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Phenotypic characteristics of thymic microenvironment in WR-638-protected rats after whole-body irradiation: Macrophages, interdigitating cells and mesenchymal stroma

KEYWORDS: Thymus Gland; Macrophages; Dendritic Cells; Stromal Cells; Whole Body Irradiation; Cystaphos; Rats, Wistar

ABSTRACT

Phenotypic changes of macrophages, interdigitating (ID) cells and mesenchymal stroma of the thymus were studied in male Wistar rats subjected to radioprotector WR-638 (358 mg/kg IP) and/or whole body X-ray irradiation 15 days after their application. They were identified in situ on cryostat thymic sections using immunoperoxidase staining by R-MC 23, 39, 41, 44, 45, ED1, ED2 and OX 39 monoclonal antibodies (mAbs). It was shown that WR-638 protects rat thymus from ionizing radiation effects, preferentially as a result of lymphoid compartment preservation. Radioprotector caused changes in number and phenotypic characteristics of macrophages and ID cells in irradiated rat thymus. It also prominently reduced changes caused by radiation in vascular structures of the thymus. This influence of WR-638 on thymic microenvironment elements probably contributed to its beneficial radioprotective effect on the rat thymus.

INTRODUCTION

The thymus is a central lymphoid organ in which thymocytes proliferate and differentiate into T cells (1,2). This process is a consequence of close interactions between developing thymocytes and thymic microenvironment composed of epithelial cells, macrophages, interdigitating (ID) cells and mesenchymal stroma. Because of the high radiosensitivity of thymocytes this organ undergoes dramatic involution after sublethal X radiation (3,4). However, owing to its regeneration potential the thymus recovers quickly and investigation of this repairing process is a useful approach for studying not only recovery from the effects of radiation but also of the mechanism of thymopoiesis in the normal organ. On the other hand sensitivity of the thymus to the ionizing radiation and anticancer drugs contributes a great deal too serious and clinically significant states of immunodeficiency, which appear after patients subjecting to these therapeutic procedures (5). Therefore, development of drugs, which can be used to provide normal tissue protection against radiation but without compromising its antitumor effect, is highly desirable. Among numerous compounds that were developed and tested aminothiols designated as WR were the most effective class of radioprotectors (6) One of the most effective aminothiol is WR-638 (aminoethylphosphorotioate), which conversely to its congener WR-2721, has been less frequently studied in protection of immune system, including thymus. Our previous investigation has shown that WR-638 accelerated the rat thymic regeneration after whole-body irradiation, due to its beneficial effects on the lymphatic tissue (7,8).
However, since mechanism of WR-638 protective action is still not known enough the aim of this study was to examine its effects on thymus microenvironment changes induced by irradiation, especially macrophages, ID cells and mesenchymal stroma using a panel of monoclonal antibodies (mAbs).

MATERIALS AND METHODS

Experiments were performed on male Wistar rats, 6 to 8 weeks old, bred at the Farm for Experimental Animals, Military Medical Academy. Irradiation procedure has been described elsewhere (8). Briefly, rats were divided in 4 groups(group I - sham-irradiated animals (controls); group II - whole body irradiated animals by 8 MeV X-rays at a dose of 3.5 Gy using linear accelerator (SL 75-20, Philips); group III - irradiated animals protected with WR-638 (358 mg/kg BW, IP, 30 min before irradiation); and group IV - sham-irradiated ones treated with WR-638 in the same way. The controls were treated with saline (1ml/kg ip IP). Rats were sacrificed 2, 4, 8 and 14 days after treatment. Phenotypic changes of macrophages, ID cells and mesenchymal stroma were identified in situ on cryostat thymic sections using immunoperoxidase staining procedure described elsewhere (9). Panel of R-MC mAbs (R-MC 23, 39, 41, 44, 45), ED series of mAbs (ED1 and ED2) with immunoreactivity previously described (9,10,11) and OX39 mAb, anti interleukin 2 receptor (IL-2R) mAb, commercially obtained from Serotec, were used.

RESULTS AND DISCUSSION

In our previous experiments it was shown that X(ray irradiation (3.5 Gy) caused cyclic changes in rat thymic cellularity manifested as(primary involution (until day 2), primary regeneration (from days 2 to 14), secondary involution (from days 14 to 21) and secondary regeneration (from days 21 to 30) (8). They were not only the consequences of destruction and regeneration of thymic lymphoid compartment but also of its microenvironmental change (12). We have also shown that WR(638 reduced the magnitude of thymocyte depletion in the primary involutive phase primarily as a result of cortical thymocyte protection, while the increased proliferation and differentiation of CD4-CD8- and CD4+CD8+ thymocytes has led to faster primary regeneration (7). However, the effects of this protector on irradiated rat thymus microenvironment were not investigated at all.
In the primary involutive phase using mAbs, which detect distinct subpopulations of macrophages it was shown that their overall number was reduced in WR-638-treated rats comparing to only-irradiated ones, although they were still more numerous than in control. Staining with R-MC 39+, R-MC 41+, R-MC 45+ and ED2+ mAbs specific for cortical and cortico-medullary zone (CMZ) macrophages revealed that in both WR-638-protected and non-protected animals these cells were large, but almost without processes extending between surrounding thymocytes what can be seen in the normal rat thymus. Expression of antigens detected with all mentioned mAbs were prominently increased on cortical macrophages of both treated groups comparing to controls. However, number of cortical and CMZ macrophages were prominently decreased in WR-638-protected rats comparing to only-irradiated animals. On the other hand, staining with ED1+, R-MC 44+ and R-MC 45+ mAbs has shown showed slight increase in the number of medullary macrophages in protected rats comparing to non-protected ones. mAb OX39

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is reactive with small number of thymocytes, as well as with medullary ID cells in the rat. Staining with this mAb revealed that number of ID cells was also reduced in protected rats comparing to only-irradiated animals. R-MC 23 mAb defines rat fibrous stroma (capsula, septa and vessels) and Colic at al. (9) have supposed that it detects collagen or molecules associated with it. More intensive staining with this mAb in all parts of the thymus of irradiated rats comparing to controls was observed. Dilated blood vessels of CMZ and medulla were also observed in these animals. All these changes were less prominent in WR-638-protected rats.

In the early primary regenerative phase number of macrophages was still significantly increased in irradiated animals, especially in the cortex, comparing to controls. This was showed by using R-MC 39, R-MC 41, ED1 and ED2 mAbs. Macrophages in the cortex are were numerous, often in the groups with still predominantly rounded shape and more prominent membrane (ED2, R-MC 39, R-MC 41) and citoplasmatic (ED1) staining then in controls. With progressive regeneration of the thymus their number are decreasing, and macrophages more and more look like on cells in intact animals. In WR-638-protected rats the number of macrophages, both in cortex and medulla, was decreased comparing to only-irradiated animals, especially in the early regenerative phase. Increased number of ID cells, as well as increased expression of antigens on them (detected by OX 39 mAb) still existed in this phase both in only-irradiated animals as well as in protected ones. Staining with R-MC 23 mAb has shown that thymus of irradiated rats becomes strongly vascularized, especially the cortex, comparing to the same animals in the primary involutive phase. This finding was less prominent in protected animals.

The results presented here clearly show that whole body X-ray irradiation, although non-lethal, has also affected thymic stromal architecture in accordance with some previous results (13). Namely, since the normal function of the macrophages in the cortex is probably phagocytosis of thymocytes after apoptosis (2) its increased activity after irradiation can be explained by massive destruction of lymphoid cells caused by X-rays (13). Increased number of these cells, especially in the cortex, during the phase of primary involution, can be explained by increased migration of macrophages to the thymus, already shown in conditions such as application of some anticancer drugs (14). Previous report proposed that rapid and massive apoptosis after radiation may induce accumulation of neutrophils, ultimately causing inflammation, and may induce the production of macrophage chemotactic factors (13). Anyway, changed morphology and antigens expression as well as increased phagocytic activity of macrophages point to their increased activity, probably as a result both of direct X-rays effect on their cellular membranes, as well as indirectly, by stimulation of phagocytosis. Extremely strong antigen expression detected by ED1 and ED2 mAbs on cortical macrophages, as well as high frequency of these cells in irradiated rats comparing to controls were also showed by other authors (15). They supposed that these changes in antigens expression and abundance of macrophages precede the occurrence of thymocyte repopulation, and that they not only took part in phagocytosis but also could promote T-cell differentiation. This was actually showed for ED2(macrophage (15). Increased number of ID cells in CMZ and medulla can also be explained by its increased phagocytic activity in irradiated thymus as already showed (13), although in normal condition the majority of these cells lack phagolysosomes or other cytoplasmic organelles typical of phagocytic cells (2). Reduced number of cortical and CMZ macrophages in WR-638-protected rats could be explained by less prominent lymphoid destruction in these animals, and therefore by less prominent migration and activation of them in situ. Staining changes with R-MC 23 mAb in the thymus of irradiated rats comparing to control indicate some changes in phenotypic characteristics of its fibrous stroma, as well as dilatation of blood vessels in CMZ and medulla. Other authors found that the density of blood vessels increases throughout the mice thymus in the primary involution phase (13). According to them it was due to reduction of the cortex, as well as active tissue response to radiation. Mizutani et al. (15) also connected vascular changes with local inflammatory reactions, including accumulation of macrophages and changes in production of pro-inflammatory cytokines. In our experiment strong vascularization was most intensive, particularly in the cortex, in the primary regenerative phase, in accordance with previous results (13). Furthermore, some authors suggest that abrupt recovery of the thymus structure after irradiation was due primarily to these changes in the vascular structure (16). Anyway, we consider that it is connected with active thymic inflammatory response to irradiation, what has been substantiated by persistent increase of both macrophages and ID cells number in our experiment. It is known that these cells produce IL-1 in vitro, as well as that X-irradiation increases production of IL-1 in macrophages both in vitro and in vivo (17). Rocha et al. (18) also showed that IL-2R(thymic dendritic cells in vitro proliferate in the presence of IL-2 and monocytes stimulated by interferon ? express IL-2R and in combination with IL-2 secrete IL-1. Increased expression of IL-2R on ID cells after irradiation was shown in our experiment. Macrophages and ID cells are obviously not only important for phagocytosis of large number of apoptotic thymocytes, but also have a prominent role in thymus regeneration thanks to secretion of various cytokines (15). Our previous results have showed that WR-638 has potentially some beneficial effects in relation to IL-2/IL-2R pathway in postirradiation regeneration of the thymus (19). Further investigations are needed to elucidate the real nature of WR-638 thymic microenvironment protection from deleterious effects of X-ray irradiation.

CONCLUSION

Therefore, WR-638-induced significant reduction of changes in the rat thymus structure caused by X-ray irradiation was preferentially the result of the lymphoid compartment protection. However, concerning that it has also caused changes of macrophages, ID cells and mesenchymal stroma phenotypic characteristics it can be concluded that these effects also contribute to WR-638 radioprotective action on thymus.

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