Anti-CD38 prevents the development of the adaptive response induced by X-rays in human lymphocytes

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Introduction

Adaptive response induced in human lymphocytes by DNA damaging agents (for review, see Wolff, 1992; Wojcik and Streffer, 1994) can be revealed by examination of survival, chromosome damage or mutation frequency. Pre-irradiated or pre-treated (adapted) cells exhibit higher survival and lower frequencies of chromosomal aberrations or mutations than the non-adapted ones upon receiving a subsequent higher (challenge) dose of the same or different DNA-damaging agent. The adapting dose usually is very low, e.g. 1 cGy of X-rays, and by itself inflicts only negligibly small damage.

Our previous studies on the adaptive response of human lymphocytes have been used to test a working hypothesis taken from Weichselbaum et al. (1991); the signalling loop model. It has been assumed that upon treatment with a DNA-damaging agent an alarm signal is generated in the nucleus. This signal is further sent to the cytosol and returned to the nucleus through an as yet undefined pathway involving calcium ions (Wojewódzka et al., 1994). Activation of a set of genes follows, that leads to de novo protein synthesis. Some of these proteins are involved in repair of the damage inflicted by the challenge dose; hence, a lower biological effect of the dose than that in the non-adapted cells.

In this work we used X-rays as the DNA damaging agent that induced the adaptive response and we applied anti-CD38 antibody as an inhibitor of ADP-ribose cyclase (Okamoto et al., 1995), to check whether cyclic ADP-ribose (cADPR) is involved in the expression of the adaptive response. The work was carried out on human T-lymphocytes (stimulated by phytohaemagglutinin) and consisted of the following steps: (i) examination of the adaptive response with the use of micronuclei frequency as the end-point; (ii) testing the effect of anti-CD38 antibody on the adaptive response; and (iii) examination of the rate of DNA repair in the adapted and non-adapted (± anti-CD38) cells after the challenge dose.

Materials and methods

Lymphocyte culture

Human whole blood was obtained by venipuncture from two healthy male donors, non-smokers, aged ~30 years. The donors were selected from the group of donors previously tested for the presence of adaptive response. X-irradiation induced a similar adaptive response in lymphocytes from these two donors. The blood was drawn into a syringe containing 1 U of heparin per 1 ml of blood and added into Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma, St Louis, MO) supplemented with 20% newborn calf serum (Calbiochem, La Jolla, CA) or fetal calf serum (Hungarpool). Stimulation was carried out by adding phytohaemagglutinin P (PHA P, Difco) to the final concentration of 0.5 μg/ml (time zero). To evoke the adaptive response, the cultures were X-irradiated (1 cGy) 6 h after PHA stimulation. Anti-CD38 antibody (CD38 Leu™-17; Becton Dickinson, Sunnyvale, CA) was given 1 h before irradiation and was present till the end of culture. In experiments with micronucleus frequency as the end-point we followed the experimental schedule described by Wojewódzka et al. (1994). Microscopic preparations were made and stained according to Penech and Morley (1985). Experiments were repeated three times.

Irradiation conditions

X-irradiation was performed using a Stabilipan-250 machine, Siemens, Erlangen, Germany. Conditions of irradiation were: 180 kV, 18 mA, 1 mm copper filter and dose rate of 1.28 Gy/min. Irradiations were performed at 37°C (adaptive dose) or at 0°C (challenge dose). For 1 cGy irradiation, the dose-rate was lowered to 0.32 Gy/min by changing irradiation conditions to 140 kV, 13 mA (filter unchanged).

'Comet' assay

The initial DNA damage and its repair rate were determined by the alkaline version of the 'comet' assay, performed as described by Singh et al. (1988) with modifications by Green et al. (1992). Image analysis of data was by the Comet v.3.0 (Kinetic Imaging Ltd, Liverpool, UK). All steps of the 'comet' assay preceding electrophoresis were performed on ice to prevent repair. Electrophoresis was carried out at 10°C; 50 cells were scored per experimental point and experiments repeated two or three times. Statistical evaluation and curve fitting for repair kinetics was done with Statistica (Statsoft, Tulsa, OK) software. The measure of damage was tail moment, that is, percentage DNA in the 'comet's tail multiplied by the tail's length.

Results

Examination of the X-ray-induced adaptive response was carried out with the frequency of micronuclei as the criterion of the response. The measure of the response was the percentage of the response expected: if it was <100% the interpretation of the results was that the adaptive response was induced in consequence of the treatment applied. As shown in Figure 1, there was a pronounced sparing effect of the initial X-ray dose on the subsequent cellular response to X-irradiation: the micronuclei frequency observed was ~30% lower than that...
the Mann-Whitney and Kolmogorov-Smirnov tests. There were no differences in the distributions and mean DNA damage levels, with the exception of the pair: control -/control + 1 cGy. Figure 4 shows that treatment with anti-CD38 does not alter the repair rate in adapted lymphocytes; nevertheless, the mean DNA damage level is significantly higher in the lymphocytes incubated with anti-CD38 at all time points.

**Discussion**

X-rays induced a similar adaptive response in lymphocytes from the two donors, i.e., ~70% of the expected micronuclei frequency (results pooled). This result was in agreement with those reported previously (reviewed in Wojcik and Streffer, 1994). The examined lymphocytes were able, therefore, to develop the adaptive response; treatment with anti-CD38 antibody concomitant with the adapting dose prevented the adaptation (Figure 1).

The relationship of the initial DNA damage and its repair rate to adaptation identified at the chromosomal level is less obvious. The kinetics of DNA repair, shown in Figures 2 and 4 are very similar. Accordingly, parameters of the equation describing DNA repair (Table I) do not significantly differ; so, adaptation of lymphocytes, seen as reduced micronucleus frequency, is not accompanied by an increased rate in DNA repair. Zhou et al. (1992) described an increased rate of rejoining of DNA strand breaks determined electrophoretically at pH 8. A study by T.Ikushima (personal communication) with the use of the comet assay, similar to ours, but with electrophoresis at neutral pH, has also confirmed the importance of repair rate in recovery from effects of X-irradiation. It is generally assumed that delay in strand break rejoining enables damage interaction, leading to illegitimate recombination and chromosome damage (Schwartz et al., 1988; Dikomey and Jung 1993). In contrast, faster rejoining would increase repair fidelity, leading to a lower chromosomal damage and hence, to a diminished lethal effect. On the other hand, Wojcik et al. (1996) failed to observe a stringent correlation of increased repair rate with decreased chromosomal aberration frequency (or vice versa) and our data are consistent with their results.

On the contrary, as summarized in Table II, there is a statistically significant difference between adapted and non-adapted lymphocytes in the initial DNA damage; anti-CD38 treatment, in itself not affecting the damage level (Figure 2), abolishes this difference. As seen in Figure 4, the treatment leaves unaltered the kinetics of DNA repair in adapted lymphocytes (Table I).

The discrepancy between the micronuclei data showing the adaptation (Figure 1) and the DNA repair data (Figures 3 and 4) may have two reasons. First, with the DNA repair rate unaltered, the fidelity of repair may vary, leading to a different proportion of damage seen at the chromosomal level. The lowered mutation frequency (i.e. increased repair fidelity) in adapted cells (reviewed in Wójcik and Streffer, 1994) points to this possibility. Secondly, cellular radiosensitivity seems to be connected with the cell’s ability to convert DNA double strand breaks into chromatin breaks (Bryant and Liu, 1994); as discussed by Cornforth and Bedford (1994) only 10–15% of breaks give rise to aberrations, possibly those breaks that are placed in the transcriptionally active chromatin. Adaptation expected for an additive effect of both X-ray doses given separately. Treatment with anti-CD38 antibody prevented the induction of the adaptive response (Figure 1). The antibody treatment did not change the growth of unirradiated lymphocytes, as judged from the mitotic index, measured at 72 h after PHA stimulation, and from the index of binucleated cells estimated at 72 h. It did not modify the response of non-adapted lymphocytes to the challenge dose.

DNA repair in non-adapted, untreated or anti-CD38 treated lymphocytes after irradiation with the challenge dose is shown in Figure 2 as best fit to the equation usually used to describe the kinetics of DNA repair. There is no alteration in the repair rate in treated versus untreated cells, as reflected in lack of a statistically significant difference in parameter b of the equation to which the data were fitted (Figure 2 and Table I). Although the equation applied is the most often used one for the description of repair kinetics, it is also possible to compare the damage level at specific time points. We compared the mean DNA damage at selected time intervals. For time zero samples statistical significance of differences between tail moment distributions was calculated by Student’s t-test with correction for variance estimation; for other samples we used the Mann-Whitney and Kolmogorov-Smirnov tests. There were no differences in the mean DNA damage at time zero and 15 min between anti-CD38-treated and untreated cells. At other time points the differences in the mean DNA damage level were statistically significant.

Figure 3 compares tail moment distribution in adapted and non-adapted lymphocytes. There were no differences in the distributions and mean DNA damage levels, with the exception of the pair: control -/control + 1 cGy. Figure 4 shows that treatment with anti-CD38 does not alter the repair rate in adapted lymphocytes; nevertheless, the mean DNA damage level is significantly higher in the lymphocytes incubated with anti-CD38 at all time points.
Anti-CD38 prevention of X-ray induced adaptive response

Fig. 3. Distribution of tail moments in adapted and nonadapted human lymphocytes irradiated with 1.5 Gy.

may involve, therefore, a qualitative change in damage repair or processing, that would not be reflected by a simple repair test, as used in this work.

Effects of anti-CD38 antibody treatment on the development of adaptation implicated the role of cADPR in this process. As already mentioned, we found that calcium ions are indispensable for adaptation induced by low radiation dose or low concentration of hydrogen peroxide (Wojewódzka et al., 1994). cADPR is a novel second messenger involved in calcium mobilization from intracellular stores (Lee et al., 1989; Lee and Aarhus, 1991). It is synthesized and hydrolyzed by CD38, lymphocyte surface antigen and ubiquitous membrane protein with a dual enzymatic activity, that of ADP-ribose cyclase and hydrolase (Takasawa et al., 1993a,b). Both activities are inhibited by treatment with the antibody (Okamoto et al., 1995). cADPR is active in insulin secretion in pancreatic cells. It has been proposed by Okamoto et al. (1995) that its synthesis declines when DNA is damaged and concomitantly, activated poly(ADP-ribose) polymerase (PARP) uses up NAD+. The latter nucleotide is substrate for both PARP and ADPR cyclase. Due to high activity of PARP the NAD+ pool becomes depleted and, therefore, not enough substrate is left for ADPR cyclase. The lack of cADPR and hence, absence of calcium mobilization, is the ultimate cause of decrease in insulin secretion and leads to diabetes. Accordingly, there is a link between DNA damage and cADPR-induced calcium mobilization, that could be a part of the signal transduced from the cytoplasm to the nucleus and affect the response to the DNA damaging agent. This kind of relationship, however, is improbable in the adaptive response, since the adapting dose, and, in consequence, the damage, are very low; hence, depletion of the NAD+ pool cannot be expected.

Another possibility is that recently described by Kontani et al. (1996); CD38 ligation induces a transmembrane signalling involving tyrosine phosphorylation of several cellular proteins, among them p120 and in HL-60 cells caused to differentiate

Table I. Parameters of the equation $y = a \exp(-bt) + c$, describing DNA repair in the adapted and non-adapted human lymphocytes, untreated or treated with anti-CD38 antibody (CD38) concomitantly with the adapting dose

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$a$ ± SD</th>
<th>$b$ ± SD</th>
<th>$c$ ± D</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) 1.5 Gy X</td>
<td>22.49 ± 7.39</td>
<td>0.1577 ± 0.1077</td>
<td>2.93 ± 1.89</td>
<td>0.9687</td>
</tr>
<tr>
<td>(2) 1 cGy + 1.5 Gy X</td>
<td>24.38 ± 6.03</td>
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<td>0.49 ± 0.45</td>
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<td>(3) CD38 + 1.5 Gy X</td>
<td>19.07 ± 7.42</td>
<td>0.0952 ± 0.0389</td>
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<td>(4) 1 cGy + CD38 + 1.5 Gy X</td>
<td>27.48 ± 5.16</td>
<td>0.0979 ± 0.0372</td>
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Fig. 4. DNA damage repair measured by the 'comet' assay in adapted human lymphocytes, untreated or treated with anti-CD38 antibody concomitantly with the adapting dose. Data computer-fitted (least squares method) to the equation indicated. See Table I for equation parameters and Table II for statistical evaluation. Bars denote SD.

Table II. Two-way analysis of variance (ANOVA) of differences in tail moments between various treatments of human lymphocytes

<table>
<thead>
<tr>
<th>Treatment for adaptation</th>
<th>Control</th>
<th>1.5 Gy (0 min repair)</th>
<th>1.5 Gy (60 min repair)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>3.57</td>
<td>28.99</td>
<td>6.51</td>
</tr>
<tr>
<td>1 cGy</td>
<td>6.73*</td>
<td>31.60*</td>
<td>7.22</td>
</tr>
<tr>
<td>CD38</td>
<td>5.08</td>
<td>28.29</td>
<td>9.22*</td>
</tr>
<tr>
<td>CD38 + 1 cGy</td>
<td>6.26*</td>
<td>35.31</td>
<td>7.83</td>
</tr>
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*Statistically significant; $P < 0.05$. 

Table L Parameters of the equation $y = a \exp(-bt) + c$, describing DNA repair in the adapted and non-adapted human lymphocytes, untreated or treated with anti-CD38 antibody (CD38) concomitantly with the adapting dose

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into granulocytes by retinoic acid. The identity of protein tyrosine kinase that responds to stimulation by CD38 ligation has not been established, nor the mechanism of stimulation. Signalling initiated by CD38 might prevent the development of the adaptive response by a mechanism different from inhibition of cADPR synthesis.

Altogether, the results reported for the X-ray-induced adaptive response are in agreement with the previously published data on hydrogen peroxide or X-ray induction of adaptation to X-rays (Wojewódzka et al., 1994). Also, they are consistent with the idea that damage inflicted by the adapting dose gives rise to an alarm signal, subsequently propagated with the (possible) involvement of ADPR cyclase and calcium ions.

Acknowledgements

The work was partly supported by the statutory grant from the State Committee of Scientific Research (KBN) and Copernicus grant no. CIPA-CT94-0129.

References


Received on April 23, 1996; accepted on July 2, 1996