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RESEARCH

Immunotoxicity Monitoring of Hospital Staff Occupationally Exposed to Cytostatic Drugs

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Abstract The aim of our study was to investigate the immunotoxicity of occupational cytostatic drug exposure, and to assess the possible effect of confounding factors, such as age and smoking. In this human study, the immunotoxic effect of antineoplastic drugs was investigated among 306 nurses working in oncology chemotherapy units. Results were compared to 98 non-exposed women. The immune status of the subjects was characterized by immune phenotyping of peripheral blood lymphocytes by flow cytometry, using monoclonal antibodies against surface antigens (CD3, CD4, CD8, CD19, CD25, CD45, CD56 and CD71). The killing ability of neutrophil leukocytes was assessed by the measurement of reactive oxygen intermediate production. Occupational exposure to antineoplastic drugs caused shifts in the major lymphocyte subpopulations, resulting in a statistically significant increase in the ratio of B cells. Cytostatic drug exposure also manifested itself in a decreased frequency of CD25 positive, activated T lymphocytes, and increased oxidative burst of neutrophil granulocytes, both of which may have a functional impact on the immune system of exposed subjects. In the younger subjects exposure also caused a shift in T cell subpopulations: a reduction in the cytotoxic T cell population lead to an elevated Th/Tc ratio. In the exposed group, smoking increased activation of T lymphocyte subpopulations. In conclusion, we have demonstrated that low dose occupational cytostatic drug exposure is immunotoxic, and age and smoking modify the effect of exposure.

Keywords CD antigens · Cytostatic drug · Immunotoxicity · Lymphocyte phenotype · Occupational exposure · Oxidative burst

Abbreviations

APC	allophycocyanin
FITC	fluorescein isothiocyanate
fMLP	N-formyl-Met-Leu-Phe
IL-2R	interleukin-2 receptor
PBS	phosphate buffered saline
PE	phycoerythrin
PerCP	peridinin-chlorophyll-protein complex
PMA	phorbol 12-myristate 13-acetate
ROI	reactive oxygen intermediates

Introduction

Occupational exposure to cytostatic drugs is a major hazard of healthcare personnel. In the last decades, a vast number of antineoplastic drugs have been introduced to the treatment of cancers. Different mixtures of cytostatic drugs are used for chemotherapy of cancer patients, so occupational exposure of oncology nurses is usually to mixed compounds. Due to the long time of service of

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healthcare personnel, physicians and nurses can be exposed to cytostatic drugs over a long period of time, to low exposure levels with accidental peaks. Occupational exposure to antineoplastic agents may occur in different ways: by inhalation of airborne antineoplastic agents, absorption through skin contact, human excreta handling, ingestion during drug preparation and administration, and during disposal of equipment [1–3].

Most cytostatic drugs have been classified as carcinogenic to humans according to their mutagenic and DNA damaging properties [4, 5]. Since many of the antineoplastic drugs are genotoxic, biomarkers for detecting genotoxicity have been used to monitor workers' exposure to these drugs, such as chromosomal aberrations, micronuclei, sister chromatid exchange, and DNA damage. Several studies have demonstrated a significant difference in these biomarkers of antineoplastic drug exposed workers, compared to control populations [6–8].

The effect of cytostatic drugs on immunological parameters has been studied in a number of cases, in patients receiving various antineoplastic drugs as treatment for cancer. These studies indicate that each chemotherapy regimen has a different effect on immunological parameters [9–11]. The results of studies on patients, however, cannot be extrapolated to occupational exposures. The concentration of drugs, and routes of administration/exposure are different between therapeutic and occupational exposures. Immunotoxicity monitoring of occupational cytostatic drug exposure has been very scarce. In one study, occupational exposure to chemotherapeutic antiblastic agents significantly increased the circulating levels of interleukin-15 in 17 health care workers compared to their age-matched controls [12].

In the year 2000, at the National Institute of Chemical Safety, Hungary, we were among the first to introduce the detection of immunotoxicological biomarkers besides genotoxicological biomarkers to monitor workers occupationally exposed to various toxic substances [13]. We have shown that chemical exposure can alter the ratio of lymphocyte subpopulations and may cause changes in the activation of lymphocytes among oil-industry, health-service and metallurgy workers [13, 14] and thus specific immunological markers can be used to assess exposure. In a previous study our research group compared geno- and immunotoxicological effect markers among nurses exposed to cytostatics [15]. The genotoxicologically affected (chromosome aberration frequency > 4% and sister chromatid exchange frequency >7.5 per mitosis) subjects showed an increase in the frequency of helper T lymphocytes (Th), activated (IL-2R positive) T and Th-cells, and transferrin receptor positive B cells compared to genotoxically non-affected subjects.

In the present study we have investigated the immunotoxicity of occupational cytostatic drug exposure in a relatively large population of exposed subjects. Their data were compared to healthy, non-exposed controls. Confounding factors i.e. age and smoking were taken into account. Based on our previous experience, we have chosen the ratio of lymphocyte subpopulations (T cells, helper T cells, cytotoxic T cells, B lymphocytes and NK cells), and the activation of lymphocytes, measured by CD25 (IL-2R) and CD71 (transferrin receptor) expression, to assess the effect of occupational exposure [14]. The ability of leukocytes to kill by oxygen-dependent mechanisms was assessed by the measurement of reactive oxygen intermediate production of neutrophils.

Materials and Methods

Subjects

Altogether 306 nurses handling cytostatic drugs were investigated, from various oncology units. The cytostatic drugs which were most frequently handled by the persons involved in the study were: Cyclophosphamide, Cisplatin, Methotrexate, Adriamycin, Vincristine, Fluorouracil, Bleomycin, Etoposide and Mitomycine-C. Results were compared to 98, occupationally not exposed healthy controls, all women. 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 subjects were considered "Smokers". "Drinkers" consumed no more than 80 g pure alcohol regularly (a liter of beer or equivalent). Heavy drinkers were excluded. With the informed subjects' written permission, blood samples were collected by venipuncture. The samples were processed for immunological analysis and, to assess health status, for a routine clinical check-up including haematology, liver and kidney function tests.

Immune Phenotyping of Peripheral Blood Lymphocytes (PBL) by Flow Cytometry

Heparinized whole blood was mixed and incubated at room temperature for 20 min with the appropriate amount of fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin-chlorophyll-protein complex (PerCP), or allophycocyanin (APC) labelled monoclonal antibodies against

surface antigens. The erythrocytes were removed by lysis with the addition of FACS Lysing solution (Becton Dickinson). After washing with phosphate buffered saline (PBS, Sigma-Aldrich), samples were analyzed within 4 h after labelling, or fixed with 2% paraformaldehyde. The studied antigens were: CD3, CD4, CD8, CD19, CD25, CD45, CD56 and CD71. The monoclonal antibodies were purchased from Becton Dickinson. Three- and four-color analysis was performed on a Becton Dickinson FACSCalibur flow cytometer. The following monoclonal antibody combinations were used: (1) CD25-FITC / CD8-PE / CD3-PerCP / CD4-APC; (2) CD56-FITC / CD3-PerCP / CD45-APC; and (3) CD71-FITC / CD3-PerCP / CD19-APC. Standard forward and side scatter gating combined with CD45 was used to separate leukocyte populations and to set the lymphocyte gate. The lymphocyte subpopulations (T lymphocytes, cytotoxic T lymphocytes, helper T lymphocytes, B and NK cells) were determined as follows: CD3 was used as T cell marker, helper T cells were characterized by CD3+/CD4+ phenotype, cytotoxic T cells by CD3+/CD8+ phenotype, NK cells as CD3-/CD56+ phenotype and B lymphocytes were characterized as CD19+ cells. Activation of cells was estimated by measuring the percent of cells expressing CD25 and CD71 activation antigens. Data for at least 10000 leukocytes per sample were acquired, CellQuest Pro Software (Becton Dickinson) was used for analysis.

Measurement of Oxidative Burst Activity of Neutrophil Granulocytes

The measurement of oxidative burst was carried out from heparinized blood samples, using the Bursttest (Phagoburst®) kit (ORPEGEN Pharma, Heidelberg, Germany), which utilizes the conversion of dihydrorhodamine 123 to rhodamine 123 by reactive oxygen intermediates produced upon stimulation of neutrophil granulocytes. The conversion of dihydrorhodamine 123 to rhodamine 123 is proportional to the production of reactive oxygen intermediates. The mean fluorescence of rhodamine 123 correlates with oxidation quantity per individual leukocyte. The test was performed according to the instructions of the Phagoburst® kit. Briefly, 100 μl heparinized whole blood was incubated with 20 μl of either opsonized E. coli (1×10^9 bacteria per ml), phorbol 12-myristate 13-acetate (PMA) (8×10^{-6} M), N-formyl-Met-Leu-Phe (fMLP) (5×10^{-6} M) or medium (negative control) at 37°C in a water bath for 10 min. 20 μl of dihydrorhodamine 123 substrate was then added and incubated at 37°C for further 10 min. The reaction was stopped and erythrocytes were lysed by adding 2 ml lysing solution, incubated at room temperature for 20 min and then washed once with 3 ml washing solution. DNA staining was performed by incubating samples with 200 μl of 40 μg/ml propidium iodide at 0°C for 10 min. Cells were analysed on a FACSCalibur (Becton Dickinson) flow cytometer. Data for at least 15000 leukocytes per sample were acquired, CellQuest Pro Software (Becton Dickinson) was used for analysis. Standard forward and side scatter gating was used to identify granulocytes, combined with live gating on leukocyte DNA to exclude debris, bacteria and aggregates.

Statistical Analysis

Statistical differences between the studied groups were analysed by Student's t-test, $p<0.05$ was considered as statistically significant.

Results

Subjects

The most important demographic data of the subjects (all women) are listed in Table 1. The subjects' average duration of exposure to cytostatic drugs was 9.0± 0.5 years. The mean age of the exposed nurses (35.6± 0.6 years) was lower than the controls (39.3±1.3 years). For analysis of confounding factors, namely age and smoking, both groups were subdivided into two subgroups according to age (≤ 35 years "young" and >35 years "old") or smoking habits (non-smokers versus active smokers). In the latter subdivision ex-smokers were not included in either group.

The General Effects of Cytostatic Drugs on Lymphocyte Subpopulations and Activation Antigens

Major lymphocyte subpopulations (total T cells, helper and cytotoxic T cells, B lymphocytes and NK cells) are expressed as percentage of total lymphocytes (see Table 2). The mean % ±SE are given for each group. Phenotypes in activation antigen studies are expressed as percentage of positive cells of the given lymphocyte subpopulation e.g. CD25+/CD3+ denotes the percent of CD25 positives among total T cells. The results of activation marker studies are presented in Table 3.

The cytostatic exposed group had a significantly ($p<$ 0.01) elevated B cell percentage (Table 2) and a decreased frequency of CD25+/CD4+ T cells (Table 3) compared to the non-exposed controls.

Cigarette smoke in itself causes exposure to more than 4000 chemicals, so to exclude the effect of smoking as a

Table 1 Main demographic data of the subjects

Groups	n	Mean age year ± SE	Smokers [a] %	Drinkers [b] %	Mean duration of exposure year ± SE
Control	98	39.3±1.3	21.4	43.9	–
Exposed	306	35.6±0.6	43.1	45.4	9.0±0.5

[a] active smokers

[b] no more than 80 g pure alcohol regularly

confounding factor, non-smoking exposed were compared to non-smoking controls. Besides the changes observed above (elevation of B cell frequency, decrease in CD25+/CD4+ cells), a reduction in CD25+/CD3+ and CD25+/CD8+ activated T cells, as well as a decrease in CD56+/CD3+ T cells was apparent in non-smoking exposed compared to non-smoking controls (Table 3).

The Effect of Cytostatic Drug Exposure on the Oxidative Burst of Neutrophil Granulocytes

Using the Bursttest assay, the neutrophil granulocytes of the cytostatic drug exposed group produced more reactive oxygen intermediates (ROI) when stimulated by opsonized E. coli or PMA than the controls, and more cells produced an oxidative burst at the E. coli stimulus compared to the controls (Table 4).

To exclude the effect of age as confounding factor, we compared "young" (≤ 35 years) exposed to "young" controls, and "old" exposed to "old" controls. The exposed showed an elevation of ROI production to the E. coli stimulus in both age groups (Table 4).

Comparing non-smoking exposed to non-smoking controls to exclude the effect of smoking, the oxidative burst of neutrophils showed the same changes as when comparing the whole groups, namely an increase in ROI production in the opsonized E. coli and PMA stimulated samples, and a rise in the percent of neutrophils producing oxidative burst in the E. coli treated samples compared to control non-smokers (Table 4).

The Effect of Age on Immune-Toxicological Parameters

In the young subjects, namely when we comparing "young" (≤ 35 years) exposed to "young" controls, we found an increase in B cell percentage, and a decrease in CD25+/CD4+ T cells, similarly to the results found when comparing the entire groups, but there was also a decrease in the cytotoxic T cell population, leading to an elevated Th/Tc ratio (Table 2) in the young exposed compared to young controls. Compared

Table 2 Lymphocyte subpopulations in cytostatic drug exposed and control subjects expressed as percentage of lymphocytes. The mean % ± SE are given for each group. $p<0.05$ was considered as statistically significant by Student's t-test. Bold characters indicate a significant difference between corresponding exposed and control groups

Group	n	T cell CD3+	T helper CD3+/CD4+	T cytotoxic CD3+/CD8+	Th/Tc CD4/CD8	B cell CD19+	NK cell CD56+/CD3-
Control	98	72.6±0.7	44.2±0.8	26.0±0.8	1.9±0.1	10.0±0.4	12.9±0.6
Exposed	306	72.5±0.4	45.1±0.4	24.8±0.4	2.0±0.0	**11.6±0.2**[a]	11.7±0.3
Control≤35	50	73.3±1.0	41.7±1.1	28.5±1.1	1.6±0.1	9.5±0.4	12.7±0.8
Exposed≤35	167	72.2±0.5	43.6±0.5	**25.5±0.5**[b]	**1.8±0.0**[b]	**11.5±0.3**[b]	11.9±0.5
Control >35	48	71.7±1.0	46.5±1.1[b]	24.0±1.2[b]	2.2±0.1[b]	10.5±0.7	13.3±1.0
Exposed >35	139	72.8±0.6	46.8±0.7[c]	24.0±0.6[c]	2.2±0.1[c]	11.6±0.3	11.6±0.5
Control non-smoker	65	72.7±0.8	44.1±1.0	25.1±0.5	2.0±0.1	9.5±0.5	13.7±0.9
Exposed non-smoker	145	71.9±0.6	43.8±0.6	24.4±0.6	1.9±0.1	**10.9±0.3**[d]	12.7±0.5
Control smoker	20	72.3±1.6	44.6±1.6	25.6±1.4	1.9±0.2	11.4±1.2	11.8±1.2
Exposed smoker	132	73.6±0.5[e]	46.7±0.6[e]	24.4±0.6	2.1±0.1[e]	12.2±0.4[e]	10.4±0.4[e]

[a] significant compared to control

[b] significant compared to control age≤35

[c] significant compared to exposed age≤35

[d] significant compared to control non-smokers

[e] significant compared to exposed non-smokers

Table 3 Lymphocyte activation in cytostatic drug exposed and control subjects. Values are given as percentage ± SE of a given subpopulation of lymphocytes. $p<0.05$ was considered as statistically significant. Bold characters indicate a significant difference between corresponding exposed and control groups

Group	n	CD25+/CD3+	CD25+/CD4+	CD25+/CD8+	CD56+/CD3+	CD71+/CD3+	CD71+/CD19+
Control	98	15.1±1.0	23.2±1.3	4.7±0.6	7.7±0.6	2.0±0.2	49.1±3.2
Exposed	306	13.8±0.6	**19.9±0.8**[a]	4.8±0.5	6.4±0.3	2.4±0.1	44.7±1.8
Control≤35	50	14.5±1.3	23.7±1.9	4.2±0.8	7.7±0.7	1.9±0.2	43.9±4.2
Exposed≤35	167	12.5±0.8	**18.1±1.1**[b]	4.7±0.7	6.4±0.4	2.4±0.2	43.6±2.6
Control >35	48	16.2±1.5	23.4±2.0	5.4±0.9	7.6±1.2	2.2±0.4	55.0±5.1
Exposed >35	139	15.4±0.9[c]	22.0±1.3[c]	4.9±0.5	6.5±0.4	2.4±0.2	45.9±2.6
Control non-smoker	65	17.3±1.2	26.4±1.6	5.6±0.8	8.7±0.9	2.4±0.3	53.0±3.8
Exposed non-smoker	145	**12.1±0.8**[d]	**17.9±1.2**[d]	**3.7±0.4**[d]	**6.3±0.4**[d]	2.1±0.2	44.3±2.7
Control smoker	20	11.4±1.8[d]	16.3±2.5[d]	2.8±1.0[d]	5.4±0.7[d]	1.1±0.1[d]	31.7±7.3[d]
Exposed smoker	132	14.8±0.9[f]	21.1±1.2	5.1±0.5[e.f]	6.3±0.5	2.5±0.2[e]	43.8±2.7

[a] significant compared to control

[b] significant compared to control age≤35

[c] significant compared to exposed age≤35

[d] significant compared to control non-smokers

[e] significant compared to control smokers

[f] significant compared to exposed non-smokers

to "old" controls, the "old" exposed showed no significant changes in the ratio of lymphocyte subpopulations or activation antigen expressing cells.

The ratio of T cell subpopulations changed in both exposed and controls when comparing "young" and "old" groups: the percentage of T helper cells increased with age, while the percentage of cytotoxic cells decreased, resulting in the elevation of Th/Tc ratio regardless of exposure (Table 2). Comparing the "young" exposed group to the "old" exposed group, the percent of CD25+/CD3+ and CD25+/CD4+ activated T cells increased significantly with age (Table 3). There were no significant changes in the ratio of activated T and B cells comparing age groups of the controls. The oxidative burst of neutrophils did not show age related changes (Table 4).

The Effect of Smoking on Immune-Toxicological Parameters

Several parameters changed when the results were compared in smokers and non-smokers among the exposed subjects. A

Table 4 Oxidative burst in neutrophil granulocytes of cytostatic drug exposed and control subjects (production of ROI and percent of ROI producing cells). Bold characters indicate a significant difference between corresponding exposed and control groups

Group	n	Mean fluorescence intensity				% of ROI producing cells			
		Control	fMLP	E. coli	PMA	Control	fMLP	E. coli	PMA
Control	44	6.5±0.9	9.4±1.0	242.3±19.9	844.8±66.9	1.7±0.2	4.7±0.8	80.7±3.2	98.5±0.3
Exposed	81	5.9±0.8	10.2±1.2	**386.9±26.6**[a]	**1037.9±61.9**[a]	1.6±0.3	4.7±0.5	**88.1±1.6**[a]	98.9±0.3
Control≤35	13	4.5±0.9	8.5±1.6	271.7±37.0	865.4±164.5	1.5±0.4	6.4±2.3	83.6±4.1	98.8±0.3
Exposed≤35	39	6.6±1.2	11.6±1.9	**428.1±43.1**[b]	1091.4±98.6	2.1±0.5	5.7±1.0	90.5±2.0	98.7±0.5
Control >35	29	7.1±1.3	9.3±1.2	208.1±19.7	815.2±69.5	1.7±0.3	3.9±0.5	78.8±4.4	98.5±0.4
Exposed >35	42	5.2±1.0	8.9±1.6	**348.7±31.5**[c]	991.1±78.1	1.2±0.1	3.7±0.4	85.8±2.3	99.2±0.2
Control non-smoker	31	6.4±1.1	9.1±1.1	211.3±20.0	828.1±64.2	1.7±0.2	4.3±0.5	79.0±3.8	99.0±0.3
Exposed non-smoker	41	5.5±1.1	9.4±1.7	**405.0±35.6**[d]	**1067.0±92.9**[d]	1.5±0.4	4.7±0.9	**91.4±1.7**[d]	98.8±0.4

[a] significant compared to control

[b] significant compared to control age≤35

[c] significant compared to control age>35

[d] significant compared to control non-smokers

significant rise was detected in the blood leukocyte count ($p<0.001$), the neutrophil count ($p<0.001$), and lymphocyte count ($p<0.001$) of smokers compared to non-smokers. The exposed smokers' leukocyte count was 7.60 G/l±0.18, the neutrophil count was 5.15 G/l±0.15, and the lymphocyte count was 2.09 G/l±0.05, while the exposed non-smokers' leukocyte count was 6.43 G/l±0.14, the neutrophil count was 4.29 G/l±0.10, and the lymphocyte count was 1.81 G/l±0.04. We also found a shift in the lymphocyte subpopulations: the ratio of T, Th and B cells increased, while the ratio of NK cells decreased in exposed smokers compared to exposed non-smokers (Table 2). The rise in CD4+ Th cells lead to an elevation of the Th/Tc ratio. There were also more CD25+/CD3+ and CD25+/CD8+ activated T cells in exposed smokers than in exposed non-smokers (Table 3).

Comparing smokers and non-smokers of the control group, a rise could be detected in the blood leukocyte count, neutrophil count and lymphocyte count between non-smokers and smokers, but this did not reach significance (data not shown). No differences were found in the major lymphocyte subpopulations (Table 2). The activation antigen expressing cells however, showed a completely different pattern of changes than in the exposed: the non-exposed, control smokers had a decreased percentage of CD25+/CD3+, CD25+/CD4+, CD25+/CD8+, CD71+/CD3+ and CD71+/CD19+ activated phenotypes, and also a reduction in the CD56+/CD3+ subset of T cells, compared to control non-smokers (Table 3).

Discussion

In the present study we have shown that low level occupational exposure to antineoplastic drugs caused shifts in the major lymphocyte subpopulations, resulting in a statistically significant increase in the ratio of B cells. In the younger subjects exposure also caused a shift in T cell subpopulations: a reduction in the cytotoxic T cell population lead to an elevated Th/Tc ratio at the group level. According to Mackall, cytotoxic antineoplastic therapy induced immunodeficiency is primarily related to T-cell depletion, with CD4 depletion generally more severe than CD8 depletion [16]. B cells also sustain profound depletion in the context of dose-intensive multiagent chemotherapy [16]. In our studies on the other hand the ratio of B cells increased, which is probably due to the different route and concentration of cytostatic drug exposure. NK cells, in contrast, appear to be relatively resistant both to cytotoxic antineoplastic therapy [17] and, as in our case, low dose occupational exposure.

In our study exposure to cytostatic drugs also caused a decrease in CD25+/CD4+ activated helper T cells. Moreover, when smoking as a confounding factor was eliminated, a significant reduction in CD25 positive activated T cells was detected, which affected not only the helper, but the cytotoxic T cell population as well. CD25 is the α chain of the high affinity IL-2 receptor (IL-2R) which is a heterotrimer comprised of three chains, IL-2Rα (CD25), IL-2Rβ, and IL-2Rγ [18, 19]. IL-2Rβ and IL-2Rγ are present constitutively in resting lymphocytes [20, 21]. In contrast, IL-2Rα is expressed on T cells following activation [22]. Interleukin-2 is crucial for the proper effector functions of conventional T cells, such as cytokine production, growth and survival. IL-2 also plays a critical role in the development and function of regulatory T cells. Accordingly, as reviewed by Lan et al., major IL-2 dysfunctions in both humans and mice are associated with the development of autoimmunity as well as immunodeficiencies, thus highlighting that the balance between the IL-2 pro- and anti-inflammatory effects is critical for an appropriate mounting and resolution of immune responses [23]. A reduction of IL-2 receptors on T cells of cytostatics exposed subjects therefore may have detrimental effects on T cell function.

We observed age-related differences between younger ($\leq$ 35 years) and older (>35 years) study participants: in the older group the percent of CD4+/CD3+ T cells increased, causing an elevation in Th/Tc ratio irrespective of exposure, i.e., both in the exposed and the control population. These findings are in good accordance with a previous study on a Hungarian population stating that the proportion of CD4 positive T cells increase slightly with aging, leading to a significantly positive trend with age in Th/Tc ratio [24].

Although the changes in lymphocyte subpopulations were similar, there were differences in the T cell activation antigen expression between the exposed and control groups according to age. Comparing the two age groups within the exposed, we found more activated T cells (CD25-expressing CD3+ total T cells, and CD25-expressing CD4+ helper T cells) in the older population. Comparing age groups of the controls, however, no significant changes in the ratio of activated T and B cells could be found.

Many studies have been published on the impact of smoking on immunological parameters. Most studies report of an increased blood leukocyte count, stating that smokers have higher counts of neutrophils, although counts of other leukocytes are conflicting in the various studies [25–28]. We also detected an increase in total white blood cells, including both neutrophils and lymphocytes, in smokers, especially among cytostatic exposed smokers compared to non-smokers. Studies investigating the influence of cigarette smoking on different lymphocyte subsets have produced conflicting data, even in recent studies using flow cytometry based analysis. Some studies state that no changes in B- or T-cell frequencies could be noted in

connection with smoking [29] and absolute numbers and percentages of circulating peripheral blood CD4+, CD8+ and CD19+ lymphocytes in smokers were not significantly different compared to non-smoking individuals [30]. Other studies found that smoking increased the number of CD4+ and CD3+ T lymphocytes [31]. In our study smoking did not lead to significant changes in the main lymphocyte populations in the control group. However, in the cytostatic exposed group smoking caused a shift in all major lymphocyte subpopulations: the percent of total T cells, CD4 positive T cells and B cells increased, while that of NK cells decreased, changes similar to the data of Schaberg et al [28]. The CD4/CD8 ratio was elevated in smokers, which is in good accordance with our previous results [13] and the data of Tanigawa et al [32].

In the exposed group smoking lead to increased activation of T lymphocytes, which reached significance in the CD25+/CD3+ and CD25+/CD8+ phenotypes. In the control group, as opposed to the exposed, smoking caused a reduction in all activated phenotypes studied. It also a caused a reduction in CD56+/CD3+ T cells, compared to non-smokers, which is in good accordance with our previous findings [13]. Thus, while smoking in the cytostatic exposed group caused activation in T cells, in the control group it caused the opposite: the percent of activated phenotypes decreased. It is therefore important to take smoking into account as a confounding factor when assessing exposure to cytostatic drugs.

Neutrophils play a fundamental role in the acute inflammatory response, destroying invading microbial pathogens. The ability to generate reactive oxygen species is essential for neutrophils to kill infectious microorganisms. Bursttest allows the quantitative determination of leukocyte oxidative burst, which is proportional to intracellular killing by oxygen-dependent mechanisms. Using the Bursttest assay, the neutrophil granulocytes of the cytostatic drug exposed group produced more reactive oxygen species when stimulated by opsonized E. coli or PMA than the controls, and more cells produced an oxidative burst at the E. coli stimulus than the controls. These results suggest that neutrophils are "primed" by exposure, and therefore react more readily to stimulation through Fc-receptors (opsonized E. coli), and protein-kinase-C (PMA). Priming refers to a process whereby the response of neutrophils to an activating stimulus is potentiated by prior exposure to a priming agent. For instance, exposure of these cells to various inflammatory mediators (e.g., LPS, TNF-α, or GM-CSF) greatly enhances subsequent agonist-induced respiratory burst activity and degranulation responses [33]. In our case antineoplastic drugs may somehow either directly, or through mediators, induce priming of the cells. While priming improves the ability of neutrophils to locate and kill invading microorganisms, it is also implicated in neutrophil-mediated tissue injury both in vitro and in vivo [34, 35]. Hence, the recruitment of large numbers of such hyperresponsive cells at an inflammatory focus may predispose to tissue injury and contribute to a variety of neutrophil-mediated diseases, including acute respiratory distress syndrome, rheumatoid arthritis, and ischemia-reperfusion injury [36].

Occupational exposure to antineoplastic drugs may cause alteration in the immune system, which can lead to health disorders. Indeed, accounts have been reported of various symptoms experienced by nurses handling chemotherapy, such as hair loss, irregular menstrual cycle, skin and eye irritation [37–39]. In a previous paper we have shown increased incidence of raised blood glucose level, anemia, thyroid alterations, myoma and other benign tumors in the cytostatic drug exposed nurses [15]. Interindividual variability of immune parameters is high in human populations, so a change in a single immune test in an individual may not indicate increased susceptibility for disease [40]. Nevertheless, even a subtle alteration in an immunity biomarker in the whole population indicates immunotoxicity [41]. In our study we have demonstrated that immunotoxicity caused by occupational cytostatic drug exposure manifests itself in the shift of major lymphocyte subpopulations, the decreased activation of T lymphocytes and the increased oxidative burst of neutrophil granulocytes, and age and smoking status of the exposed must be taken into account when assessing occupational exposures.

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References

1. McDevitt JJ, Lees PS, McDiarmid MA (1993) Exposure of hospital pharmacists and nurses to antineoplastic agents. J Occup Med 35:57–60
2. Neal AW, Wadden RA, Chlou WL (1983) Exposure of hospital workers to airborne antineoplastic agents. Am J Hosp Pharm 40:597–601
3. Hirst M, Mills DG, Tse S, Levin L, White DF (1984) Occupational exposure to cyclophosphamide. Lancet 1:186–188
4. IARC monographs on the evaluation of carcinogenic risks to humans, volume 76, 2000. Some antiviral and antineoplastic drugs, and other pharmaceutical agents, WHO International Agency for Research on Cancer
5. Sorsa M, Hemminki K, Vainio H (1985) Occupational exposure to anticancer drugs—potential and real hazards. Mutat Res 154:135–149
6. Jakab MG, Major J, Tompa A (2001) Follow-up genotoxicological monitoring of nurses handling antineoplastic drugs. J Toxicol Environ Health 62:307–318

7. Connor TH, McDiarmid MA (2006) Preventing occupational exposures to antineoplastic drugs in health care settings. CA Cancer J Clin 56:354–365
8. Sorsa M, Anderson D (1996) Monitoring of occupational exposure to cytostatic anticancer agents. Mutat Res 355:253–261
9. Sabbioni ME, Castiglione M, Hurny C, Siegrist HP, Bacchi M, Bernhard J et al (1999) Interaction of tamoxifen with concurrent cytotoxic adjuvant treatment affects lymphocytes and lymphocyte subsets counts in breast cancer patients. Support Care Cancer 7:149–153
10. Tong AW, Seamour B, Lawson JM, Ordonez G, Vukelja S, Hyman W et al (2000) Cellular immune profile of patients with advanced cancer before and after taxane treatment. Am J Clin Oncol 23:463–472
11. Zielinski CC, Muller C, Kubista E, Staffen A, Eibl MM (1990) Effects of adjuvant chemotherapy on specific and non-specific immune mechanisms. Acta Med Austriaca 17:11–14
12. Spatari G, Fenga C, Minciullo PL, Di Pasquale G, Cacciola A, Ventura-Spagnolo E et al (2005) Modification of interleukin-15 serum levels in workers exposed to chemotherapeutic agents. Mediators Inflamm 1:60–62
13. Biro A, Pállinger É, Major J, Jakab MG, Klupp T, Falus A, Tompa A (2002) Lymphocyte phenotype analysis and chromosome aberration frequency of workers occupationally exposed to styrene, benzene, polycyclic aromatic carbohydrates or mixed solvents. Immunol Lett 81:133–140
14. Biro A, Pállinger E, Falus A, Tompa A (2004) Characterization of chemically exposed groups by immunotoxicological methods. Hungarian Oncology 48:137–139
15. Tompa A, Jakab M, Biro A, Magyar B, Fodor Z, Klupp T, Major J (2006) Chemical safety and health conditions among Hungarian hospital nurses. Ann N Y Acad Sci 1076:635–648
16. Mackall CL (2000) T-cell immunodeficiency following cytotoxic antineoplastic therapy: a review. Stem Cells 18:10–18
17. Mackall CL, Fleisher TA, Brown MR, Magrath IT, Shad AT, Horowitz ME et al (1994) Lymphocyte depletion during treatment with intensive chemotherapy for cancer. Blood 84:2221–2228
18. Smith KA (1988) Interleukin-2, inception, impact, and implications. Science 240:1169–1176
19. Takeshita T, Asao H, Ohtani K, Ishii N, Kumaki S, Tanaka N et al (1992) Cloning of the gamma chain of the human IL-2 receptor. Science 257:379–382
20. Siegel JP, Sharon M, Smith PL, Leonard WJ (1987) The IL-2 receptor beta chain (p70): role in mediating signals for LAK, NK, and proliferative activities. Science 238:75–78
21. Kondo M, Takeshita T, Higuchi M, Nakamura M, Sudo T, Nishikawa S et al (1994) Functional participation of the IL-2 receptor gamma chain in IL-7 receptor complexes. Science 263:1453–1454
22. Leonard WJ, Kronke M, Peffer NJ, Depper JM, Greene WC (1985) Interleukin 2 receptor gene expression in normal human T lymphocytes. Proc Natl Acad Sci USA 82:6281–6285
23. Lan RY, Selmi C, Gershwin ME (2008) The regulatory, inflammatory, and T cell programming roles of interleukin-2 (IL-2). J Autoimmun 31:7–12
24. Regeczy N, Gorog G, Paloczi K (2001) Developing an expert system for immunophenotypical diagnosis in immunodeficiency. Age-related reference values of peripheral blood lymphocyte subpopulations in Hungary. Immunol Lett 77:47–54
25. Freedman DS, Flanders WD, Barboriak JJ, Malarcher AM, Gates L (1996) Cigarette smoking and leukocyte subpopulations in men. Ann Epidemiol 6:299–306
26. Schwartz J, Weiss ST (1994) Cigarette smoking and peripheral blood leukocyte differentials. Ann Epidemiol 4:236–242
27. Hughes DA, Haslam PL, Townsend PJ, Turner-Warwick M (1985) Numerical and functional alterations in circulatory lymphocytes in cigarette smokers. Clin Exp Immunol 61:459–466
28. Schaberg T, Theilacker C, Nitschke OT, Lode H (1997) Lymphocyte subsets in peripheral blood and smoking habits. Lung 175:387–394
29. Zavitz CCJ, Gaschler GJ, Robbins CS, Botelho FM, Cox PG, Stampfli MR (2008) Impact of cigarette smoke on T and B cell responsiveness. Cell Immunol 253:38–44
30. Bijl M, Horst G, Limburg PC, Kallenberg CGM (2001) Effects of smoking on activation markers, Fas expression and apoptosis of peripheral blood lymphocytes. Eur J Clin Invest 31:550–553
31. Tanigawa T, Araki S, Sata F, Nakata A, Araki T (1998) Effects of smoking, aromatic amines, and chromates on CD4+ and CD8+ T lymphocytes in male workers. Environ Res, Section A 78:59–63
32. Tanigawa T, Araki S, Nakata A, Sakurai S (1998) Increase in the helper inducer (CD4+CD29+) T lymphocytes in smokers. Ind Health 36:78–81
33. Condliffe AM, Hawkins PT, Stephens LR, Haslett C, Chilvers ER (1998) Priming of human neutrophil superoxide generation by tumour necrosis factor-α is signalled by enhanced phosphatidylinositol 3, 4, 5-trisphosphate but not inositol 1, 4, 5-trisphosphate accumulation. FEBS Lett 439:147
34. Smedly LA, Tonnesen MG, Sandhaus RA, Haslett C, Guthrie LA, Johnston RB Jr et al (1986) Neutrophil-mediated injury to endothelial cells: enhancement by endotoxin and essential role of neutrophil elastase. J Clin Invest 77:1233
35. Worthen GS, Haslett C, Smedly LA, Rees AJ, Gumbay RS, Henson JE et al (1986) Lung vascular injury induced by chemotactic factors: enhancement by bacterial endotoxins. Fed Proc 45:7
36. Lee WL, Downey GP (2001) Neutrophil activation and acute lung injury. Curr Opin Crit Care 7:1
37. Shortridge LA, Lemasters GK, Valanis B, Hertzberg V (1995) Menstrual cycles in nurses handling antineoplastic drugs. Cancer Nurs 18:439–444
38. Valanis B, Vollmer W, Labuhn K, Glass A (1997) Occupational exposure to antineoplastic agents and self-reported infertility among nurses and pharmacists. J Occup Environ Med 39:574–580
39. Krstev S, Perunicic B, Vidakovic A (2003) Work practice and some adverse health effects in nurses handling antineoplastic drugs. Med Lav 94:432–439
40. Schwenk M, Sack U, Esser C, Klein R (2007) Diagnostic relevance of the determination of lymphocyte subpopulations in environmental medicineInt. J Hyg Environ Health 210:177–198
41. Environmental Health Criteria 180: Principles and methods for assessing direct immunotoxicity associated with exposure to chemicals. World Health Organization, Geneva, 1992