CD27+ Peripheral Blood B-Cells Are a Useful Biodosimetric Marker in vitro

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Summary
The CD8+ natural killer (NK) subpopulation has recently been identified as a fast and reliable biodosimetric indicator within human peripheral blood mononuclear cells (PBMC) in vitro. In irradiated and subsequently cultivated PBMC, a decrease of the relative number of intact CD3−CD8+ lymphocytes 16 and 48 h after treatment has allowed for estimating the received dose in the range of 0 - 10 Gy and lethal/sublethal dose discrimination, respectively. Here we show that suitable biodosimeters can also be found in the peripheral blood B-cell compartment. Multiparameter flow cytometric analysis of irradiated and subsequently cultivated human PBMC revealed that both the CD27+ and CD21- B-cell subpopulations can be used as biodosimeters and the CD19+CD27+ lymphocytes have proved useful for retrospective determination of the received dose in the range of 0 - 6 Gy. In addition, several CD19+ lymphocyte subsets characterized by co-expression of CD21, CD27 and CD38 have been shown to bear biodosimetric potential, too. However, when important parameters like the original size within the CD19+ compartment, its radiation-induced changes and data variation had been taken into account, the CD27+ subpopulation proved superior to the other B-cell subpopulations and subsets. It appears that, in the dose range of 0 - 6 Gy, the relative decrease of CD27+ B lymphocytes provides more sensitive and reliable data than that of CD8+ NK-cells due mainly to lower data variation. In contrast to CD27+ B-cells, the proportions of CD27+ subpopulations of T-cells were not affected by irradiation. We have also proposed a simple experimental protocol based on full blood cultivation and three-color CD27/CD3/CD19 immunophenotyping as a time-saving and inexpensive approach for practical biodosimetric evaluations on simple, three-to-four color flow cytometers.

Key words
B-cells • CD27 • Biodosimetric marker • γ-irradiation

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Introduction
One of the main tasks of biodosimetry is to identify naturally-occurring indicators (e.g. biopolymers, soluble factors or cell populations) generally called biodosimetric markers that can be used for determining the received dose in individuals exposed to ionizing radiation (Straume et al. 1992, Pass et al. 1997, Mori et al. 2005, Marchetti et al. 2006). In most cases when accidentally exposed victims do not bear personal dosimeters body fluids and cells are the only available materials that can help with dose estimates and subsequent selection of post-irradiation treatment. It has
been documented that allogeneic bone marrow transplantation at short notice can save the life at intermediate-to-high doses (4 - 10 Gy) (Weisdorf et al. 2006); at lower expositions, hematopoietic growth factor administration is often sufficient as supportive care while histoincompatible grafting can have adverse effects due to the graft versus host reaction (Baranov et al. 1989). In addition to their sensitivity in the range of 0 - 10 Gy, appropriate biodosimetric markers should be easily accessible and reliable and the dose estimate should be available within the first day after irradiation as time is a crucial factor in triage of irradiated patients in case of accidental irradiations and mass casualties (Young 1987). The simplicity and costs of the detection system may also play a role, especially when a mass number of suspected sufferers are to be examined (Walker and Cerveny 1989).

Peripheral blood represents an easily accessible source of cells and soluble substances. It can be repeatedly collected at well-defined time intervals after irradiation with essentially no impact on the examined individual. In addition, hematopoiesis belongs to the most radiosensitive systems within the body and peripheral blood leukocytes as well as their subpopulations and subsets have been well characterized in terms of their phenotype and function. Lymphocytes are well recognized as the most radiosensitive white blood cell population (Harrington et al. 1997, Chambers et al. 1998) and they have been thoroughly investigated as a possible source of biodosimetric information (Gridley et al. 2002). Several biodosimetric assays including micronuclei formation (Lee et al. 2000), dicentric chromosome enumeration (Gotoh et al. 2005, Belloni et al. 2005), detection of chromosomal translocations after chromosome painting by fluorescence in situ hybridization (Lucas et al. 1995, Szeles et al. 2006) or mutation frequency determination (Saenko et al. 1998) have been used in the attempt to retrospectively determine the dose received by an individual. The estimation of doses received by victims of the Chernobyl radiation accident by chromosomal aberrations in mitogen-stimulated peripheral blood lymphocytes correlated well with the decrease of both granulocyte numbers and further clinical course (Gale et al. 1993) and provided the lowest dose detection limit of 0.25 Gy. Such biodosimetric approaches have proved reliable and provided good dose estimates but they are labored and time consuming. It is thus obvious that faster and easier protocols with high reliability of results are required.

Lymphocyte populations, subpopulations and subsets differ in their sensitivity to ionizing radiation, which can be used for biodosimetric purposes (Crompton and Ozsahin 1997, Harrington et al. 1997, Mori et al. 2005). Higher radiosensitivity of B-cells than T-cells was documented in mice (Anderson et al. 1977, Durum and Gengozian 1978), rats (Bazin and Plateau 1986, Bazin et al. 1986) and humans (Schwartz et al. 1983, Ashwell et al. 1986) a long time ago. Surface immunophenotyping combined with Annexin V binding to superficially exposed phosphatidylserine (PS) has proved an effective approach in studies focused on γ-radiation-induced apoptosis in lymphocyte subpopulations and subsets characterized by surface marker expression (Koopman et al. 1994, Hertveldt et al. 1997, Philippé et al. 1997, Kern et al. 1999). Hence, Schmitz et al. (2003) found B-cells the most radiosensitive lymphocyte population in a large set of human PBMC samples and higher radiosensitivity of CD19+ lymphocytes (B-cells) than their CD3− counterparts (T-cells) was confirmed by Philippé et al. (1997). Higher radiosensitivity of CD4 + T-cells than the CD8+ ones has been documented by several groups (Seki et al. 1995, Wilkins et al. 2002, Schmitz et al. 2003, Vokurková et al. 2006). When T-lymphocyte subsets were analyzed in more detail, CD4-positive and CD45RA-negative cells were more prone to apoptosis than the CD8-positive and CD45RA-positive ones (Philippé et al. 1997). When compared to T-lymphocytes, B-cell subpopulations and subsets have received less attention. Riggs et al. (1988) showed that, in irradiated mice, splenic B-cells with the sIgM+ / sIgDdim surface phenotype are more radiosensitive than B-cells bearing much less sIgM than IgD on the surface. This finding was supported by histological analysis confirming enhanced radiosensitivity of the marginal zone B-cells. A similar conclusion was made from observations in rats irradiated with doses equal to or higher than 3.5 Gy where the non-circulating marginal zone B-cell population appeared more susceptible than circulating cells from the follicular compartment (Bazin and Plateau, 1984, Bazin et al. 1986). Interestingly, selective resistance of peritoneal B-1 cells to radiation-induced apoptosis, which was not paralleled by either B-1 or B-2 splenocytes has recently been published (Otero et al. 2006).

We have recently identified a population of relatively radiosensitive small CD3+CD8− PBMC with a biodosimetric potential in the range of 0 - 10 Gy (Vokurková et al. 2006). We have also shown that no significant increase in the proportion of Annexin V-
binding cells occurs in irradiated PBMC during first 6 h of *in vitro* cultivation, which may represent a useful time window for sample collection and immunophenotypic characterization of lymphocyte subsets before cells start dying. In this work we have focused on the B-cell compartment and studied relative radioresistance of CD19+ peripheral blood B-cell subpopulations and subsets in the attempt to identify another reliable, easily accessible and highly sensitive biodosimetric markers.

**Materials and Methods**

**Cell isolation**

In three sequential experiments, seven peripheral blood mononuclear cell (PBMC) preparations (2, 3 and 2) and five full blood samples (0, 3 and 2) were analyzed. Heparin-treated blood obtained from seven different volunteer donors was diluted 1:1 with phosphate buffered saline (PBS) and either used directly (full blood samples) or PBMC were isolated by 400 g centrifugation through the Ficoll-Histopaque (density 1.077, Sigma, St. Luis, MO) cushion (20 min, 20°C). PBMC collected from the Ficoll/plasma interface were washed twice in sterile RPMI 1640 (GIBCO Life Technologies, Grand Island, NY) supplemented with 20 % FCS, 2 mM glutamine, 100 UI/ml penicillin and 0.1 mg/ml streptomycin (all products from Sigma) and their density was finally set to 10^6 cells/ml.

**Irradiation and cultivation**

500 µl and 1 ml aliquots of PBMC and PBS-diluted blood, respectively, were transferred into a 24-well polystyrene cultivation plate (Nunc). Individual plates were sham-treated or irradiated with the doses of 3, 6 or 10 Gy delivered with a rate of 1.29 Gy/min at a distance of 1.23 m from the Co^{60} source. Subsequently, the plates were transferred into a humidified incubator and cultivated for 16 h at 37 °C. After cultivation, the cells were harvested by thorough pipetting and centrifuged (400 g for 10 min at 4 °C). Red cells were removed from full blood samples by 10 min exposure of the resuspended pellet to the EasyLyse solution (Dako, Glostrup, DK) and remaining peripheral blood leukocytes (PBL) were centrifuged. PBMC and PBL pellets were resuspended and washed twice in ice-cold washing and staining buffer (WSB, PBS containing 0.2 % gelatin from cold water fish skin and 0.1 % sodium azide, all reagents from Sigma) and their density was set to 5x 10^6/ml. Until data acquisition, the cells were kept in cold and at the presence of sodium azide to prevent apoptosis progression.

**Flow cytometry**

Anti-CD19/Pacific blue (anti-CD19/PB, clone HD37, Dako), anti-CD5/Fluorescein isothiocyanate (anti-CD5/FITC, clone DK23, Dako), anti-CD27/FITC (clone M-T271, Dako), anti-CD3/R-Phycocerythin (anti-CD3/RPE, clone UCHT1, Immunotech), anti-CD21/RPE (clone B-ly4, Becton Dickinson), anti-CD138/RPE (clone BB4, Immunotech), anti-CD19/RPE-cyanin 5 (anti-CD19/PC5, clone HD37, Dako) and anti-CD38/PC5 (clone LS198-4-3, Immunotech) conjugates of mouse anti-human monoclonal antibodies (moAbs) were used in the following cocktails for multicolor immunophenotyping: CD19/PB + CD27/FITC + CD21/RPE + CD38/PC5, CD19/PB + CD5/FITC + CD138/RPE + CD38/PC5 and CD27/FITC + CD3/RPE + CD19/PC5. 100 µl of cell suspension (5 x 10^5 cells) was incubated with the moAbs cocktail for 30 min on ice. Then the cells were washed twice in ice-cold WSB and data were acquired on a nine-color flow cytometer CyAn ADP (Dako). Propidium iodide (PI) was added just before acquisition to the final concentration of 0.1 µg/ml. Acquisition and analysis were performed using the Summit software (Dako). The detector sensitivity and off-line compensation of FITC, RPE and PC5 emission spectra overlaps were set using unstained and single-color stained samples and the automatic compensation software utility. The emission of PB excited by the violet (405 nm) laser was not necessary to compensate with the set of fluorochromes used.

**Statistical analysis**

To eliminate differences between individual blood donors, we have defined “irradiated versus non-irradiated ratio” (IVNIR) by dividing the relative size of a selected cell population in the irradiated sample by its respective number in the non-irradiated control. IVNIR cohorts were statistically evaluated and results are given as mean ± SD. In experiments on the dose-dependence, differences between samples were assessed by the Student’s t-test.

**Results**

**Surface marker selection and gate logic**

Since the main task of our work has been to identify readily accessible, easily detectable and reliable
biodosimetric markers within the B lymphoid compartment we have chosen a panel of fluorochrome-labeled mAbs directed against differentiation antigens present on the surface of peripheral blood B-cells. We have omitted anti-Ig heavy chain immunoreagents as extensive washing of blood leukocytes is required before staining to prevent blocking the anti-Ig binding sites by serum-born Ig, which would unnecessarily prolong sample processing. In addition, Fc-receptor bound Ig may represent a potential source of false positive signal in terms of B lineage identification. Last but not least, polyclonal antisera are traditionally used for Ig subclasses detection and we wanted to prevent batch-to-batch variation in long-term experiments and in practical ramifications in radiobiology. Therefore, anti-CD19 mAb was used to distinguish B-cells from other PBMC lineages and anti-CD5, anti-CD21, anti-CD27, anti-CD38 and anti-CD138 were selected for visualizing B-cell subpopulations and subsets.

We and others have previously demonstrated that superficial phosphatidyl serine exposure at the early stage of apoptosis is rapidly followed by cell shrinking and decreased surface marker expression (Hertveldt et al. 1997, Vokurková et al. 2006). The great majority (> 95%) of Annexin V-binding cells in the intermediate and late stages of apoptosis can thus be excluded from analysis by gating them out using a region closely fitting the predominant population of small mononuclear cells clearly visible in irradiated as well as sham-treated PBMC cultures (region R1 in Fig. 1A). The left margin of R1 (the vertical bar in Fig. 1A, B and C) was set to exclude the majority of PI+ events and cells with diminished CD19 expression (Fig. 1B and 1C, respectively) from analysis. Immunophenotyping within the “R1 AND R2 AND R3” logical gate has made it possible to determine IVNIR for subpopulations and subsets of small (R1) and propidium iodide-negative (R3) lymphocytes with unimpaired CD19 expression (R4). This approach has provided us a platform for identification of suitable biodosimetric markers within the peripheral blood B-cell compartment.

**B-cell subpopulation and subset definition and their biodosimetric potential**

To reveal putative biodosimetric candidates with short response time among B-cell subpopulations and subsets, a high lethal dose irradiation (10 Gy) followed by mid term (16 h) cultivation and four-color immunostaining was chosen as the first step. Hereafter,
all CD19−PI− events in R1 (Fig. 1A) with comparable expression of one of the differentiation markers studied will be referred to as a subpopulation of small intact B-cells. We also define a subset of small intact B-cells as all CD19−PI− events in R1 characterized by surface expression of two differentiation markers. Fig. 2 shows an example of CD21, CD27 and CD38 co-expression profiles on intact small B lymphocytes in sham-treated (A, B, C) and 10 Gy irradiated (E, F, G) PBMC cultivated for 16 h after treatment. We disregarded fine resolution of surface marker density and defined the positive and negative B-cell subpopulations for every single marker as shown by quadrant statistics in Fig. 2. For example we refer to CD21+ and CD21− B subpopulations as the sum of the subsets mapping to the upper and lower quadrants, respectively, in Fig. 2A, D. In the same dot plots, CD27− B subpopulation is represented by the two subsets in both of the right quadrants while its CD27+ counterpart can be found in the left quadrants only. Analogously, the CD21−CD38+ single positive B-cell subset is defined as all events in the upper left quadrants in Fig. 2B and 2E. Double positive and double negative B-cell subsets for all marker combinations analyzed in Fig. 2 can thus be found in upper right and lower left quadrants, respectively. Inter-donor differences in proportions of B-cell subpopulations and subsets in peripheral blood have been eliminated by introducing IVNIR, the number reflecting relative radiosensitivity of individual B-cell subpopulations and subsets.

Summary of γ-radiation induced changes in the relative numbers of B-cell subpopulations and subsets are given as IVNIR statistics in Fig. 3A and 3B, respectively. In contrast to CD27− B-cells, the relative number of which obviously decreases in irradiated cultures in vitro, the CD21+ population of B-cells relatively increases its size at the expense of their negative counterparts (Fig. 3A). The overall changes in the CD38-defined compartments are less pronounced and the CD38− and CD38+ subpopulations appear similarly radioreistant, although the CD19−CD38+ subpopulation can be slightly more radiosensitive. Results shown in Fig. 3A suggest that it is the CD21+ and CD27− subpopulations of B-cells that could represent promising radiation biomarkers since prominent changes in their relative size with reasonably low data variation can be observed in a relatively short time after high dose γ-irradiation. When the two subsets are compared, the CD19−CD27− B-cells appear to represent the superior biomarker for the following reasons. First, the relative numbers of CD27+ B-cells exceeded the size of the CD21− subpopulation in all non-irradiated samples studied: 18 - 35 % small CD19− lymphocytes were CD27− while the relative numbers of CD19− CD21+ B-cells were in the range of 15 - 23 %. Second, IVNIR variation of the CD27− subpopulation was lower than that of CD21− B-cells. Third, the positive and negative populations are easier to distinguish for CD27 than for CD21 (Fig. 2). Experiments with anti-CD5 and anti-CD138 moAbs did not reveal any significant differences between irradiated and non-irradiated samples and these markers appear useless for biodosimetric purposes in peripheral blood (data not shown).
**Fig. 2.** B-cell subpopulation and subset definition. An example of CD27 versus CD21 (A and D), CD38 versus CD21 (B and E) and CD38 versus CD27 (C and F) two dimensional expression profiles on small intact B-cells (CD19⁺PI events in R1 in Fig. 1) is shown in sham-treated (A, B, C) or 10 Gy-irradiated (D, E, F) PBMC cultivated for 16 h. Spectral overlap between FITC and RPE was compensated using single stained controls. A limited number of 3 000 events are shown in all dot plots to optimize visualization of differences in cell subset proportions between the irradiated and non-irradiated sample. For selected marker combinations, the quadrant statistics in individual dot plots define the double positive (upper right quadrant), double negative (lower left quadrant) and two single positive (upper left or lower right quadrants) subsets, the relative numbers of which are given.
Two parameter immunophenotyping within the B-cell compartment (Fig. 3B) has shed some light on the relative radiosensitivity of individual CD19+ lymphocyte subsets. As follows from subpopulation analysis (Fig. 3A), CD27+ or CD21+ B-cells represent most promising single parameter biodosimetric candidates. Thus not surprisingly, when both the relative decrease in irradiated samples and the data variation have been taken into account, the CD21+CD27+, CD27+CD38+ and CD21-CD38+ immunophenotypes appear to be worth testing as radiation biomarkers. In addition, the CD21/CD27 double positive and double negative subsets bear some biodosimetric potential, too. Importantly, in the process of subdividing major B-cell subpopulations into subsets, the proportion of the cells of interest decreases and small number errors may begin playing a role. Moreover, because the overall cell numbers in irradiated samples significantly decrease with the dose and time of cultivation, experimental data may be negatively influenced when very small populations are analyzed. In this respect, the CD21+CD27+, CD21-CD27 and CD27+CD38− B-cell subsets with their relative size of 15 - 28 %, 15 - 22 % and 11 - 24 %, respectively, can provide more reliable data than the smaller CD21+CD38+ (8 - 16 %) and CD21+CD27+ (3 - 6 %) ones.

Interestingly, data from Ficoll-isolated PBMC have been confirmed by a pilot study of CD19 versus CD27 expression on B-cells in irradiated and sham-treated PBL preparations. Similar to IVNIR in PBMC (0.65 ± 0.05, see the left column in the middle of Fig. 3A), the values of 0.53 ± 0.05 have been obtained for IVNIR of CD19+CD27+ small intact lymphocytes in PBL preparations. This finding suggests that full blood cultivation ex vivo may represent a convenient alternative to more labored and time-consuming density gradient PBMC isolation.

The CD27+ B-cell subset as a radiation dose indicator

A convenient biodosimeter should be able to distinguish between low (sublethal) and high (lethal) dose of irradiation as lethal versus sublethal case discrimination is one of the most important parameters in terms of the treatment of an irradiated individual. We have thus tested the reporter capacity of CD19+CD27+ lymphocytes in 0, 3, 6 and 10 Gy-irradiated PBMC samples. We have also asked a question if it is specifically the CD27 surface glycoprotein that is sensitive to irradiation irrespective of its presence on the surface of B- or T-cells or the peripheral blood CD27+ B-cells represent a radiation-sensitive population, the disappearance of which indicates previous exposure to γ-rays. Last but not least, we have considered that the practical assay should be as simple as possible for being performed on simple and inexpensive analyzers. We have therefore used a modified staining protocol with the cocktail of anti-CD27/FITC, anti-CD3/RPE and anti-CD19/PC5 moAbs, which is convenient for data acquisition on standard flow cytometers equipped with a single blue laser and three fluorescence channels. When the fourth channel is available it can be used for propidium iodide or 7-aminoactinomycin D-based live/dead discrimination, although Figure 1 shows that thorough gating in the FSC/SSC dot plot provides a relatively pure population of intact cells and PI exclusion need not be absolutely required for reliable biodosimetric data acquisition.

Dose dependent relative changes in major lymphocyte populations and their CD27+ subpopulations in PBMC are illustrated in Fig. 4. As expected, peripheral B-cells are more radiosensitive than T-cells, which is reflected by the increasing T : B cell ratio (Fig. 4A). This, however, cannot be used as a reliable biodosimetric parameter due mainly to a relatively large data variation. On the other hand, the CD27+ T-cell subpopulation does not change its relative size within the T-cell compartment after irradiation (Fig. 4B), which means that CD27+ and CD27− T-cells are equally radiosensitive. Similarly, when CD27++ T-cells (with CD27 expression higher than that on B-cells), and their CD27− counterparts (CD27 expression comparable to B-cells) were analyzed separately no difference in radiosensitivity between CD27++, CD27+ and CD27− T-cell subpopulations was observed within the range of doses studied (data not shown). In striking contrast, the CD19+CD27+ lymphocyte subpopulation has confirmed its biodosimetric potential (Fig. 4C) in a dose-dependent manner. Irradiation with Co60 γ-rays followed by in vitro cultivation of PBMC for 16 h has resulted in about 25 % and 50 % reduction of IVNIR at 3 and 6 Gy, respectively. The differences in IVNIR in the range of 0 - 6 Gy were highly statistically significant. No significant differences in the relative size of the CD19+CD27+ lymphocyte subpopulation were observed between 6 and 10 Gy-irradiated samples. The IVNIR data variation indicate that the relative decrease shown in Figure 4C could provide a reliable estimate of the received dose in the range of 0 - 6 Gy in in vitro cultures.
Differences in the response to ionizing radiation between and within white lineages of hematopoiesis have been documented by many authors (Schwartz et al., 1983, Ashwell et al. 1986, Koopman et al. 1994, Seki et al. 1995, Hertveldt et al. 1997, Philippé et al., 1997, Kern et al. 1999, Wilkins et al. 2002, Schmitz et al. 2003, Mori et al. 2005). In contrast to relatively radioresistant myelomonocytic cells (granulocytes and monocytes) lymphocytes represent highly radiosensitive blood leukocytes (Dutreix et al., 1987, Mori and Desaintes 2004, our unpublished observations in vitro and ex vivo) and, in general, belong to most radiosensitive quiescent cells within the mammalian body. Thus not surprisingly, lymphocytes have traditionally been regarded as possible biodosimetric markers both for retrospective determination of the received dose (Gale et al. 1993, Crompton and Oszahin 1997, Menz et al. 1997) and as indicators of individual’s radioresistance (Crompton and Oszahin 1997, Crompton et al. 1999, Oszahin et al., 2005). Although some authors originally concluded that major lymphocyte populations, their subpopulations and subsets were equally prone to radiation-induced apoptosis (Dutreix et al. 1987, Clave et al. 1995), other groups have observed that the impact of in vivo or in vitro γ-irradiation on a lymphoid cell strongly depends both on its type and differentiation status. Therefore, B-lymphocytes have generally been considered the most radiosensitive lymphocyte population (Anderson et al. 1977, Durum and Gengozian 1978, Schwartz et al. 1983, Ashwell et al. 1986, Philippé et al. 1997, Pecaut et al. 2001) and, in the more radioresistant T-cell compartment, CD4+ T-cells appeared less radiosensitive than their CD8+ counterparts (Seki et al. 1995, Wilkins et al. 2002, Schmitz et al. 2003) although some groups have concluded the opposite (Farnsworth et al. 1988). Most controversial data exist about the radiosensitivity of NK-cells. While some authors (Ban and Cologne, 1992, Clave et al., 1995, Philippé et al. 1997, Louagie et al. 1999, Pecaut et al. 2001) described NK-cells as a relatively radioresistant population, other groups (Farnsworth et al. 1988, Rana et al. 1990 and Seki et al. 1995) have emphasized NK-cell radiosensitivity. Experiments focused on the radioresistance of B- and T-cell subsets are scarce and provide only fragmentary information about well-defined populations. Rather surprisingly, even though high radiosensitivity and differential radioresistance within the lymphoid compartment appear to offer an opportunity to design fast and relatively simple biodosimetric protocols, only a few groups have successfully used lymphocyte subpopulations and subsets as biodosimeters (Crompton and Oszahin, 1997, Menz et al. 1997, Vokurková et al. 2006).

In our opinion, the difficulties with identifying convenient biodosimeters in the lymphoid compartment have originated from the focus on major lymphocyte populations and their most popular subpopulations like
CD4+ or CD8+ T-cells (Kunosoki et al. 1994, Clave et al. 1995, Philippé et al. 1997, Crompton and Ozashin, 1997) or subsets expressing different isoforms of CD45 (Clave et al. 1995, Philippé et al. 1997). The individual’s radioresistance has also been expected to represent a bottleneck in biodosimetric studies within the outbred human population (Terzoidi et al. 2006).

We have recently tested the hypothesis that cultivation of irradiated PBMC and appropriate lymphoid subset selection can provide a clue for successful biodosimetry in vitro. We have confirmed that it is neither major lymphocyte populations (T- and B-cells) nor the most popular subpopulations (e.g. CD4+ or CD8+ T-cells) that would represent reliable biodosimetric markers (data presented here, Vokurková et al. 2006). This is due mainly to their heterogeneity – even a relatively simple compartment like peripheral blood where essentially no cycling, differentiating or apoptotic cells are present under normal conditions consists of numerous stages of T, B and NK cell development ranking from naïve recent emigrants from primary centers to fully differentiated and effectors stages including memory, cytolytic and antibody secreting lymphocytes. The selection of a uniform lymphocyte subset and careful data analysis have recently revealed highly radiosensitive CD27+ B-cells, the existence of one radioresistant (CD21+CD27-) subset radioresistance has not been the purpose of our work. Moreover, we feel that we do not have enough experimental data to make any general conclusions concerning relative radioresistance of peripheral blood B-cell subsets, although our results showing high radiosensitivity in the CD27+ compartment of activated/memory peripheral blood B-cells (Klein et al. 1998) is in a good agreement with lessons learnt from animal models (Bazin and Platteau 1984, Bazin and Platteau 1986, Bazin et al. 1986). At this level of understanding we have

Subset analysis within the CD19+PT lymphoid compartment in 10 Gy irradiated PBMC confirmed the usefulness of the CD27+ and CD21+ subpopulations as biodosimetric indicators. The CD38+ and CD38- subsets of CD27+ B-cells were negatively affected by irradiation but relatively high data variation made them less convenient for biodosimetric purposes. On the other hand, the CD21-CD38+ subset seemed to be fully responsible for radiation-induced reduction of CD21+ B-cell numbers and as such it qualifies for more detailed investigation. When CD21 expression was studied versus CD27, the existence of one radiosensitive (CD21’CD27’) and three radiosensitive (CD21’CD27+, CD21’CD27+ and CD21’CD27+) subsets became apparent.

We do not show here the results of three-dimensional subset analysis of CD19+ B-cells as further subdivision makes some populations too small to prevent small number errors and detailed description of individual subset radioresistance has not been the purpose of our work. Moreover, we feel that we do not have enough experimental data to make any general conclusions concerning relative radioresistance of peripheral blood B-cell subsets, although our results showing high radiosensitivity in the CD27+ compartment of activated/memory peripheral blood B-cells (Klein et al. 1998) is in a good agreement with lessons learnt from animal models (Bazin and Platteau 1984, Bazin and Platteau 1986, Bazin et al. 1986). At this level of understanding we have
concluded that the CD19 versus CD27 immunophenotypic comparison of freshly isolated peripheral blood lymphocytes with the samples cultivated for 16 h may represent a convenient, fast and simple biodosimetric assay, especially when applied to full blood samples when neither density gradient isolation nor humidified atmosphere during cultivation is absolutely required.

References


Conflict of Interest

There is no conflict of interest.

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