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Research Article

Changes in Immune Phenotypes of Peripheral Blood Lymphocytes (PBLs) among Occupationally Cytostatic Exposed Hospital Employees

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Abstract

Health professionals who had been working in different health care units and who were chronically exposed to cytostatic drugs were examined during the last 25 years. In their working area of the oncology outpatient and hospital units cytostatic drug exposure regularly exceeded safety limits due to the lack of proper safety devices. Their health condition was annually investigated by clinics, and it was found that they developed more hematological, immunological and reproductive alterations. In the present study we assessed changes in immune phenotypes based on the CD4/CD8 T-cells ratio, B and NK cell percentages measured by FACS methodology. The investigations were carried out in 550 subjects exposed to different cytostatic agents in oncology departments. Data were compared to age matched 83 healthy and non-exposed female controls. Biomarkers were measured by routine clinical laboratory tests, completed with immune phenotyping and measured in peripheral blood lymphocytes (PBL). Health personnel exposed to different agents showed increased ratio of CD4/CD8 T-cell and a decrease in activated T-cells. Beside these parameters we found an elevation of B-cells and a decrease in NK-cells. Within the exposed groups smokers showed even stronger decreased NK-cell counts compared to non-smokers. These changes in immune-phenotypes may give us a new biomarker immune suppression of PBL-cells among nurses after toxic exposures in their working environment.

Keywords: *Occupational Exposure of Cytostatic Drugs; Immune Depression; CD4/CD8 Ratio; NK-Cells; B-Cells*

Introduction

It is widely accepted that cytostatic drugs for the treatment of cancer or autoimmune diseases have immunosuppressive effects on treated patients. However, much less is known about the effect of these drugs on the immune state of health care workers. Several studies are available which establish the importance of B, T and NK-cells in immune suppression caused by environmental or occupational stressors [1,7,10]. Our investigations were carried out in 550 subjects exposed to cytostatic drugs, and their data were compared to 83 healthy, non-exposed controls. Biomarkers were measured by routine clinical laboratory tests, completed with immune phenotyping measured by FACS methods. The primary prevention of occupational diseases using a gene-and immune toxicological monitoring system was developed by our laboratory, recently the most powerful tool of health protection in occupational cancer prevention [4,17-19]. In our previous studies, we have investigated the gene- and immune-toxicity of occupational exposures of different chemicals and drugs developing a follow-up monitoring study called "Hungarian Nurse Study" (2,3,15) Beside immune toxicity we have examined the chromosomal aberrations, as well as the reduction of DNA repair and increased apoptotic deletion of proliferating peripheral blood lymphocytes and clarified immune suppression caused by these agents [9,16,17]. The purpose of the present study is to determine the immunomodulatory effects of occupational cytostatic exposure among hospital nurses.

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Materials and Methods

Subjects

Altogether 550 subjects exposed to cytostatic drugs were investigated. The results were compared to 83 healthy controls, occupationally not exposed to known substances. Cytostatic drugs were handled by staff only with gloves and surgical mask, but they have no more safety devices during the administration of cytostatic infusion containing Cyclophodphamide, cisplatin, Methotrexate, Adriamycin, Vincristine, Fluorouracil, Bleomycin, Etoposide and Mitomycin-C.

All subjects were interviewed by a physician to collect data on age, medication, smoking and drinking habits, as well as medical and work histories including exposure to known or suspected toxicants, occupational history including duration of exposure and the use of protective devices during work. Active smoker and ex-smoker subjects were considered as "Ever smokers".

Immune phenotyping

For immune-toxicological investigations, flow cytometry analysis of surface antigens was performed on peripheral blood lymphocytes. Heparinized whole blood was incubated at room temperature for 20 minutes with the appropriate amount of FITC, PE, PerCP or APC labeled monoclonal antibodies (Becton Dickinson) against surface antigens. The erythrocytes were removed by lysis through the addition of FACS Lysing solution (Becton Dickinson). Samples were analysed within 4 hours after labeling or being fixed with 2% paraformaldehyde. Four-color analysis was performed on a Becton Dickinson FACS Calibur flow cytometer. Standard forward and side scatter gating combined with CD45 was used to separate leukocyte populations and to set the lymphocyte gate. CD3 was used as a T cell marker, helper T cells were characterized by CD3+/CD4+ phenotype, cytotoxic T cells by CD3+/CD8+ phenotype and B-lymphocytes were characterized as CD19+ cells. The studied antigens were CD3, CD4, CD8, CD19, CD25, CD45, CD56 and CD71. Data for at least 10,000 cells per sample were acquired, and CellQuest Software 3.1 was used for analysis.

Statistical analysis

Statistical analyses were performed by Student's t-test with the GraphPad Prism 3.02 software; $P < 0.05$ was considered significant. To describe sample characteristics, descriptive statistics (frequency, mean and standard deviation) were used. Normality of variables were tested by one-sample Kolmogorov-Smirnov test. Due to non-normality of variables only non-parametric tests (Mann-Whitney) were used to compare control and exposed groups. The level of significance was set at 5%, and one-sided tests were implemented. A priori sample size calculation for Mann-Whitney tests had shown a sample need of 53 subjects per each group (control and exposed), All statistical analyses were performed by using SPSS software version 25.0.

Results

Main demographic data of the investigated donors (all females) are summarized in table 1. In our study we examined 550 persons exposed to cytostatic agents for more than 15 years while working in different medical units in Hungary. The majority of these workers included nurses, assistants and medical doctors. The control group consisted of 83 non-exposed individuals. The average age of the controls was 37.25 years and in the exposed groups it was very similar (35.5 years). In the control group the rate of ever-smoked was 30.3%, which corresponded to the general smoking rate in Hungary. The rate of ever-smokers in the cytostatic exposed group was very high (56.2%).

Groups	No	Age yrs.	Ever smoked %
Controls	83	37.25 ± 1,32	30.3
Cytostatic	550	35.50 ± 0,36	56.2

Table 1: *Demographic data of controls and exposed health care workers.*

Changes in immune phenotype

In this study we also compared the CD4/CD8 ratio and quantified the cytotoxic T and helper cells in 83 controls and in the exposed group. The results are seen in table 2. We found a significant increase in CD4/CD8 ratio due to the increase in helper (CD4+). T-cells , and a decrease in cytotoxic CD8+ T-cells in the exposed group. That result was similar to the findings of Koch., *et al*. where they demonstrated a radical increase in this ratio in older people as a good sign of immune depression. The average ratio of CD4/CD8 ratio among 550 cytostatic exposed nurses rose from 1.72 to 1.99 compared to 83 non-exposed controls. We have also measured the NK and B cell percents by FACS methodology. In this table we show the changes in B cells which also rose from 9.87 to 11.41 percent although the amount of NK-cells in the same group decreased from 13.62 to 11.55 on average. Mann-Whitney non-parametrical statistical test proved all these changes significant.

Markers Groups		N	Mean	Std. Deviation	Z value	sign.
CD4/CD8	Controls	83	1.72	0.66	-3.024	0.002
	Cytostatic Exposed	550	1.99	0.78		
B cells	Controls	82	9.87	3.68	-3.876	0.000
	Cytostatic Exposed	557	11.41	3.57		
NK cells	Controls	81	13.62	6.12	-2.89	0.004
	Cytostatic Exposed	549	11.55	5.42		

Table 2: *Immunological markers showing significant changes in cytostatic exposed donors compared to non-exposed controls.*

Discussion

In the present study we have assessed the specific changes of immune phenotypes among health workers who were exposed to cytostatic drugs, based on the changes of CD4/CD8 ratio and the percent of B and NK cells in their peripheral blood lymphocytes.

B-lymphocytes can regulate the immune response by their surface molecules and by secreting different cytokines, like IL-10, IL-35 or TGF-β causing immunosuppression *in vivo*. This role of B-cells is very important in the immune tolerance and in the protection against the harmful allergic reactions [18]. High level of immune suppressive B-lymphocytes may promote chronic infections and inflammatory processes in different organs [19]. However, this immune suppression is mediated by a large population of immune-competent subsets of lymphocytes regulating inflammation and antibody production, too. This mechanism is responsible for preventing "cytokine storm" during infections and is related to antigen- specific interaction with CD8 and CD4 T-cells through MHC class I and II molecules produced by B cells. This interaction is probably present toward NKT-cells by surface expression of CD1d molecules, too [19].

Environmental or occupational genotoxic agents cause not only mutations of somatic cells but cellular stress of PBL cells which lead to immune suppression resulting in a rapid decline of immune activity. This condition will increase susceptibility to infections and higher incidences of many chronic diseases e.g. osteoporosis, cardiovascular diseases, chronic inflammation of joints, lung, liver and kidney caused by elevated production of pro-inflammatory cytokines by damaged T-cells [5,14]. Lewis., *et al*. [11] demonstrated the mechanism of T-cell

receptor independent inhibition of transcription by tumor necrosis factor alpha and CD80, which modulate CD28 expression. During immune suppression, the immune system gradually undergoes a remodeling caused by genetic and different environmental toxic and infective, e.g. viral factors. The first sign of these changes is the increased ratio of CD4/CD8 cells combined with lower production of IL-2 cytokine. These processes are highly influenced by individual susceptibility and environmental epigenetic or genotoxic agents [11,12].

Psychological and physical stress can boost clonal depression of T-cells resulting in the elevation of apoptosis, although *in vitro* studies showed certain resistance to apoptosis in the altered T-cells [6]. This contradiction is due to the differences between *in vivo* and *in vitro* systems. *In vivo* several other unknown factors may influence these processes. One of the key elements of human immune suppression is the quality of prompt immunological response to different environmental, occupational and infective agents. In humans several pathological conditions, e.g. cancer, atherosclerosis, osteoporosis and fertility problems are related to damaged immune surveillance. The actual condition of the immune response is slowed down providing a good endpoint of immune suppression. Environmental and occupational chemical and physical exposure damages the immune system. Epidemiology and the use of biomarkers in analytical epidemiology may help to bridge the gap between human and animal studies. In our study we have demonstrated the elevation of CD4/CD8 ratio from 1.72 to 1.99, the B-cell population increased from 9.87 to 11.41 and the NK-cell population decreased from 13.65 to 11.55. These parameters play a significant role in immune suppression because their function and cytokine production is able to modify the immune response to different environmental stressors, e.g. genotoxic chemicals. Recent article [20] reported significantly higher rate of impaired fertility and oral ulcers among nurses handling antineoplastic drugs. Other study [21] valuated *in vitro* genotoxicity of human lymphocytes treated with mixtures of cytostatic drugs, relevant to occupational settings in their concentration. Our study may show a new way of risk assessment of occupationally caused immune suppression, which may be the first ethological step of occupational disease development [21].

Conclusion

Healthcare workers, handling cytotoxic drugs, are probably exposed to them during preparation, administration, waste handling, and cleaning. In the present study we assessed changes in immune phenotypes based on the CD4/CD8 T-cells ratio, B and NK cell amount measured by FACS methodology. The investigations were carried out in 550 subjects exposed to different cytostatic agents in oncology departments. Data were compared to age matched 83 healthy and non-exposed female controls. Biomarkers were measured by routine clinical laboratory tests and completed with immune phenotyping and measured in peripheral blood lymphocytes (PBL). Health personnel exposed to different agents showed an increased CD4/CD8 T-cell ratio, and an elevation in the percentage of B-cells and a decrease in NK-cells. Within the exposed groups smokers showed even stronger decreased NK-cell counts compared to non-smokers. These changes in immune-phenotypes may give us a new biomarker of immune suppression using PBL-cells among nurses after toxic exposures in their working environment. To prevent potential exposure, extensive safety precautions necessary to taken in hospitals using cytostatic drugs for treatments. The current technique does not prevent release of cytostatic drugs during administration while the use of different safety devices my let a significant reduce of environmental contamination.

Competing Interests

The authors declare that they have no competing interests.

Author's Contributions

Anna Tompa was responsible for the conception and design of the studies, took part in the analysis and interpretation of the data, wrote most of the manuscript. Anna Biro took part in the analysis and interpretation of the data, wrote some parts of the manuscript. Miklós Zrínyi carried out the statistical analysis.

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