifty telegrams were sent to individuals with CBP and 50 to individuals without CBP. Among the individuals in the CBP group, there was one formal refusal, two deaths and

Table 1
Means of leucocyte counts and its sub-types between groups with CBP and control

	Cases	Controls
Leucocytes	3244	6668
Neutrophils	1400	3892
Lymphocytes	1375	2091
Monocytes	234	344
Eosinophils	206	309

eight individuals did not come for the examination; 15 individuals did not reply to the telegrams. In the control group, there was one refusal, three did not come to the exam and 22 did not reply to the telegram.

The study population was composed of 48 individuals, 24 with CBP and 24 without CBP. In both groups, there were 22 men and two women. The mean age was 51.1 years in the CBP group, ranging from 36 to 66 years of age, and 50.7 years in the control group, ranging from 36 to 71 years of age ($p = 0.866$). In general, workers were employed in chemical companies and the mean time of exposure to benzene was 140 months, varying from 24 to 276 months. When this study was conducted, many workers had already been withdrawn from the exposure; the mean time was 112 months varying from 26 to 192 months.

No individual from either of the groups mentioned a history of disease related to hypersensitivity, immunodeficiency or self-immunity, or involvement of the immune system. Of the 48 individuals, only one belonging to the control group reported a recent history of diabetes mellitus.

4.2. Description of the leucograms

The peripheral blood smear analysis of cases and controls was included in this study, and no qualitative alterations were shown in the red cells, leucocytes or platelets.

Table 1 describes the means of the absolute numbers of leucocytes of the groups with and without CBP. For the comparison of the means between groups, the value of the mean percentage of each group was used due to the heterogeneity of the groups in relation to the number of cells. Significant differences were found for the neutrophils (42.6 ± 8.3 for the group with CBP and 56.1 ± 11.3 for the control group) and monocytes (7.3 ± 1.6 for the group with CBP and 5.3 ± 1.3 for the control group) (Table 2).

4.3. Phenotype analysis of lymphocyte sub-populations

Table 3 describes the means of the absolute numbers of lymphocyte sub-populations of the groups with and without CBP. The comparison of the means between groups, however, was done with the value of the mean percentage of each group due to the heterogeneity of the groups in relation to the number of cells. Differences were found in the lymphocyte sub-population T $CD3^+CD8^+$ (27.9 ± 11.3 for the

Table 2
Distribution of the mean percentage and standard deviation of the number of neutrophils, lymphocytes, monocytes and eosinophils

	Cases		Controls		P-value
	$\overline{X}$ (%)	DP*	$\overline{X}$ (%)	DP*	
Neutrophils	42.6	8.3	56.1	11.3	0.001
Lymphocytes	59.8	82.0	33.4	9.8	0.124
Monocytes	7.3	1.6	5.3	1.3	0.001
Eosinophils	6.0	4.6	4.6	4.1	0.250

* Standard deviation.

Table 3
Means of lymphocyte sub-population counts of the groups with CBP and control

Sub-population	CD marker profile	Cases (cells/μL)	Controls (cells/μL)
Cell Th	CD3+CD4+	518	825
Cell Tc	CD3+CD8+	388	500
Cell B	CD19+	143	228
Cell NK	CD56+	297	375
LTh activated	CD4+CD25+	41	75
LTc activated	CD8+CD25+	24	7
LTh memory	CD4+CD45R0	432	769
LTc memory	CD8+CD45R0	301	472

group with CBP and 19.4 ± 5.9 for the control) and in the T memory helper cells (31.2 ± 9.4 for the group with CBP and 37.0 ± 5.9 for the control); data available in Table 4.

5. Discussion

The carcinogenic effect of benzene is recognized by the scientific community. However, studies about the immuno-toxic action are rare, generally carried out in animals and are related to the term of exposure. In the literature, no papers analyzing the immunological pattern of individuals with a history of occupational exposure to benzene after exposure had ceased were found.

In one of the rare studies carried out in Brazil [40], the authors evaluated the peripheral hematological condition of patients with CBP after 5 years of having been removed from exposure and showed that 48% of the patients had not nor-malized their blood cell counts. In the present study, the data

demonstrated that leucopenia was maintained even after a period of approximately 11 years after exposure had ceased. This finding may suggest a long-term effect.

The half-life of exhaled benzene in humans varies depend-ing on the benzene exposure concentration and duration. Exposure to 99 ppm for 1 h resulted in an initial phase half-life of 42 min, and exposure to 6.4 ppm for 8 h resulted in an initial phase half-life of 72 min, with a terminal phase half-life (from 10 to 100 h after exposure) of 23–31 h. Because of this short half-life, it is impossible to evaluate past expo-sure to benzene using biomarkers. In spite of this limitation, chronic health effects caused by an old exposure, such as low blood cell counting, immunological impairments, chro-mosome aberrations and cancer can be evaluated many years after exposure cessation.

With the delineation and execution of this stage, one is unable to understand the mechanism that leads to the increase in the population of monocytes in peripheral blood, although one recognizes the role of this important phagocyte cell that has the characteristic potential of an antigen-presenting cell. This cell may compensate the decrease of the other important phagocyte type, the neutrophil, in the individual's defense.

Immunotoxicity studies carried out in animals have shown a reduction in the functions of lymphocytes. These include: reduction in the T and B response to mytogens; in the pro-duction of IL-2 by T helper cells; in cytotoxic activity by T cytotoxic cells; production of antibodies by the B lympho-cytes; and in the resistance of the macrophage to intracellular infections [41,29]. In studies in humans [36,37], there was evaluated the number of leucocytes in workers during the period of exposure, and found a reduction in the percent-ages and absolute numbers of total T lymphocytes, lym-phocytes TCD4, TCD8, NK cells and an increase in the monocytes count. With these findings, the authors suggest that the depressive effect of benzene on the T cells may be a factor of the probable carcinogenic activity of benzene via the immune system.

The most recent immunophenotyping study using flow cytometry was done in Hungary by Biró and colaborators [39], but as with the other studies it was also carried out dur-ing the term of exposure. In this study, no decrease was found in the numbers relative to the lymphocyte sub-populations (CD3, CD4, CD8, CD19, CD25, CD45RO, CD56); the

Table 4
Distribution of the mean percentage and standard deviation of the lymphocyte sub-population of the case and control groups

Sub-population	CD marker profile	Mean percentage, group with CBP	DP	Mean percentage, control group	DP	P-value
Cell Th	CD3+CD4+	38.7	12.4	39.8	10.3	0.739
Cell Tc	CD3+CD8+	27.9	11.3	19.4	5.9	0.002
Cell B	CD19+	12.1	5.9	11.0	3.7	0.447
Cell NK	CD56+	21.4	9.6	17.7	5.4	0.106
LTh activated	CD4+CD25+	3.1	2.7	3.7	2.4	0.427
LTc activated	CD8+CD25+	1.8	5.3	0.3	0.5	0.180
LTh memory	CD4+CD45R0	31.2	9.4	37.0	5.9	0.015
LTc memory	CD8+CD45R0	21.6	8.6	21.7	7.7	0.973

authors point out a super expression in the activation markers like CD4$^+$CD25$^+$, drawing attention to their importance for maintaining self-tolerance. In this study, there was a difference in the mean percentage of lymphocyte sub-populations for the cytotoxic T cells (CD3$^+$CD8$^+$) and T memory helper cells (CD4$^+$CD45RO).

This study shows an increase in the values of lymphocytes TCD3$^+$CD8$^+$, although no alterations had been noted in the NK cell values. Probably, these lymphocytes interceded in a specific way in possible cell alterations to maintain the homeostasis of these individuals, which may, at least partly, justify the non-reporting of serious clinical conditions referred to by the patients.

On the other hand, the memory cells, fundamental for maintaining the individual's health and balance, are responsible for the protection under secondary challenge by giving the defense system an increased response to the first stimulus with different qualitative and quantitative characteristics. Thus, this cellular phenotype gives and provides long-lasting immunity in different pathological processes. Little is known about the mechanisms involved in the "appearance" of long-life memory cells. Even less is known about the proportions and reference values of the peripheral blood of normal individuals or those involved in pathological processes. Only over the last few years has scientific information appeared, which has culminated in associating the individual's clinical condition, as well as markers and consequently, the use of methodologies that enable these phenotypes in the blood and tissues of human beings to be characterized.

A reduction was found in the number of B lymphocytes during exposure [38]. However, the results of this study did not show differences between the groups with regard to the percentage of lymphocytes. These results should be analyzed with caution, so that one does not precipitately infer that the lymphocytes are not the target of immunotoxicity to benzene. A study was carried out by Sul and colaborators [42] showed damage to the DNA of B lymphocytes in workers exposed to low levels of benzene. The authors suggested the molecular assessment of the B lymphocytes for the bio-monitoring of human exposure to benzene at low levels.

No serious systemic dysfunctions were identified or were associated with immunodeficiency in the patients of the present study. It is known, however, that there is a well-documented correlation in man between qualitative and quantitative changes in the immune function and the appearance of clinical signs of immunodeficiencies.

Many factors, including age and nutritional status, have considerable influence on the immunological competence and harm to the immune system. The characteristics of the group with CBP and control were similar in the present study, and there were no significant differences with regard to age and sex. Therefore, the reduction in the numbers of lymphocytes and sub-populations did not occur as a result of immune system aging, but probably because of exposure to benzene.

Some investigators demonstrated that the relative number of the lymphocyte sub-populations does not reflect their true size and are of limited value, and diagnosis of immunological alterations based on absolute numbers are more recommendable [43]. The absence of reference values for lymphocyte phenotypes in the healthy population reinforces the need for population studies.

Within the limitation of the studied sample, and respecting the characteristics of the present studies, the results observed enable one to draw the following conclusions: workers with a history of hematological alteration due to CBP maintained the leucopenia after a long period of occupational exposure having ceased; the patients in the CBP group presented immunological impairment with alterations in the relative numbers only of the LT cytotoxic and LT memory helper phenotypes; the analysis of the phenotype profile of the lymphocytes and their sub-populations may be useful for monitoring exposed patients.

These results may contribute towards the understanding of the action of benzene on the immune system. Further studies are required to check the cellular immune function.

Acknowledgement

References

[1] Mendes R. Exposição ocupacional ao benzeno e seus efeitos sobre a saúde dos trabalhadores: estado atual do problema e questões não resolvidas. Rev Assoc Med Bras 1993;39(4):249–56.

[2] Snyder R, Witz G, Goldstein BD. The toxicology of benzene. Environ Health Perspect 1993;100:293–306.

[3] Hricko A. Rings of controversy around benzene. Environ Health Perspect 1994;102(3):276–81.

[4] Landrigan PF. Benzene and blood: one hundred years of evidence. Am J Ind Med 1996;29:225–6.

[5] Vigliani EC, Saita G. Benzene and leukemia. N Engl J Med 1964; 22(17):872–6.

[6] (a) Aksoy M, Dinçol K, Erdem S, Dinçol G. Acute leukemia due to chronic exposure to benzene. Am J Med 1972;52:160–6;
(b) Aksoy M, et al. Acute leukaemia in two generations following chronic exposure to benzene. Hum Hered 1974;24:70–4;
(c) Aksoy M, Erdem S, Dinçol G. Types of leukemia in chronic benzene poisoning. A study in thirty-four patients. Acta Haematol 1976;55:65–72.

[7] Vigliani EC, Forni A. Benzene and leukemia. Environ Res 1976; 11:122–7.

[8] Infante PF, et al. Leukaemia in benzene workers. Lancet 1977; 9:76–8.

[9] Sawahata T, Rickert DE, Greenlee WF. Metabolism of benzene and its metabolites in bone marrow. In: Irons RD, editor. Toxicology of the blood and bone marrow. New York: Raven Press; 1985. p. 141–8.

[10] Rinsky RA, et al. Benzene and leukaemia. An epidemiologic risk assessment. N Engl J Med 1987;23:1044–9.

[11] Yin SN, et al. Leukaemia in benzene workers: a retrospective cohort study. Br J Ind Med 1987;44:124–8.

[12] Austin H, Delzell E, Cole P. Benzene and leukemia. A review of the literature and a risk assessment. Am J Epidemiol 1988;127(3):419–37.

[13] Paci E, et al. Aplastic anemia, leukemia and other cancer mortality in a cohort of shoe workers exposed to benzene. Scand J Work Environ Health 1989;15:313–8.

[14] Selling L. Preliminary report of some cases of purpura haemorrhagica due to benzol poisoning. Bull J Hopkins Hosp 1910;21:33–7.

[15] Hektoen L. The effect of benzene on the production of antibodies. J Infect Dis 1916;19:69–84.

[16] Legge TM. Chronic benzol poisoning. J Ind Hyg 1919/1920;1:539–41.

[17] Smith AR. Chronic benzol poisoning among women industrial workers: a study of the women exposed to benzol fumes in six factories. J Ind Hyg 1928;10(3):73–93.

[18] Young N. Benzene and lymphoma. Am J Ind Med 1989;15(5):495–8 [Editorial].

[19] Fritschi L, Siemiatycki J. Lymphoma myeloma and occupation: results of a case-control study. Int J Cancer 1996;67:498–503.

[20] Rêgo MAV. Non-Hodgkin's lymphoma risk derived from exposure to organic solvents: a review of epidemiologic studies. Cad Saúde Públ 1998;14(3):41–66.

[21] Greenberg PL. Myelodysplastic syndrome. In: Hoffman R, Benz Jr EJ, Shattil SJ, Furie B, Cohen IIJ, Silberstein LE, editors. Hematology. 2nd ed. New York: Churchill Livingstone; 1995. p. 1098–121 [Chapter 71].

[22] McConnell EE. Benzene. Environ Health Crit 1993;150:110–1.

[23] Snyder R, Hedli CC. An overview of benzene metabolism. Environ Health Perspect 1996;104(6):1165–71.

[24] Young NS, Maciejewshi JP. Aplastic anemia. In: Hoffman R, Benz Jr EJ, Shattil J, Furie B, Cohen HJ, Silberstein LE, McGlace P, editors. Hematology. 3rd ed. New York: Churcill Livingstone; 2000. p. 314–5 [Chapter 19].

[25] (a) Snyder CA, et al. The inhalation toxicology of benzene: incidence of hematopoietic neoplasms and hematotocicity in AKR/J and C57BL/6J mice. Toxicol Appl Pharmacol 1980;54:323–31;
(b) Snyder CA, et al. The pharmacokinetics of repetitive benzene exposures at 300 and 100 ppm in AKR mice and Sprague–Dawley rats. Toxicol Appl Pharmacol 1981;57:164–71.

[26] Rozen MG, Snyder CA. Protracted exposure of C57BL/6 mice to 300 ppm benzene depresses B- and T-lymphocyte numbers and mitogen responses. Evidence for thymic and bone marrow proliferation in response to the exposures. Toxicology 1985;37:13–26.

[27] Rosenthal GJ, Snyder CA. Inhaled benzene reduces aspects of cell-mediated tumor surveillance in mice. Toxicol Appl Pharmacol 1987;88:35–43.

[28] Thomas ML. The leukocyte common antigen family. Annu Rev Immunol 1989;7:339–69.

[29] Hsieh III GC, et al. Effects of repeated oral exposure to combinations of benzene and toluene on immunologic responses in mice. Arch Toxicol 1990;64:320–8.

[30] McMurry ST. Acute effects of benzene and cyclosphosphamide exposure on cellular and humoral immunity of cotton rats, *sigmond hispidus*. Bull Environ Contam Toxicol 1991;46:937–45.

[31] Renz JF, Kalf GF. Role for interleukin-1 (IL-1) in benzene-induced hematotoxicity: Inhibition of conversion of pre-IL-1α to mature cytokine in murine macrophages by hydroquinone and prevention of benzene-induced hematotoxicity in mice by IL-1α. Blood 1991;78(4):938–44.

[32] Burns LA, et al. Immunotoxicity of nitrobenzene in female B6C3F1 mice. Drug Chem Toxicol 1994;17(3):271–315.

[33] Farris GM, et al. Benzene-induced hematotoxicity and bone marrow compensation in B6C3F1 mice. Fundam Appl Toxicol 1997;36:119–29.

[34] Robinson SN, et al. Immunotoxicological effects of benzene inhalation in male Sprague–Dawley rats. Toxicology 1997;119:227–37.

[35] Daiker DH, et al. Effect of CYP2E1 induction by ethanol on the immunotoxicity and genotoxicity of extended low-level benzene exposure. J Toxicol Environ Health, Part A 2000;59:181–96.

[36] Moszczynski P, Lisiewicz J. Histochemical evaluation of lysosomes in lymphocytes of workers having contact with organic solvents containing benzene and its homologues. Rev Esp Oncol 1982;1(29):49–55.

[37] Luan FJ. A study on lymphocyte subpopulations and immunologic status of female workers exposed to benzene. Zhonghua Yu Fang Yi Xue Za Zhi 1992;26(2):77–9.

[38] Bogadi-Sare A, et al. Study of some immunological parameters in workers occupationally exposed to benzene. Int Arch Occup Environ Health 2000;73(6):397–400.

[39] Biró A, Pállinger E, Major J, et al. Lymphocyte phenotype analysis and chromosome aberration frequency of workers occupationally exposed to styrene, benzene, polycyclic aromatic hydrocarbons or mixed solvents. Immunol Lett 2002;81:133–40.

[40] Augusto LGS, Vigoritto AC, Souza CA. Alterações histológicas da medula óssea secundárias à exposição ao benzeno e à evolução hematológica do sangue periférico em pacientes acometidos. Rev Bras Saúde Ocup 1993;21(78):85–92.

[41] Rosenthal GJ, Snyder CA. Modulation of the immune response to *Listeria monocytogeneses* by benzene inhalation. Toxicol Appl Pharmacol 1985;80:502–10.

[42] Sul D, et al. Single stand DNA breaks in T- and B-lymphocytes and granulocytes in workers exposed to benzene. Toxicol Lett 2002;134:87–95.

[43] Comans WMB. Immunophenotyping of blood lymphocytes in childhood. J Pediatr 1997;130(3):1–8.