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Radiation exposure leads to a risk for long-term deterministic and stochastic late effects. Two individuals exposed to protracted photon radiation in the radiological accident at the Lilo Military site in Georgia in 1997 received follow-up treatment and resection of several chronic radiation ulcers in the Bundeswehr Hospital Ulm, Germany, in 2003. Multi-parameter analysis revealed that spermatogenetic arrest and serum hormone levels in both patients had recovered compared to the status in 1997. However, we observed a persistence of altered T-cell ratios, increased ICAM1 and β1-integrin expression, and aberrant bone marrow cells and lymphocytes with significantly increased translocations 6 years after the accident. This investigation thus identified altered end points still detectable years after the accident that suggest persistent genomic damage as well as epigenetic effects in these individuals, which may be associated with an elevated risk for the development of further late effects. Our observations further suggest the development of a chronic radiation syndrome and indicate follow-up parameters in radiation victims.

INTRODUCTION

Total-body irradiation (TBI) in humans leads to manifestation of systemic effects within several days, known as the acute radiation syndrome (*1*), which comprises involvement of several organs and organ systems (such as the hematopoietic, gastrointestinal, neurovascular and cutaneous systems) depending on both the extent of damage of the exposed organs and the radiosensitivity of the specific organ. Currently, the concept of the organ response to TBI is gradually shifting toward the concept of a radiation-induced multi-organ involvement and failure as the general denominator of the clinical course of the radiation reaction (*2*). Diagnosis and treatment of TBI is still problematic. Despite the fact that advances in the treatment of the acute radiation syndrome and the categorization of acute health effects after exposure to ionizing radiation have been made (*1*), there is still a clear lack of standardized protocols for the long-term follow-up of radiation accident victims.

Here we report on the clinical follow-up in two patients, patient 6 (B.Z.) and patient 9 (I.D.), who were accidentally exposed to γ radiation by contact with abandoned radioactive (^{137}Cs and ^{226}Ra) sources left by the former Soviet Army on a site that later became the Lilo Training Center of the Georgian Army (*3*). In October 1997, 11 soldiers had developed local radiation-induced skin lesions on various parts of their bodies. The Government of Georgia contacted the IAEA for assistance in handling of this accident. At that time all patients displayed various symptoms of a cutaneous radiation syndrome with one or several erythemas on different parts of the body together with general symptoms attributable to the acute radiation syndrome. Exposure to ionizing radiation was very inhomogeneous and likely occurred over approximately 60–300 days (*3*).

In October 1997 these patients were transferred and treated in WHO/REMPAN collaboration centers, seven of them in the Bundeswehr Hospital Ulm and two each in the Percy Military Hospital and Institute Curie in Paris. Patients B.Z. and I.D. returned to the Bundeswehr Hospital Ulm at the end of 2003 for medical follow-up due to persistent complaints such as lassitude, fatigue, radiation ulcers and frequent infections during the 6 years after the accidental radiation exposure. Re-examination of these patients revealed the following results.

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MATERIALS AND METHODS

Flow Cytometry, Lymphocyte Typing and Cell Adhesion Markers

The determination of three-part differential and percentages of mature lymphocyte subsets in whole blood from Georgian patients and controls was done using the Simultest™ IMK-Lymphocyte Kit (Becton Dickinson, Heidelberg, Germany). The kit enumerates the percentages of mature human leukocyte subsets in erythrocyte-lysed whole blood [T ($CD3^+$) lymphocytes, B ($CD19^+$) lymphocytes, T-helper ($CD3^+CD4^+$) lymphocytes, T-suppressor ($CD3^+CD8^+$) lymphocytes, and natural killer ($CD3^-CD16^+$ and/or $CD56^+$) lymphocytes] and allows determination of the helper/suppressor T-lymphocyte ratio ($CD3^+CD4^+/CD3^+CD8^+$). Stained samples were run on a Becton Dickinson FACSCalibur flow cytometer and analyzed using Simultest IMK-Lymphocyte Software (Becton Dickinson). The three-part differential was obtained by expressing lymphocytes, granulocytes and monocytes as percentages of leukocytes.

The expression of adhesion molecules ICAM1 (CD54) and β1-integrin (CD29) on granulocytes was measured by staining with an FITC-conjugated monoclonal mouse anti-human ICAM1 antibody (clone 15.2, Chemicon) and an FITC-conjugated monoclonal mouse anti-human CD29 antibody (clone TDM29, Chemicon) as described above. Flow cytometry analysis was performed using CellQuest software (Becton Dickinson). The fluorescence intensity of the FITC emission of gated granulocytes was quantified, and the mean channel fluorescence (MCF) served as an indicator of the amount of ICAM1 or β1-integrin.

Assay of Dicentric Chromosomes

The assay of dicentric chromosomes was performed essentially as described previously (*4*). Phytohemagglutinin-M (Life Technologies)-stimulated whole blood cultures of lymphocytes were set up in the presence of 5-bromodeoxyuridine (Sigma) to enable discrimination between the first and subsequent cell cycle metaphases. Cells were harvested after addition of Colcemid and 48 h of culture according to standard procedures. Chromosome preparations were prepared by standard acetic acid/methanol fixation (1/3) and subjected to FPG staining. Air-dried preparations were covered with Eukitt (Merck) before analysis using a Leica microscope. Metaphase spreads were located and analyzed by two investigators. Unstable chromosome aberrations (dicentric chromosomes, acentric fragments and centric rings) were scored in at least 1000 metaphases of each individual. For the dose estimates, a dose–effect relationship was fitted to published chromosome aberration data from blood lymphocytes irradiated *in vitro* at a dose rate of 0.5 Gy/min with ^{60}Co rays (*4*).

Three-Color FISH Painting

Three-color FISH with chromosome 1, 4 and 12 paint probes and a pancentromere probe was done according to Bauchinger *et al.* (*5*).

Irradiation Conditions

For the comet and the γ-H2AX focus assays, *ex vivo* irradiation of blood and lymphoblastoid cells was done at room temperature with single doses of 240 kV X rays (Isovolt 320/10; Seifert, Ahrensberg, Germany) filtered with 3 mm beryllium; absorbed dose was measured with a Duplex dosimeter (PTW; Freiburg, Germany). The dose rate was ~1 Gy/min at 13 mA. Control cells were sham-irradiated.

Single-Cell Gel Electrophoresis

Single-cell gel electrophoresis (comet assay) was performed according to Singh *et al.* (*6*). A total of 100 randomly captured comets per individual and per time (50 comets on each of two replicates) were examined with a 25× magnification objective lens using an epifluorescence microscope (Leica DMIRB) connected through a black and white camera (COHU) to the image analysis system Comet 4.0 (Kinetic Imaging Ltd, Liverpool, UK). For each cell, the percentage of DNA in the tail was determined, and the data were expressed as mean values. Data from different individuals and times (4 Gy/0 min repair time, 4 Gy/120 min repair time, and nonirradiated cells) were compared by the nonparametric Mann-Whitney *U* test with the aid of statistical analysis software (SigmaStat 2.0, Jandel, Erkrath, Germany).

DNA Repair Analysis by γ-H2AX Foci

γ-H2AX focus analysis was done using method of Rothkamm and Löbrich (*7*) with the following modifications. Cells of lymphoblastoid cells lines were cultured in RPMI 1640 medium and irradiated in culture flasks. Subsequently, cells were harvested by centrifugation at room temperature and washed once in PBS. Cells were then resuspended in 200 μl 70% ethanol and 50 μl was spun onto cleaned glass slides using a cytocentrifuge (Shandon Thermo). The preparations were briefly air-dried and extracted with 0.2% Triton X-100 (Serva), PBS for 20 min at 0°C. Immunofluorescence labeling with a monoclonal anti-γ-H2AX antibody (Upstate) was done as described (*8*).

Blood and Bone Marrow Cell Assessment

The concentrations of cells in the peripheral blood were determined using conventional laboratory methods and blood smears. Bone marrow aspirates were obtained from the right spina iliaca posterior superior in both patients and processed for standard bone marrow smears and bone marrow sections. The latter were examined with respect to the functional activity and differentiation as well as proliferation. In a separate step, the bone marrow smears were screened for mitotically connected cellular abnormalities such as binucleated cells, perturbed mitotic figures, cytoplasmic bridges, and karyomeres (*9*).

RTQ-PCR

For expression analyses peripheral blood was collected into PAXgene tubes (Qiagen), total RNA was isolated by the RNeasy Mini Kit (Qiagen), and the remaining DNA was digested (RNase-free DNase Set, Qiagen). Quality control was done as described by Stassen *et al.* (*10*). Aliquots of total RNA (1 μg) were reverse transcribed using Multiscribe™ reverse transcriptase and thermal cycled according to a two-step PCR protocol (TaqMan Gold RT-PCR Kit). The resulting cDNA was diluted in water (1μg/μl), stored at −20°C, and used as template for subsequent PCR reactions. Intron-spanning primer and probe designs (PPDs) of five gene targets (*PSME3, GLUT1, MRP8, ISGF3G* and *PKCI1*) were used (*6*). Further PPDs to *TNFSF6* (Fas-ligand), *TNFSF10* (TNF-ligand), Clusterin, *BCL2, BAX, TP53,* p21, *PCNA, GADD45A, RAB2, NFKB* and 18S rRNA were applied. DNA-PK was detected by the primer pair: forward: ATTGATTAAATTGTGCTACGATGCA; reverse: CAGATATGGCGCA GTTGTATGC), and a FAM-labeled MGB probe (TTACAGAGAACAT GGCAGGAG) was used for TaqMan^R chemistry. The specificity of the amplicon sequence designed was controlled using three independent methods [(*10*), M. Abend *et al.,* unpublished results]. The PCR reaction typically included TaqMan Master Mix containing hot start AmpliTaqGold DNA polymerase, 300 n*M* forward and 300 n*M* reverse primer, 200 n*M* FAM-labeled probe, and 10 ng cDNA. Forty PCR cycles were driven with annealing and elongation of primers and probes occurring at 60°C over 1 min, followed by a 1-min denaturation step at 95°C. A relative standard curve derived from sequential eightfold dilutions of stock cDNA of known quantity (yielding 0.5 ng–7.6 fg cDNA) was used for a linear regression analysis of unknown samples, thus allowing us to convert the so-called threshold cycles (C_T) of the PCR reaction of unknown samples into nanograms of cDNA. The dynamic range of linearity lasted over 6 log units. The slope of the standard curves was nearly constant throughout the experiments (range 3.4–3.6, which corresponds to a PCR efficiency ≥90%). Furthermore, 18S rRNA controls were run in parallel to control the amount of cDNA added to each PCR reaction. Expression analysis was done using a GeneAmp 5700 Sequence Detection System (SDS, Version 1.3, TaqMan, Applied Biosystems).

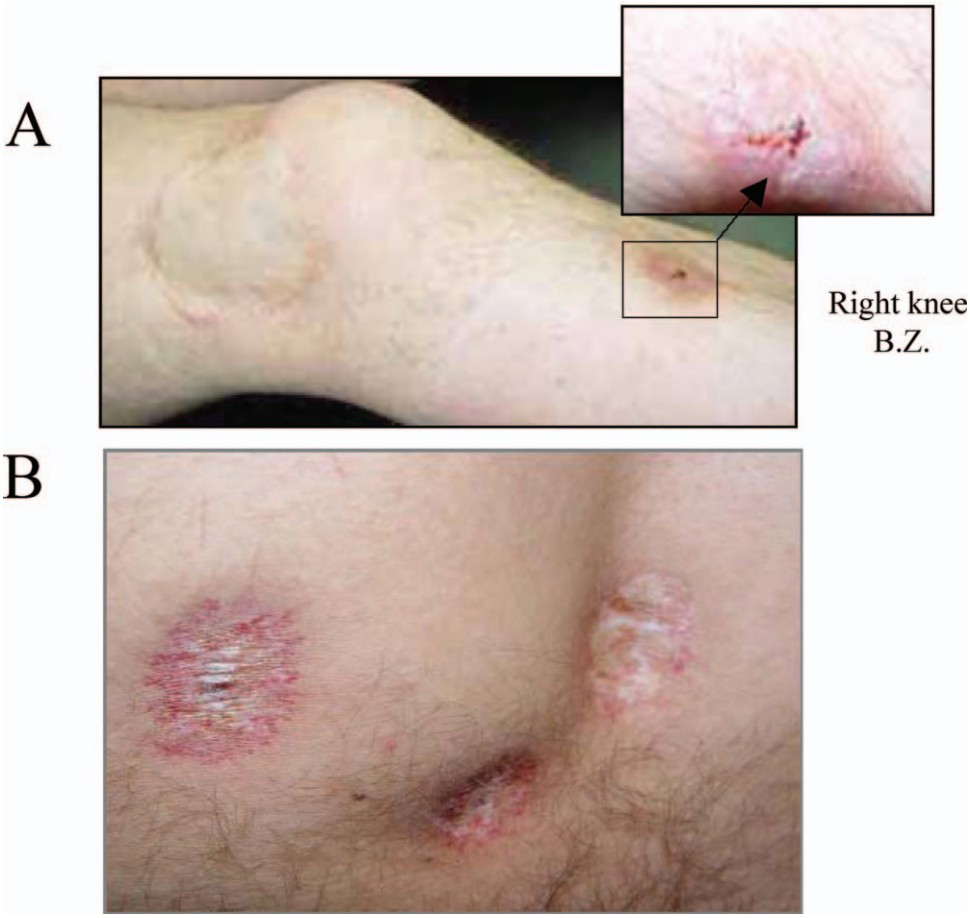

FIG. 1. Panel A: Lower part of the right leg of patient B.Z. showing a fibrotic and erythematic area 2 cm diameter in 2003. Panel B: Back of patient I.D. showing multiple poikilodermatic atrophic sclerodermiformic areas with teleangiectasias before treatment in November 2003.

RESULTS

Patient B.Z. [patient 6 (*3*)] had a 7 cm × 2-cm ulcer on the right thigh above the knee joint. A white macule of 1.2 cm × 5 cm and an ulcer of 1 cm × 0.8 cm covered by a central hemorrhagic scab were found in the lower extremity of the left leg. The patient also suffered from a radiation-induced synovitits of the right knee and associated complications that had been treated in four surgical interventions between October 1997 and April 1998. In 2003, the patient showed a fibrotic and erythematic area 2 cm in diameter on the lower extremity of the right leg (Fig. 1A). Flexing of the right knee joint was painfully limited (0/0/135), and crepitus and light swelling were seen. NMR examination of the knee joint showed degeneration and advanced active inflammatory processes, while X-ray examination showed a medial gonarthritis with a narrowing of the medial joint space and osteophytic extensions.

Patient I.D. [patient 9 (*3*)] had multiple poikilodermatic atrophic sklerodermiformic areas of the skin with teleangiectasias on his back. He had 11 white maculae of a diameter of about 2.5 cm with fine lamellar desquamation on his chest, abdomen and back (Fig. 1B) and an ulcer of 2.2 cm × 2 cm in the area of the lower thoracic back. Contrast NMR examination of the cerebral, thoracic and lumbar spine revealed discrete subcutaneous indurations in the area of the previous ulcers. Two ulcers, one on the back and one in the right gluteal region, and one ulcer on the left shoulder had been treated surgically in 1997 and 1998, respectively.

Patients I.D. and B.Z. both received treatment for their persistent, potentially precancerous radiation-induced skin lesions. All ulcers were completely removed. Normal skin grafts successfully covered the defects and healed without any disturbances, suggesting an *in sano* removal of radiation-damaged skin.

Andrological Parameters

Immediately after the radiation accident, azoospermia was diagnosed in both patients (*3*). Six years after the accidental radiation exposure, analysis of ejaculate revealed normal concentrations, motility and morphology of spermatozoa (data not shown). Sonography and analysis of the hormones in the sera demonstrated normal endocrinological parameters (testosterone, LH, FSH, SHBG, prolactin, estradiol, DHEA-S, PSA and TSH) (data not shown). There

were no pathological microbiological findings. Taken together, these data indicate that radiation-induced impairment of spermatogenesis underwent complete recovery during the 6 years after exposure. These results are in agreement with the finding of Rowley *et al.* that a dose of not more than 4.5 Gy to the testis can lead to a reversible azoospermia (*11*).

Hematological Status

Analysis of blood cells revealed concentrations in the normal range, reminiscent of the findings in 1997 (*3*). Bone marrow smears from both patients showed normal maturation of all cell lineages of erythropoiesis, granulocytopoiesis and megakaryocytopoiesis. However, close inspection of the morphology of numerous bone marrow cells of all three cell lineages indicated the persistence of mitotically abnormal cells such as binucleated cells and anaphase bridges (Fig. 2A) that were reminiscent of the findings in 1997. Such abnormalities have also been observed in other radiation accident victims and the atomic bomb survivors in Japan (*9, 12*) and are indicative of the persistence of genomically damaged hematopoietic progenitor cells in the bone marrow of the radiation victims 6 years after the exposure. Moreover, these findings also indicate that there is a persisting mal-differentiation within the hematopoetic system of the two patients, who thus were continuously at risk for the development of leukemia.

Immunological Status

Two-color immunofluorescence flow cytometry in the two patients and two controls revealed normal levels of lymphocytes, monocytes and granulocytes as well as normal percentages of antibody-stained T lymphocytes, B lymphocytes and NK cells (not shown). However, the ratios of the percentage of $CD4^+$ T-helper cells and the percentages of $CD8^+$ T-suppressor cells were significantly reduced in both radiation victims compared to the controls (Table 1), which could represent a persistent radiation-related effect since it has also been noted in A-bomb survivors (*13*).

The analysis of adhesion molecule expression on gated granulocytes revealed an elevated expression of ICAM1 (B.Z. 3.7-fold, I.D. fivefold) and β1-integrin (B.Z. 3.4-fold, I.D. 4.3-fold) compared to nonexposed controls (Table 1). These data suggest increased inflammatory activity in both patients 6 years after the radiation accident, which may indicate long-term radiation-induced effects at the level of the immune system.

Unstable Chromosome Aberrations 6 Years after Exposure

Analysis of the frequency of unstable chromosome aberrations (dicentrics, rings and fragments), done as described (*4*), revealed that the frequencies of dicentrics in patient I.D.'s lymphocytes were significantly elevated above control; this was not the case in patient B.Z. (Table 2). Dose estimation based on the hypothesis of a protracted irradiation led to an estimated integrated whole-body dose for patient I.D. of about 3.7 Gy, which mirrors the values arrived at previously (*3*). The decline of unstable chromosome aberrations in B.Z.'s lymphocytes was not unexpected 6 years after a protracted inhomogeneous partial-body exposure, which precluded a dose estimation.

FISH Painting Dosimetry

To overcome the decline of unstable chromosome aberrations in B.Z., we performed multicolor FISH painting with target chromosomes 1, 4 and 12 and simultaneous staining of all centromeres in metaphase preparations from both patients (Fig. 2B), a technique that has the potential to reveal exposures even years after irradiation (*14–16*). The combined frequencies of reciprocal and one-way translocations were used to estimate dose by reference to an *in vitro* calibration curve obtained with ^{137}Cs γ rays at 0.5 Gy/min (*17, 18*). Scoring of the painted genome fraction in 206 metaphases of I.D. revealed six translocations and seven dicentrics, with the ratio suggesting a concomitant decline of dicentrics and translocations. This assumption was prompted by the observed overdispersion for the common distribution of dicentrics and translocations (Rao's u test, $u = 3.66$, $P < 0.01$) and could reflect the decline after a whole-body dose or a partial-body irradiation, with the latter scenario being more likely for both patients (*3*). Calculation of aberration frequencies based on a 6-year decline was performed according to a bi-exponential model (*17*) and revealed a mean whole-body dose of 3.2 Gy for I.D., which is similar to the dicentric data for I.D. (Table 2).

FISH evaluation of the painted genome fraction in 190 metaphases of B.Z. revealed seven translocations and one dicentric. Assuming a protracted irradiation, suggesting that there was a decline in dicentric frequency compared to the more stable translocation rate, leads to a reconstructed acute protracted whole-body dose of 3.7 Gy (Table 2). Compared to the whole-body integrated dose of 0.6 Gy estimated in 1997 (*3*), the present retrospective translocation analysis suggests that B.Z. likely received a higher dose than initially estimated by dicentric analysis. Given similar general symptoms and the presence of aberrant mitotic cells in both patients, and a bone marrow edema in B.Z. in 1997 (*3*), it is possible that the dose reconstructed by translocation analysis is the more likely one. Our findings agree with the view that FISH painting is more appropriate method for dose reconstruction in cases of protracted exposure (*19*) and should thus be applied when this type of exposure has occurred.

Lymphocytes of the Georgian Patients Exhibit Normal DNA Repair Capacity

To see whether previous protracted radiation exposure left an imprint in the DNA repair response and capacity in the patients' lymphocytes, we measured short-term DNA

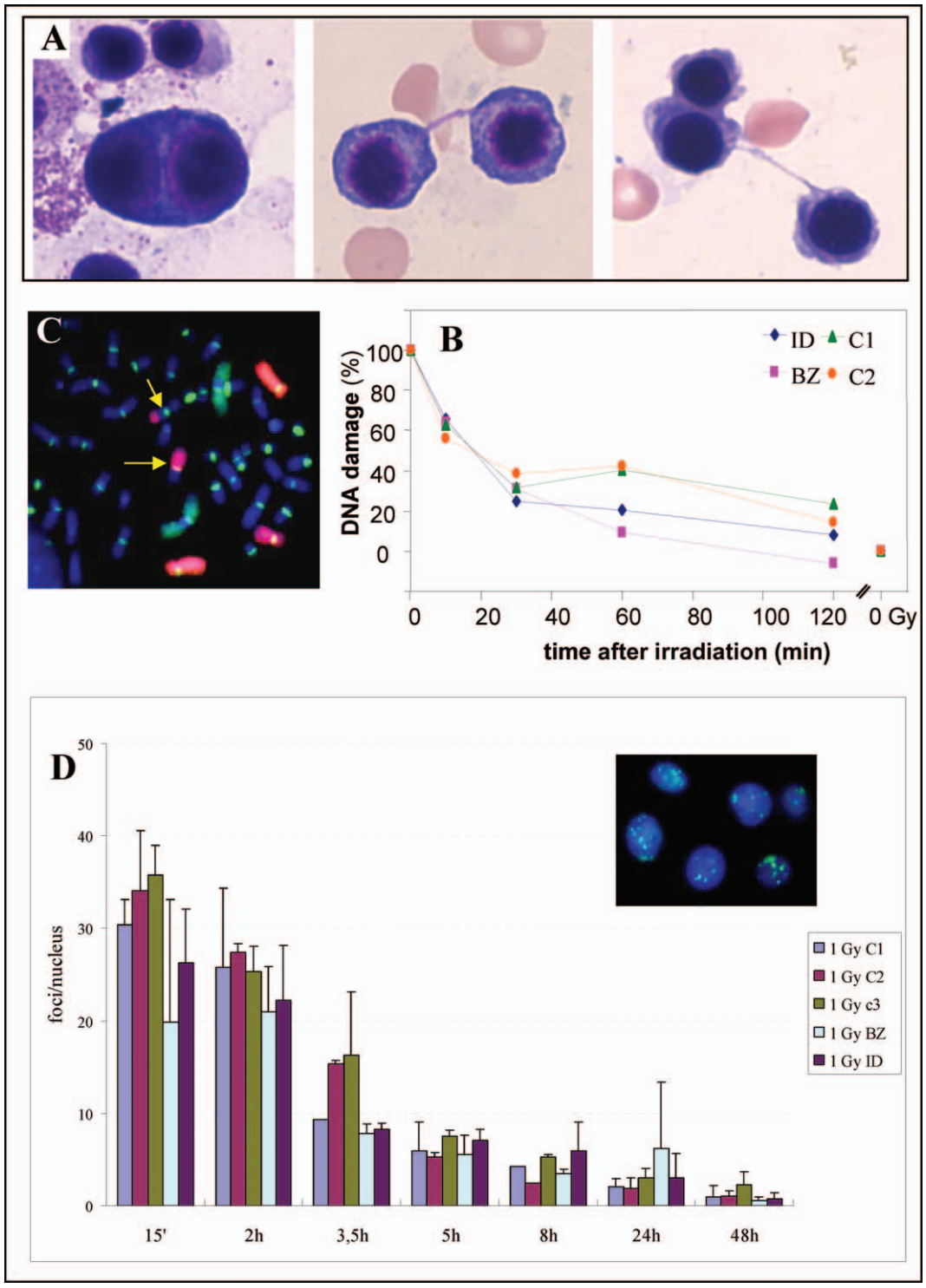

FIG. 2. Panel A: Abnormal cells observed in the bone marrow smears of both patients. Left, binucleated cell; center and right detail, anaphase and cytoplasmic bridges in erythropoietic precursor cells. Panel B: Comet assay shows similar short-term repair kinetics in peripheral blood lymphocytes of patients B.Z. and I.D. and controls, expressed as percentage tail DNA damage. Panel C: FISH painting analysis for chromosomes 1 (green), 4 (orange) and 12 (red). Metaphase spread with reciprocal translocation involving chromosome 12 (arrows). Centromeres are labeled with FITC (green), and chromosomes are counterstained with DAPI (blue). 0′ 0 Gy, DNA damage in blood cells before irradiation. Panel D: Radiation-induced γ-H2AX foci in lymphoblastoid cell lines of patients I.D. and B.Z. and controls (C1–3). Mean focus number per cell was determined at the indicated times after irradiation of lymphoblastoid cell cultures. Means and SD of at least three experiments are shown. The responses of lymphoblastoid cells of the Georgian patients were similar to those of control lymphoblastoid cells after X irradiation. Similar observations were made after 0.2 Gy X irradiation (data not shown).

TABLE 1
Mean Channel Fluorescence Flow Cytometry of Adhesion Molecules

	B.Z.[a]	I.D.	Control 1	Control 2
ICAM 1	34.57 (3.7-fold)[a]	46.61 (5-fold)	9.02	9.64 (mean: 9.33)
CD29	69.20 (3.4-fold)	88.20 (4.3-fold)	19.67	21.59 (mean: 20.63)
CD8/CD3 flow cytometry				
$CD4^+$ T-helper/suppressor lymphocytes	0.52	0.81	2.18	1.84

[a] Fold increase relative to control mean.

repair by alkaline single-cell gel electrophoresis (comet assay) (*6*). Peripheral blood lymphocytes of the two Georgian patients and of two volunteers without radiation history were irradiated *ex vivo* with 4 Gy of 240 kV X rays and subjected to the comet assay in triplicate. Analyses of tail DNA formation and repair in lymphocytes of patients I.D. and B.Z. showed similar repair capacities relative to the two controls. Likewise, repair kinetics were similar at different times (0, 10, 30, 60 and 120 min) after *in vitro* irradiation (Fig. 2C).

Next we determined the capacity for repair of radiation-induced double-strand breaks (DSBs) in B-lymphocyte cell lines derived from the patients' blood by the γ-H2AX focus assay (*7*) after *in vitro* X irradiation (240 kV, 1 Gy/min) (Fig. 2C). γ-H2AX focus formation and disappearance have been shown to correlate with the progress of DSB repair (*7, 20*). In the patients' lymphoblastoid cell lines, the induction and disappearance of γ-H2AX and 53BP1 foci were similar to that of the three control lines of matched sex (Fig. 2D and data not shown). These results agree with those of the comet assay and suggest that a previous radiation exposure left no imprint on the repair capacity and DSB damage response, at least in the cell lines derived from the exposed individuals.

Gene Expression Analysis

To test for potential effects of previous radiation exposure at the gene expression level, a set of genes known to be modulated by ionizing radiation [(*10, 21*), M. Abend, unpublished observations] was measured by replicate Q-PCR in lymphocytes isolated from peripheral blood of the two patients and three healthy individuals without radiation exposure. The genes monitored belong to four functional groups: (1) cell death (FAS-ligand, TNF-ligand, Clusterin, *BCL2* and *BAX*), (2) repair/proliferation (*TP53,* p21, *PCNA, GADD45A* and DNA-PK), (3) signal transduction (*RAB2, PKCI1, ISGF3G* and *NFKB*), and (4) metabolism (*MRP8, GLUT1* and *PSME3*). It was found that 12 of the 17 monitored genes were down-regulated in patient B.Z. and nearly all monitored genes showed reduced expression in I.D.'s blood cells, with down-regulation in most cases being two- to fivefold relative to the mean of the expression from the cells of three control individuals (Fig. 3). Monitoring of late radiation-induced effects by Q-PCR offers a novel approach that must be validated in bigger cohorts of radiation accident victims. It should be noted that the current study monitored only two DNA repair genes, which, given the large number of genes involved in DNA repair processes,

TABLE 2
Cytogenetic Findings

	B.Z.	I.D.
Dose estimates based on the hypothesis of a protracted irradiation based on dicentric analysis		
Dicentrics/centric rings per cell	0.001	0.0077
Acute whole-body equivalent dose (Gy)	—[a]	0.78
95% confidence interval	—[a]	0.38–1.2
Protracted mean dose (Gy)	—[a]	3.7
Estimated dose (1997, dicentric analysis)[b]	0.6	4.1
FISH painting dose reconstruction (based on the hypothesis of a protracted irradiation)		
Translocations/dicentrics	7/1	6/7
Acute whole-body equivalent dose by FISH (Gy)	1.3	1.1
95% confidence interval	0.7–2.1	0.5–1.9
Estimated dose (Gy)	3.7[c]	3.2[d]

[a] Decline of unstable chromosome aberrations in B.Z.'s lymphocytes prevented dose reconstruction.
[b] Dose estimate based on dicentrics and rings (*3*).
[c] Protracted dose (based on translocations using the linear coefficient of the calibration curve only).
[d] Acute reconstructed dose (based on dicentrics).

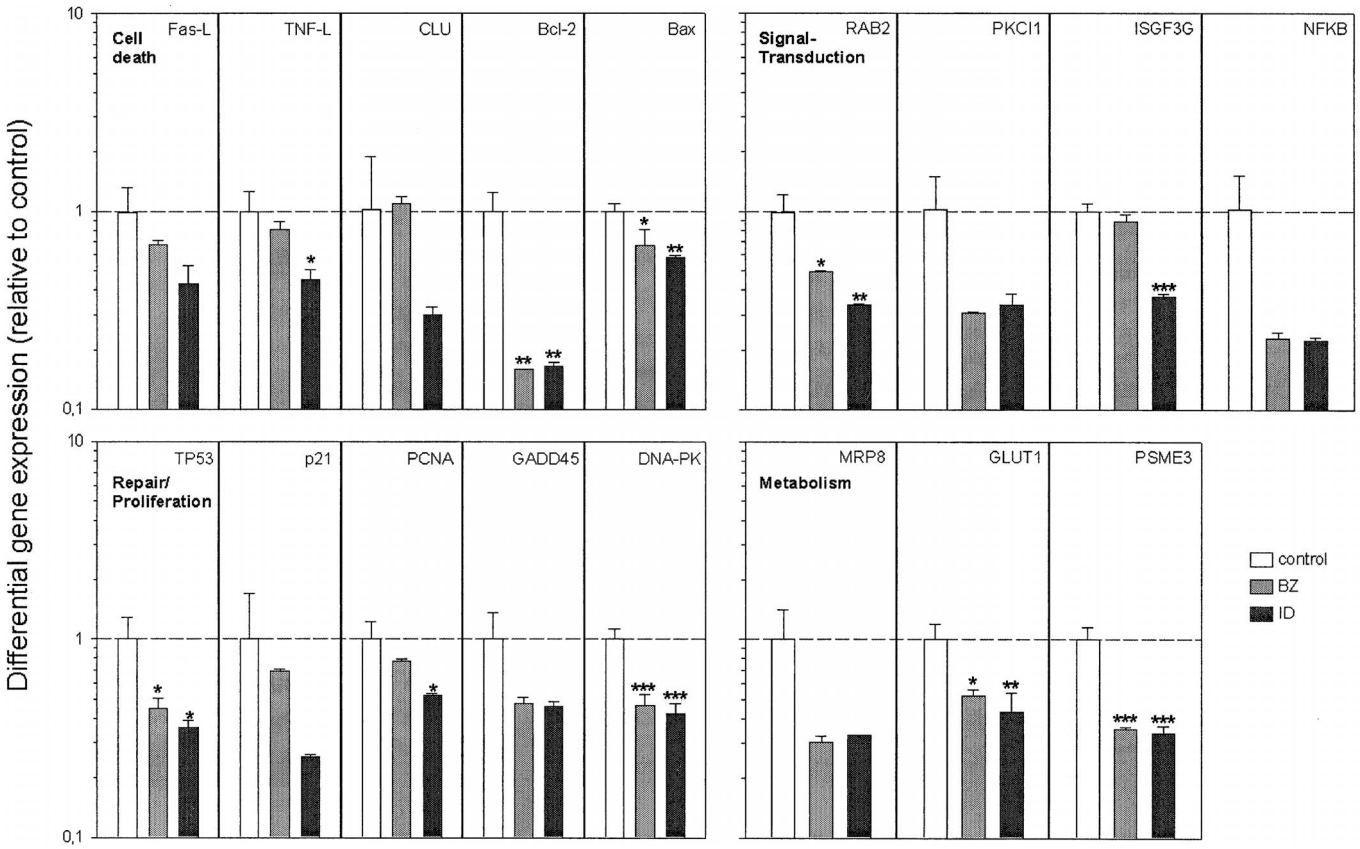

FIG. 3. Differential gene expression as measured by Q-PCR after *in vitro* X irradiation of lymphocytes. Bar graphs represent the mean differential expression of the indicated genes in patients B.Z. (gray bars), I.D. (dark gray bars) and a control (white bars). Error bars for the patients represent the maximum values of three replicate experiments. Error bars for the controls are the upper SEM of three healthy individuals (measurements done in duplicate) and SD of three replicate experiments for the two patients. Significance levels between control and patient values at $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

precludes speculation on an interrelationship of expression levels and repair efficiency. DNA repair, according to the comet and γ-H2AX assay data, occurred normally in the patients' blood cells.

Conclusions

Individuals accidentally exposed to ionizing radiation are often severely affected and are at high risk for the development of late effects, including cancer. The medical follow-up of such patients therefore is still a challenge, since up to now there is no accepted international standard. The present follow-up analysis of a chronic radiation syndrome in two patients 6 years after radiation exposure in the Lilo radiation accident illustrates a potential follow-up strategy using a combination of established and novel methods for the analysis of radiation-induced late effects at both the clinical and the molecular level.

We noted increased adhesion molecule (ICAM1 and β1-integrin) expression on gated granulocytes in both patients as well as an increased ratio of $CD4^+$ T-helper to $CD8^+$ T-suppressor cells, which together may indicate chronic radiation-induced inflammatory processes at the level of the immune system. These observations are in agreement with a reduction of the $CD4^+$ T-cell population in A-bomb survivors even years after exposure (*13*).

In contrast, andrological parameters and blood cell counts were normal, suggesting that a highly localized, protracted irradiation did not deplete the stem cells that contributed to recovery of these highly proliferative tissues. The relatively modest effects on the bone marrow, despite the clinical signs of a cutaneous syndrome, are typical for an accidental event involving inhomogeneous partial-body exposure. However, the hematopoetic and immune systems of the two patients display persisting genomic injury, as reflected by anaphase and chromatin bridges as well as high translocation rates in peripheral blood lymphocytes that are characteristic of previous ionizing radiation injury. Chromatin bridges are also known from the bone marrow of other radiation accident victims (*9*). For instance, cytological bone marrow markers were still present 3.5 years after exposure in the Oak Ridge accident of 1958, and stable and unstable lymphocyte markers were seen 8 years after exposure in the Chernobyl accident (*22*). The Lilo accident follow-up again indicates that the bone marrow is a long-lasting sensitive indicator of radiation exposure, although it may not disclose the whole clinical image of organ involvement after protracted partial-body exposure.

Our observations suggest that these patients could be at risk to develop neoplasms. Thus it will be important to follow them further. The anomalies detected in bone marrow cells of the two patients correlate with persistent translocation rates that suggest a dose estimate of 3.2 Gy for I.D., being reminiscent of that of 1997, while translocation rates in B.Z.'s lymphocytes now suggest a protracted dose of about 3.7 Gy, which is at odds with the initial dicentric analysis (*3*). This discrepancy may be explained by a localized and protracted irradiation that occurred over several months and induced stable aberrations in blood stem cells, which were not subject to selection. Additionally, it is possible that there was a longer time between the last exposure and the dicentric analysis for B.Z., and that a postirradiation decline in the rates of dicentrics in B.Z.'s peripheral blood lymphocytes may have led to an initial underestimation of the dose received. Alternatively, I.D.'s elevated levels of dicentrics could have been induced by a later genomic insult; however, to our knowledge, there is no indication of such an insult. It will thus be interesting to repeat this biomonitoring in a future investigation. In any case, our findings indicate, in agreement with previous suggestions (*17*), that retrospective biomonitoring should include, in addition to an assay of dicentrics, a translocation analysis if a protracted, inhomogeneous partial-body exposure is suspected.

The DNA repair capacities of the patients' lymphocytes and lymphocyte cell lines derived thereof were not compromised, suggesting that the exposure did not alter the repair capacity of the cell types examined. Gene expression analysis, on the other hand, indicates that radiation exposure may have led to an altered expression response in their lymphocytes, possibly representing a kind of genomic scar. Whether this extends to a larger set of genes or can be traced back to other circumstances remains to be determined. Although our findings need validation in a larger cohort of patients, preferably with controls from the same geographic region, the results of the long-term follow-up presented here nevertheless reveal end points that are still detectable years after the accident and that therefore may serve as indicators of a chronic radiation syndrome. A minimal version of a standardized follow-up protocol may contain the evaluation of CD54 and CD29 adhesion molecule expression and determination of $CD4^+$ T-helper to $CD8^+$ T-suppressor cell ratios in combination with dicentric/translocation analysis. Investigations in larger cohorts may also increase the understanding of the pathogenesis of radiation-induced late effects.

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