Purine Metabolizing Enzyme Activities in Radiosensitive Tissues of Mice after Sublethal Whole-body Irradiation

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Abstract. The activities of adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) were determined between days 1—14 in the spleen, thymus and femoral bone marrow of mice subjected to whole-body gama irradiation with a dose of 5.5 Gy. In control animals, the highest activity of ADA (as related to 10^6 cells) was recorded in the thymus (58.9 pmol . s^{-1}), the lowest one in the femur (34.8 pmol . s^{-1}), the PNP activity was the lowest in the thymus (14.5 pmol . s^{-1}) and the highest in the femur (96.0 pmol . s^{-1}). In the spleen, an elevation of ADA activity (up to 379 %) was observed during the first postirradiation days; PNP activity was reduced (to 58 %) on postirradiation day 3, followed by the return and even elevation on day 14 (265 %). In the thymus, a parallel reduction of the activities of both enzymes appeared during the first postirradiation days, with a subsequent increase during the regeneration phase. In the femoral bone marrow, ADA and PNP activities were increased on postirradiation day 1 (275 % and 201 %, respectively). Reference is made to the possible relationship between the observed characteristic changes in activities and the degree of damage and/or renewal of cell population in the hemopoietic tissues after irradiation.

Key words: Adenosine deaminase — Purine nucleoside phosphorylase — Whole-body irradiation — Radiosensitive tissues — Mice

Introduction

The purine metabolizing enzymes, adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP), are involved in nucleic acid catabolism and have been demonstrated to be closely related to the functional state of lymphocytes (Giblett 1985; Barton and Goldschneider 1979). Insufficient activity of these enzymes leads to immunodeficiency states. PNP inhibition is manifested

by a functional defect of T lymphocytes, whereas ADA deficiency causes disturbances in the function of both T and B lymphocytes (Watts 1983). Characteristic changes in both ADA and PNP activities in lymphocytes and peripheral blood erythrocytes have been also found to be associated with defects of lymphocyte proliferation, and both enzymes are being employed to differentiate between various forms of leukemic diseases (van Laarhoven et al. 1983). Significant changes in ADA activity have been observed also in patients suffering from acquired immune deficiency syndrome, in which mainly the function of helper T-cells is disturbed (Murray et al. 1985). Hemopoietic tissues, in which lymphocytes differentiate and mature, are particularly sensitive to the effects of ionizing radiation. Whole-body irradiation with sublethal doses of gamma rays results in a considerable depletion of these tissues which then gradually regenerate in dependence on the renewal of the individual cell populations (Takada et al. 1971). Defects of purine metabolism resulting from interventions in the activities of the enzymes under study may cause impaired renewal of the radiation damaged tissues, as demonstrated with rat thymus after the application of an ADA inhibitor to irradiated animals (Barton 1985). The present work was aimed at investigating the general pattern of changes in ADA and PNP activities after whole-body irradiation of mice with sublethal doses of gamma rays. The enzyme activities in radiosensitive tissues (spleen, thymus and bone marrow) were monitored through day 14 after irradiation, this interval covering the phase of initial damage and initial regeneration.

Materials and Methods

The experiments were performed on hybrid (CBA × C57Bl) F_1 mice aged 12 weeks. The animals were caged in groups of 20, and had free access to food (Larsen diet) and water. The animals were whole-body irradiated with a Chisostat apparatus (Chirana). The dose was 5.5 Gy, at a dose rate 0.52 Gy/min. Spleens, thymi and femora of the irradiated groups were investigated at various intervals after irradiation (on days 1, 3, 7, 10 and 14). The bone marrow was obtained by repeated flushing of femora with Isotone (Coulter), the spleens and the thymi were homogenized with a glass rod through a fine silk sieve. After filtration and washing, the erythrocytes were lysed with a 0.75 % solution of ammonium chloride in 17.5 mmol Tris pH 7.2. After another washing, the cells were lysed by double freezing to −70 °C. The enzyme activities were determined by means of high performance liquid chromatography using a microtechnique. To obtain the enzyme reaction, 100 µl of lysate was incubated (15 min at 37 °C) in a mixture of 0.2 mol/l TRIS buffer pH 7.2 and adenosine or in a mixture of 0.2 mol/l phosphate buffer pH 7.4 and inosine for ADA and/or PNP respectively. The reaction was stoped by boiling, and after the addition of 1 ml mobile phase and subsequent centrifugation, 20 µl aliquots, were applied onto the chromatographic column by means of a syringe loading valve injector (Rheodyne 7125). ADA activity was quantified from the substrate (adenosine) loss, and PNP activity was assessed from the enzyme reaction product (hypoxanthine) determined by means of CI 100 integrator (Laboratory Instruments, Prague). Chromatographic conditions:

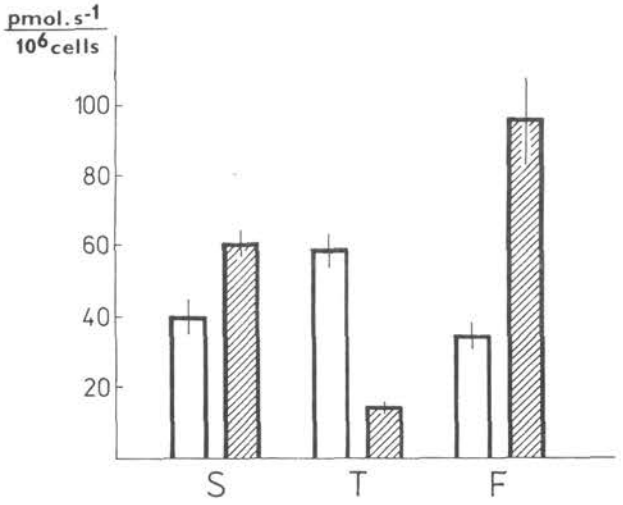

Fig. 1. The activities of ADA (open bars) and PNP (hatched bars) in control animals related to 10^6 cells. S — spleen, T — thymus, F — femoral bone marrow. The vertical lines indicate S. E. M.

high pressure pump HPP 4001, analytical glass column Separon SIX C-18, 5 μm, geometry 3.2 × 75 mm, UV detector LCD-254 nm (Laboratory Instruments, Prague), mobile phase 20 mmol/l potassium dihydrogen phosphate (pH 4.4); 10 % and 4 % methanol was added to the mobile phase to determine the activities of ADA and PNP, respectively. The results shown are means for 8 to 12 animals; reference control values were obtained from 10 to 20 animals. Unless otherwise indicated, the term activity is used to indicate specific activity related to 10^6 cells. Student's t-test was used for statistical processing of the results.

Results

In control, intact animals the activity levels of both enzymes and their ratios were characteristic of the individual hemopoietic organs (Fig. 1). A conspicuously high activity of ADA and a low PNP level (ADA : PNP ratio 4.1) were observed in the thymus; the ratio for the femoral bone marrow was approximately inverse (ADA : PNP = 0.36). The activities of both enzymes in the spleen were less pronounced with the ADA activity being lower than that of PNP (ADA : PNP = 0.66). Monitoring the activities of both enzymes after whole-body irradiation has shown that, in the period of damage and regeneration of radiosensitive tissues, splenic, thymic and femoral bone marrow ADA and PNP activities do not change in parallel (Fig. 2,3). In the spleen, ADA activity was greatly increased during the first days after irradiation (380 %, $p < 0.01$), and

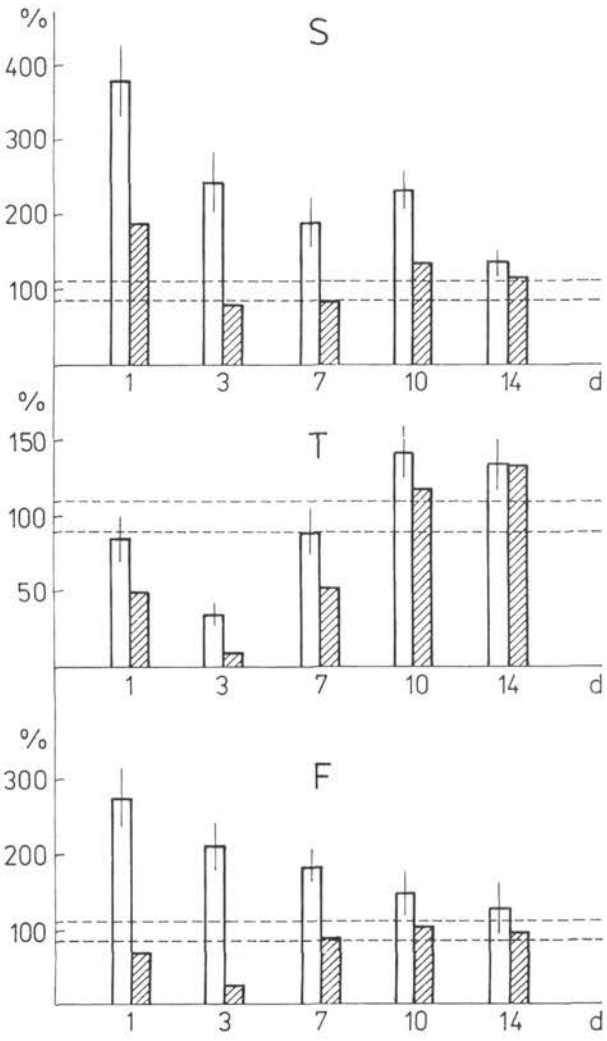

Fig. 2. Changes in ADA activity expressed per 10^6 cells (open bars) and per organ (hatched bars). Percentages of the respective control values, for various days after whole-body irradiation (5.5 Gy). For other symbols see legend to Fig. 1.

gradually returned to the control level. The PNP activity exhibited a drop on day 3 (59 %, $p < 0.05$); between post-irradiation days 7 and 14 it markedly increased reaching up to 265 % of the control level. In the thymus, coincident changes of activities were found for both enzymes: a drop during the first days (minimum values for ADA and PNP on day 3 and 1, $p < 0.01$ and 0.05 respectively) followed by an increase during the regeneration phase and reach-

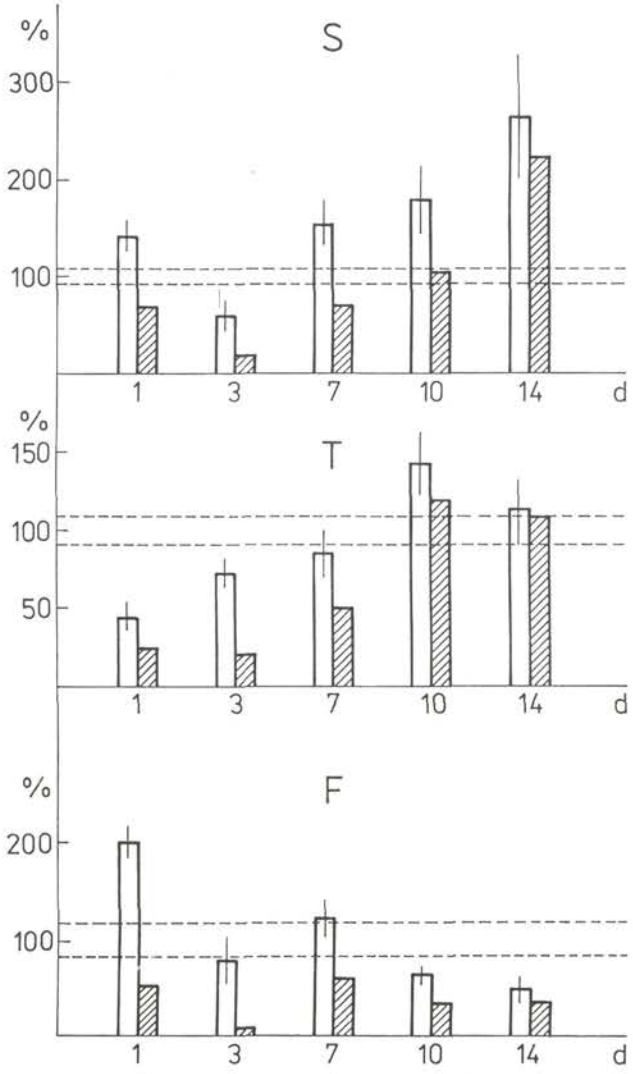

Fig. 3. Changes in PNP activity as related to 10^6 cells (open bars) and to the individual organs (hatched bars). Percentages of the control values, for various day after whole-body irradiation (5.5 Gy). For other symbols see legend to Fig. 1.

ing its maximum on post-irradiation day 10. In the femoral bone marrow, the pattern of ADA activity was similar to that seen in the spleen: an initial increase (275 %, $p < 0.05$) followed by a gradual decrease to the control level; PNP activity was elevated on day 1 (201 %, $p < 0.05$), it subsequently oscillated around the control level until falling to approximately 50 % of the control values on day 14.

Table 1. Changes in the masses of spleen, thymus and in femoral bone marrow cellularity on various days after whole-body irradiation of mice with a dose of 5.5 Gy

Days after irradiation	Spleen	Thymus	Bone marrow cellularity
	mg ± S.E.M.		$n.10^{-6}$ ± S.E.M.
1	39.1 ± 2.2	30.2 ± 3.1	3.6 ± 0.3
3	26.5 ± 1.1	15.4 ± 1.2	1.7 ± 0.2
7	35.4 ± 2.7	31.1 ± 3.7	6.7 ± 0.3
10	46.5 ± 6.4	44.0 ± 2.9	9.6 ± 0.3
14	66.4 ± 5.2	51.0 ± 2.4	10.2 ± 0.3
Controls	78.5 ± 1.2	52.5 ± 3.7	13.5 ± 0.5

Figs. 2 and 3 show values of activities related to 10^6 cells together with the activity calculated for the respective organ as a whole. On post-irradiation day 3, a drop of the total activity of both enzymes was observed in all organs, with the exception of ADA in the spleen. During the regeneration of the organs, changes in total activities roughly paralled those related to 10^6 cells; the total activities were elevated only in the thymus for both enzymes, and in the spleen for PNP. Changes in total mass of spleen and thymus and in femoral bone marrow cellularity (organ mass was employed to estimate total enzymatic activity) are shown in Table 1 for the individual postirradiation intervals.

Discussion

The enzyme activities of ADA and PNP measured in the spleen, thymus and femoral bone marrow of intact (CBA × C57Bl) F_1 mice proved to be organ-specific, not only as far as the individual levels are concerned; but in particular the organ-specifity was suggested by the mutual activity ratios. This finding agrees with the results of other authors obtained for BALB/c mice (Kizaki et al. 1983; Freire-Moar et al. 1984) and for rats (Peters et al. 1982). Sidi et al. (1982) studied the activity ratios of both enzymes in thymus cell subpopulations. High ADA activities and low PNP levels were demonstrated in cortical cells, with smaller differences in medullary cells. An inverse activity ratio was found in mononuclear cells of the peripheral blood (Vertongen et al. 1984) and in T lymphocytes of the spleen (Kizaki et al. 1983). According to Barton and coworkers, the activities of the two enzymes undergo changes during the differentiation of T cells and are mutually reciprocal (Barton and Goldschneider 1979). Almost by an order lower ADA activities than in T lymphocytes were measured

in various lines of B cells including human peripheral lymphocytes (Chechik et al. 1981). Also human erythrocytes exhibited lower ADA activities than did lymphocytes (Vertongen et al. 1984); we also could observe lower PNP and ADA activities in murine erythrocytes (unpublished results). Based on the above results we can suggest that the differences in ADA and PNP activities between the spleen, the thymus and the femoral bone marrow are due to different representation of the various cell populations in the respective tissues, and to different levels of activities of purine enzymes in these populations. The frequently considerable interspecies differences should also be considered (Borgers and Thone 1978; Peters et al. 1982).

The differing time-courses of ADA an PNP activities in the spleen after whole-body irradiation reflect the heterogeneity of the splenic cell populations and the different degree of their damage. The major cell populations, T and B lymphocytes, differ in their radiosensitivities (Anderson 1976). According to some authors, whole-body irradiation with doses similar to that used in our experiments results in an almost complete disappearance of B lymphocytes with a reappearance as late as after day 15, and in a reduction of T lymphocytes to between 30 and 50 % of the controls with a gradual increase only after post-irradiation day 6 (Anderson 1976; Bazin and Platteau 1986). In regard to PNP activity levels comparable for both cell populations (Kizaki et al. 1983), the initial drop of PNP may well be a reflection of the damage caused to the sensitive subpopulations. In addition, the population of splenic T cells is not homogenous in respect of radiosensitivity, either (Kataoka and Sado 1975), just as in respect of the ADA activity level (Shohat et al. 1984). During the postirradiation first days the splenic ADA activity goes on account of cells with high activity levels. These cells seem to be represented by a portion of the surviving cells rather than by newly formed cells, since the proliferative activity in the spleen is very low at this postirradiation period (Pospíšil et al. 1981). Radioresistant spleen cells include also macrophages and plasma cells (Anderson 1976) in addition to a part of the T cell population. During the regeneration period studied by us, intensive renewal and maturation of nuclear cells, especially T lymphocytes, occurs in the spleen, as well as a progress of erythropoiesis, as suggested by the results on incorporation of radioactive iodine and iron (Pospíšil et al. 1981; our own unpublished results) and by morphological studies (Bazin and Platteau 1986). This period is characterized by a marked enhancement of PNP activity along with a gradual reduction of ADA activity.

The very pronounced decrease in both specific and total activities of the two enzymes in the thymus reflects the damage to the radiosensitive thymic population, represented mainly by cortical cells (Anderson 1976). The parallelism of the time-courses of ADA and PNP acitivities agrees well with the fact that the susceptible cortex population represents the major part of the thymus. As

reported by Huiskamp and van Ewijk (1985), regeneration of the thymus after sublethal irradiation with neutrons as well as after X-ray irradiation begins with the null cells which probably represent a radioresistant population in both the cortex and the medulla of the thymus. The levels of activity of purine enzymes in this thymic subpopulation are not known, but ADA and PNP activities in the null cells of human peripheral blood reached approximately half the values measured in T cells (MacDermott et al. 1980).

Enhanced activities of ADA and PNP in the femoral bone marrow on postirradiation day 1 parallel, to a certain degree the activity changes seen in the spleen; these activities go on the account of the surviving radioresistant cell population. As a matter of fact, the renewal of bone marrow cells starts at an earlier postirradiational interval than that in the spleen, with the maximum proliferating activity around day 3 after irradiation and subsequent drop to the control level (Pospíšil et al. 1981). It is open to question how the ADA and PNP activities are influenced by the rapidly dividing pool of stem cells and their differentiation. According to Freire-Moar et al. (1984), e.g. T cell precusors in the femoral bone marrow of BALB/c mice have ADA activity thrice lower than that of non-fractionated bone marrow cells. Moreover, the considerable migration of cells from the bone marrow to the other hemopoietic organs should also be considered. From Figs. 2 and 3 it is evident that no enhancement of ADA or PNP activities occurs through the regeneration period in femoral bone marrow.

A reduction of the purine metabolizing enzyme activities resulting from radiation damage during the first postirradiation days is of a transient nature and distinct from genetically determined ADA or PNP deficiency. Inhibition of purine enzymes affects adversely the postirradiational regeneration of hemopoietic tissues. In this respect the question arises whether ADA and PNP activities can be modified by some chemotherapeutic agents which would then modify postiiradiation damage and renewal.

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