Acta Veterinaria (Beograd), Vol. 55, No. 2-3, 85-100, 2005.

UDK 619:612.014.481.1

PHENOTYPIC CHARACTERISTICS OF THYMIC MICROENVIRONMENT IN WR-638-PROTECTED RATS AFTER WHOLE-BODY IRRADIATION: EPITHELIAL CELLS

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(Received 16. October 2004)

Phenotypic changes of thymic epithelial cells (TEC) were studied in male Wistar rats subjected to radioprotector WR-638 (358 mg/kg b.w. ip) and/or whole body X-ray irradiation (3.5 Gy) 15 days after its application. Phenotypic changes were identified in situ on cryostat thymic sections using immunohistochemical staining by a panel of monoclonal anti-cytokeratin antibodies. It was shown that WR-638 significantly reduced changes in the structure of the thymus caused by X-ray irradiation. In the primary involution phase some phenotypic characteristic changes of TEC can be explained mostly by lymphoid destruction, although factors directly connected to the protector can not be excluded. However, in the primary regenerative phase WR-638 caused prominent changes of antigen expression on subcapsular and medullary TEC in irradiated rats. Therefore, WR-638 not only protects thymocytes, but also causes alterations of phenotypic characteristics of TEC which may contribute to its beneficial radioprotective effect on the irradiated rat thymus.

Key words: epithelial cells, radiation, radioprotection, rats, thymus,WR-638

INTRODUCTION

The thymus is the major site for generation of immunocompetent T lymphocytes. Their development is a consequence of complex interactions of immature lymphocytes with epithelial cells, macrophages, dendritic cells and mesenchymal stroma (Boyd *et al*., 1993; Nikolić-Žugić, 1994; Zinkernagel and Althage, 1999; Hudrisier *et al*., 2003). Thymic epithelial cells (TEC), as a major component of its microenvironment, provide inductive signals for normal differentiation and maturation of thymocytes through direct cellular contacts with them, as well as by production of spectrum of soluble factors, such as hormones, growth factors and lymphokines. These cells are heterogenous in terms of their ultrastructure, reactivity with different anti-TEC monoclonal antibodies (mAbs), cytokeratin (CK) polipeptide expression and probably function (Boyd *et al*., 1993; Schuurman *et al*., 1997). On the other hand, the normal thymic architecture can be dramatically changed by experimental manipulations such as single doses of

whole-body irradiation (Anderson and Warner, 1976; Huiskamp and Ewijk, 1985; Fujikura *et al*., 1997; Arduchelvan *et al*., 2000; Mizutani *et al*., 2002). It has been shown that ionizing radiation has been a very useful tool for depleting the thymus of dividing immature thymocyte subsets and to sequence thymocyte differentiation events occurring from radiation-resistant precursors. Distinct phenotypical sequencing in expressing cell surface differentiation antigens was observed in regenerating thymocyte populations. This experimental manipulation also provides a useful model in which the bidirectional interplay between the thymic stromal cells and lymphocytes can be investigated, since it has been shown that observed changes in thymic microenvironment after irradiation have been related with T cell differentiation processes in the regenerating thymus (Huiskamp *et al*., 1985; Adkins *et al*., 1988; Arudchelvan *et al*., 2000; Mizutani *et al*., 2002).

The major goal of radiobiology is the development of drugs which can be used to provide protection against radiation injury (Giambarresi and Jacobs, 1987). On the other hand, in contemporary clinical oncology development of drugs used to protect normal tissue from noxious effects of radiation, but without compromising its antitumor effect, is highly desirable (Giambarresi and Jacobs, 1987; Dragojević-Simić and Dobrić, 1996). The most effective group of radioprotectors, developed by the United States Army, are aminothiols, designated as WR. WR-638 (aminoethylphosphorotioate), conversely to its congener WR-2721, now known as amifostine, has been less frequently studied in the protection of the immune system, including the thymus. Our previous investigations have shown that WR-638 accelerates the rat's thymic regeneration after whole-body irradiation, due to its beneficial effects on the lymphatic tissue (Dragojević-Simić *et al*., 1993; Dragojević-Simić *et al*., 1994). However, the effect of WR-638 on TEC changes induced by irradiation, as well as its potential contribution to the obtained protection of the lymphoid compartment, was not examined so far.

A panel of anti-CK monoclonal antibodies (mAbs) defining various TEC subsets was used in order to describe phenotypic changes of these cells in rats after application of WR-638 and/or irradiation as well as to relate changes in the thymic microenvironment after treatment. The response of T cell differentiation process in the regenerating thymus was studied as well.

MATERIALS AND METHODS

Animals

Experiments were performed on male Wistar rats, 6 to 8 weeks old, bred at the Farm for Experimental Animals, Military Medical Academy. Animals were sacrified by aether anaesthesia. Thymi were immediately removed and frozen in liquid nitrogen. Cryostat sections were cut, air dried for 2h and fixed in acetone for 10 min.

Radiation procedure

Irradiation procedure has been described by Dragojević-Simić *et al*., (1994). Rats were divided in to 4 groups: I – sham-irradiated animals (control), II – whole body irradiated ones, which were treated with 8 MeV X-rays at a dose of 3.5 Gy by using a linear accelerator (SL 75-20, Philips), III – irradiated ones protected with WR-638 (358 mg/kg b.w. *ip*, 30 min before irradiation) and IV – sham-irradiated ones pretreated with WR-638 in the same way. WR-638 (Chemical Department, Military Technical Institute, Belgrade, Serbia and Montenegro) was dissolved in saline immediately prior to injection. The controls were treated with saline (1 ml/kg b.w. *ip*). Rats were sacrificed 2, 4, 8 and 14 days after treatment. The study protocol was based on the Guidelines for Animal Studies N^0 282-12/2002 (Ethics Committee of Military Medical Academy, Belgrade, Serbia and Montenegro).

Antibodies and reagents

Five mouse mAbs detecting subcomponents of human keratins were used for immunostaining of thymic sections (Table 1). Antibodies reacting with single polypeptide CK 7, 8, 18 and 19 were commercially obtained from Amersham International, U.K. while KL1 mAb specific for CK pair 3/10 were obtained from Serotec, U.K. Secondary antibodies and reagents (sheep anti-mouse Ig conjugated with peroxidase and goat anti-mouse IgG subclass specific biotynilated antibodies and streptavidin coupled with peroxidase) were also purchased from Amersham International.

Table 1. Characteristics of anti-cytokeratin (CK) monoclonal antibodies (mAbs) reactive with rat thymic epithelial cells (TEC)

React with	mAbs	Isotype	Dilution	Reactivity patterns
CK 8	K8	IgG_1	1:5	All epithelium (+)
Simple epithelia	K18	IgG_{2a}	1:5	Cortex TEC (+) Medullary TEC (+, b)
Glandular epithelia	K7	IgG_1	1:5	Subcapsular/subtrabecular TEC (+, a) Medullary TEC (+, b)
CK19	K19	IgG_{2b}	1:5	Subcapsular/subtrabecular TEC (+) Medullary TEC (+, b) Hassall's corpuscles (± , c)
CK 3 and 10	KL1	IgG_1	1:20	Subcapsular/subtrabecular TEC (–, p) Medullary TEC (+, b) Hassall's corpuscles (+)

+ = strong positivity; ± = weak positivity; – = negative; a = approx. 50 – 75% TEC positive; b = approx. 25 – 50% TEC positive; c = some Hassall's corpuscles (HC) positive or a peripheral layer of many HC positive, p = patches of positive TEC seen only on some sections

Immunohistochemistry

Detailed procedure has been described by Čolić *et al*. (1988a, 1990; 1990b). Briefly, after fixing in acetone sections were incubated for 60 min with mAbs. This was followed by blocking endogenous peroxidase activity with 1% H_2O_2 diluted in methanol for 15 min. After that, sections were covered with sheep anti-mouse Ig conjugated with peroxidase (1:20) in Tris-buffered saline (TBS) for 30 min. Revelation of the peroxidase activity was performed by 3'3 diaminobenzidine (DAB) and 0,01% H_2O_2.

For a more detailed analysis the cryostat sections were, after incubation with the same mAbs, treated with goat anti-mouse IgG subclass specific biotynilated antibodies (1:100) and streptavidin-peroxidase (1:100). Revelation of the peroxidase activity was performed by 10 min. incubation of sections with 0, 06% DAB in 0, 01% H_2O_2. Finally, all slides were counterstained with haematoxylin and mounted in gelatin/glycerol mounting medium.

RESULTS

In light microscope analysis of the thymic sections (H & E) it was found that whole-body X-ray irradiation caused cyclic changes in the thymic structures and 4 phases can be clearly distinguished: primary involution (until day 2), primary regeneration (from days 2 until 14), secondary involution (from days 14 until 21) and secondary regeneration (from days 21 until 30) (not shown). Radioprotector WR-638 reduced the magnitude of lymphatic tissue depopulation during the involutive phases and increased its cellularity during regeneration. Using mAbs specific for single CK polypeptides or CK pairs we described the phenotypic changes of rat TEC in the first two phases after application of WR-638 and/or irradiation.

Primary involution

Radiation has severe effects on the thymus structure during primary involution. Namely, at day 2 after the procedure the number of cortical thymocytes was much lower on comparison to non-irradiated rats, and many apoptotic cells were observed. Incubation of frozen thymic sections with K8 and K18 mAbs shows that epithelial cells were collapsed mainly due to loss of thymocytes, but without significant differences in antigen expression (Figure 1; Figure 2). In the WR-638-protected rats changes in antigen expression on cortical epithelial cells were not observed either, although staining was less confluent due to prominent preservation of cortical thymocytes (Figure 1; Table 2). On the other hand, staining with mAbs which recognize CK 7 and 19, enabled us to observe that the subcapsular flat epithelial cell layer, is thickened convoluted and more intensively stained in irradiated rats (Figure 3). This finding is less prominent in WR-638-protected animals (Table 2). Mab KL1 shows reactivity with subcapsular epithelial cells in irradiated as well as in protected animals, contrary to control rats in which it can be seen very rarely (Table 1; Figure 4). In comparison to the control animals the medullar atrophy in irradiated rats was less prominent than, cortical atrophy

(Figure 1; Figure 2). The density of $K19^+$ medullar epithelial cells has increased to some extent in irradiated animals (Figure 5). In this phase larger and more numerous Hassall's corpuscles (HC) stained with K8 and KL1 mAbs were also present in irradiated animals. The interesting fact is that most of them expressed CK 19, seldomly seen in HC of non-irradaited rats. Even more, there are HC stained with K 7, not present in the control animals at all. Less prominent medular atrophy and slightly decreased number of desribed HC in the medulla of the WR-638-protected rat in comparison to irradiated ones can be seen (Figure 1; Table 2).

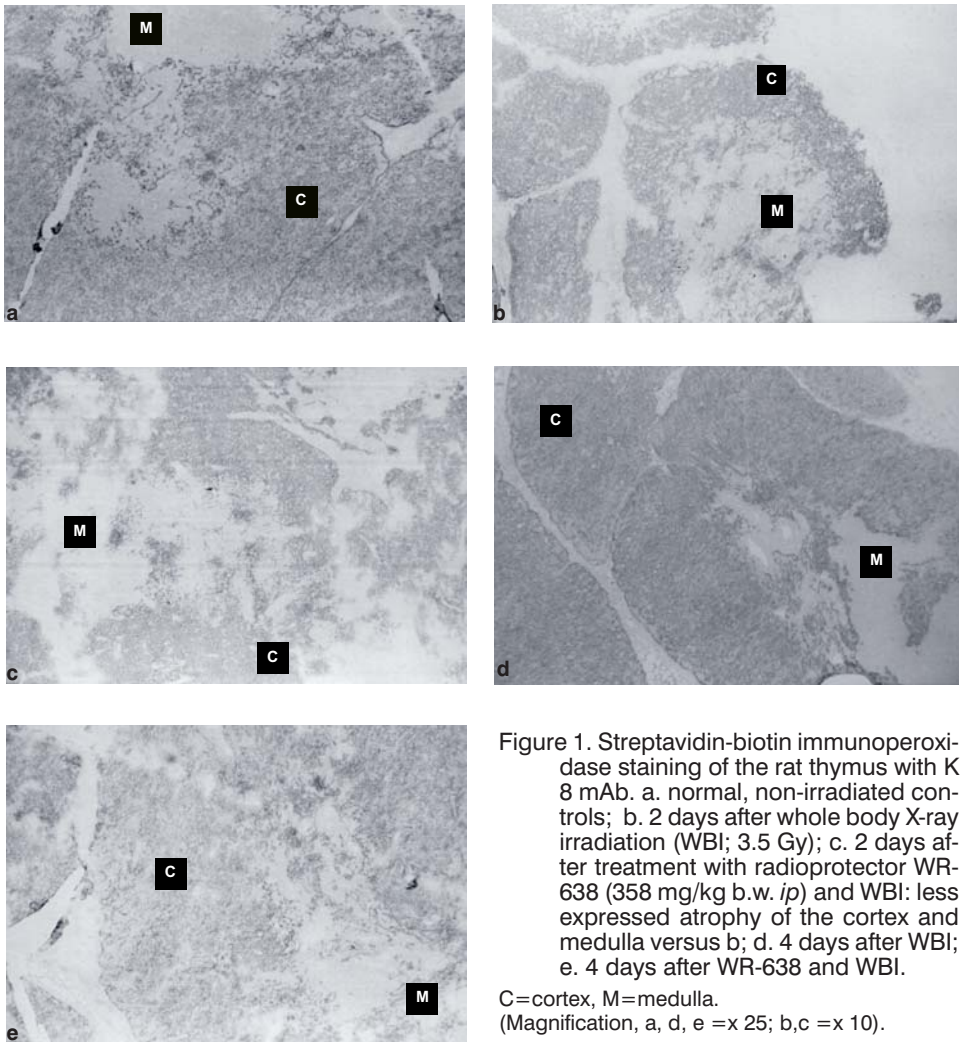

Figure 1. Streptavidin-biotin immunoperoxidase staining of the rat thymus with K 8 mAb. a. normal, non-irradiated controls; b. 2 days after whole body X-ray irradiation (WBI; 3.5 Gy); c. 2 days after treatment with radioprotector WR-638 (358 mg/kg b.w. *ip*) and WBI: less expressed atrophy of the cortex and medulla versus b; d. 4 days after WBI; e. 4 days after WR-638 and WBI.

C=cortex, M=medulla.
(Magnification, a, d, e =x 25; b,c =x 10).

Table 2. Immunohistochemical characterization of TEC in rats subjected to radioprotector WR-638 (358 mg/kg b.w. *ip*) and whole body X-ray irradiation (3.5 Gy)

Monoclonal antibodies	Immunoreactivity	Primary Involution*	Primary Regeneration*
K8, K7, K19	Subcapsular TEC (STEC)	Decreased intensity of staining and convolution of STEC	More prominent decrement of STEC staining intensity
K8, K18	Cortical TEC	Prominently less expressed atrophy of the cortex	Less prominent cortical regeneration in the early phase, less prominent epithelial- free areas
K8, K18, K7, K19, KL1	Medullar TEC	Less expressed medullar atrophy	Decreased progression of medullar atrophy with prominent changes in antigen characteristics
K8, K19, KL1	Hassall's corpuscles	Less prominent increment of number and size	Less prominent increment of number and size

* Alterations *vs* only-irradiated rats

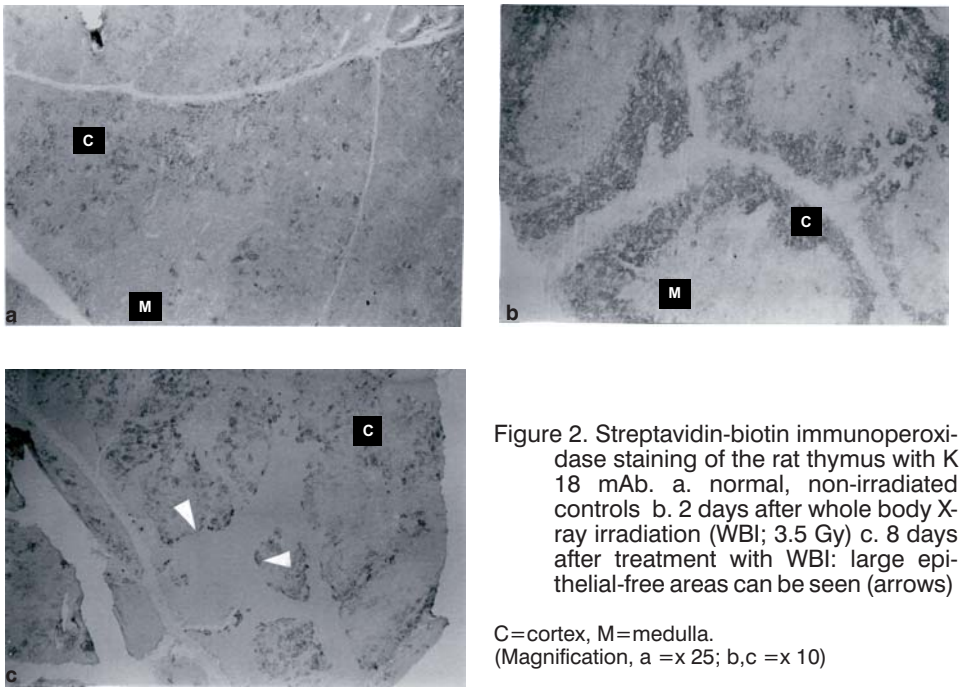

Figure 2. Streptavidin-biotin immunoperoxidase staining of the rat thymus with K 18 mAb. a. normal, non-irradiated controls b. 2 days after whole body X-ray irradiation (WBI; 3.5 Gy) c. 8 days after treatment with WBI: large epithelial-free areas can be seen (arrows)

C=cortex, M=medulla.
(Magnification, a =x 25; b,c =x 10)

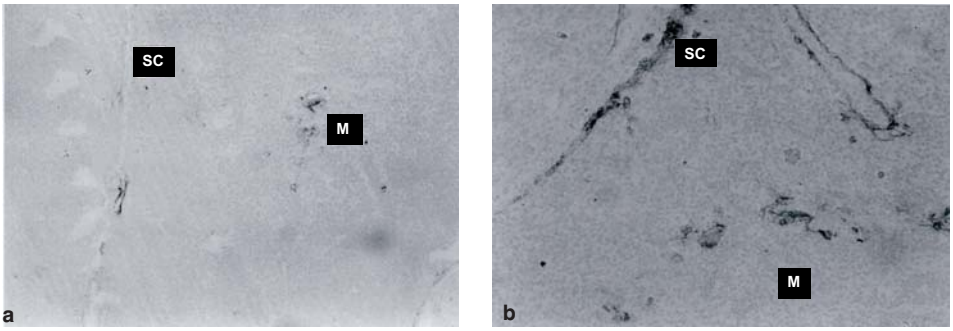

Figure 3. Streptavidin-biotin immunoperoxidase staining of the rat thymus with K 7 mAb. a. normal, non-irradiated controls b. 2 days after whole body X-ray irradiation (WBI; 3.5 Gy): Increased intensity of staining and convolution of subcapsullar flat epithelial cell layer (SC) , M=medulla.
(Magnification, a, b = x 25).

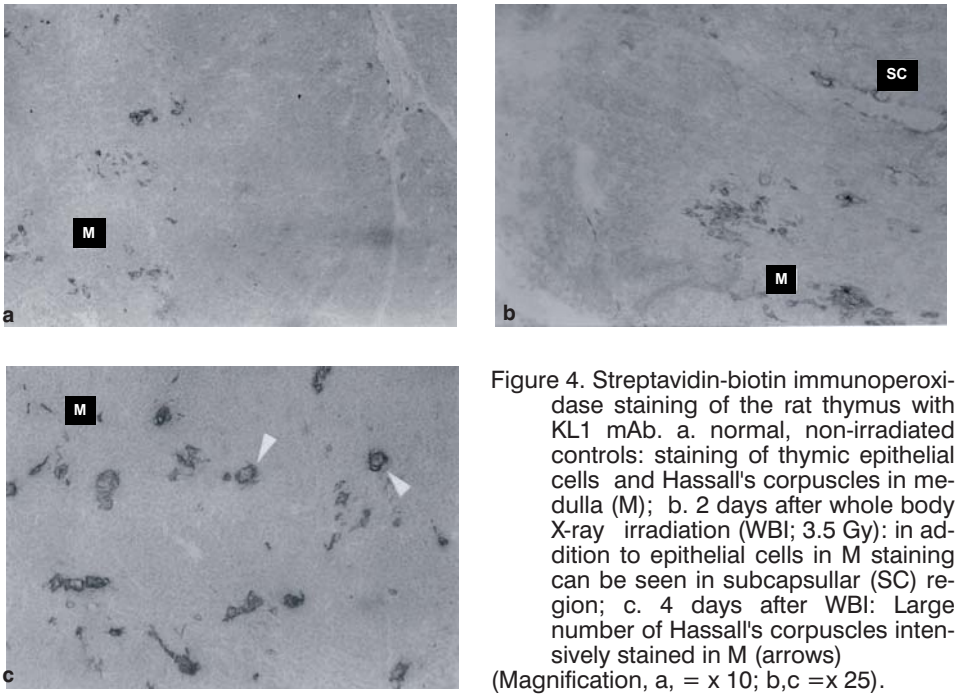

Figure 4. Streptavidin-biotin immunoperoxidase staining of the rat thymus with KL1 mAb. a. normal, non-irradiated controls: staining of thymic epithelial cells and Hassall's corpuscles in medulla (M); b. 2 days after whole body X-ray irradiation (WBI; 3.5 Gy): in addition to epithelial cells in M staining can be seen in subcapsullar (SC) region; c. 4 days after WBI: Large number of Hassall's corpuscles intensively stained in M (arrows)
(Magnification, a, = x 10; b,c =x 25).

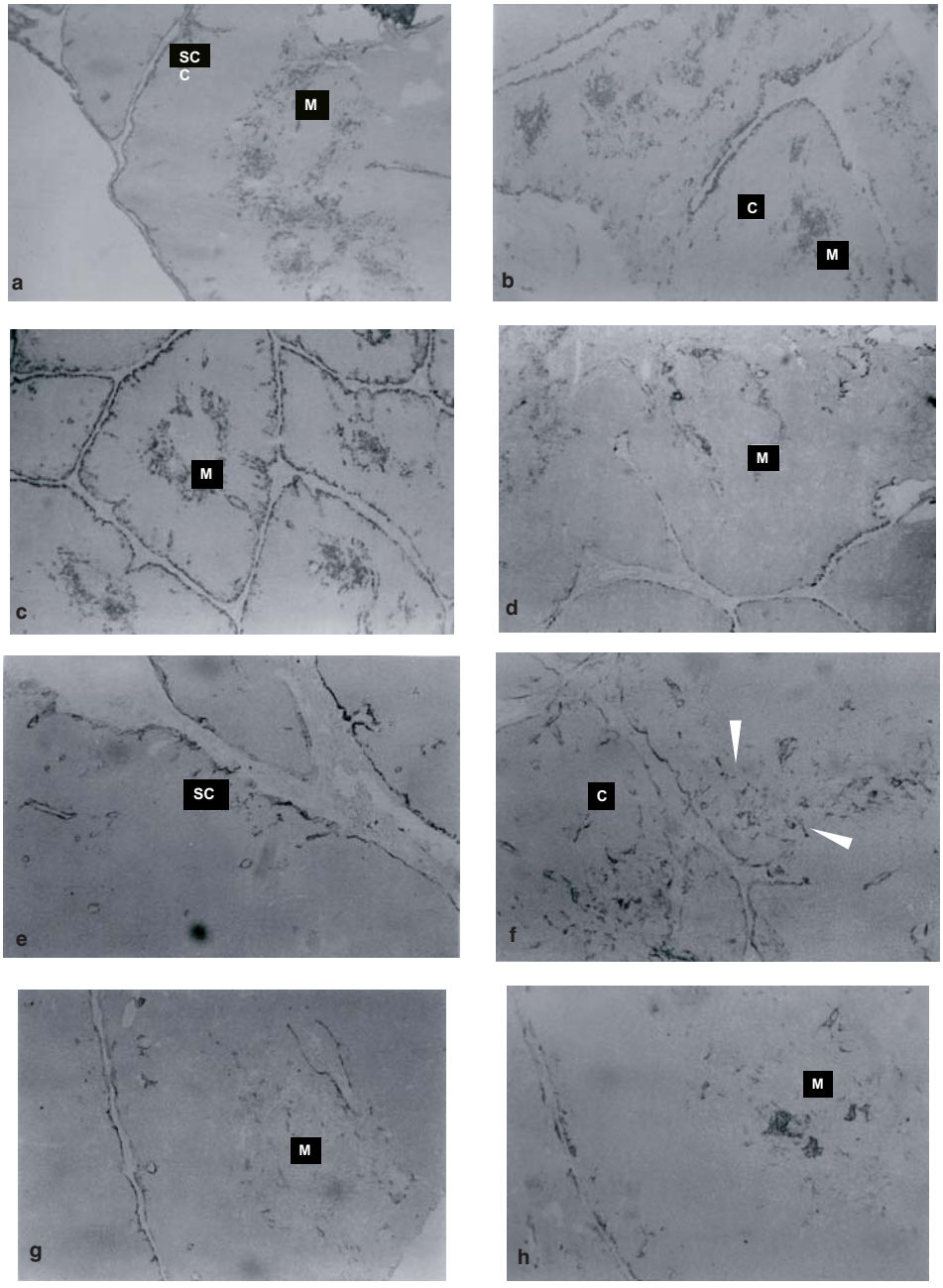

Figure 5. Streptavidin-biotin immunoperoxidase staining of the rat thymus with K 19 mAb.

Figure 5. continued
a. normal, non-irradiated controls; b. 2 days after whole body X-ray irradiation (WBI; 3.5 Gy); c. 4 days after WBI: Decreased number of thymic epithelial cells (TEC) in the medulla (M) *versus b*; d. 4 days after treatment with radioprotector WR-638 (358 mg/kg b.w. *ip*) and WBI: Reduction of TEC number in M *versus c*; e. 8 days after WBI: reduction of antigen expression in subcapsullar (SC) region *versus b*, small subset of cortical TEC expresses CK 19 (arrow), f. 8 days after WR-638 and WBI: Increased number of cortical TEC stains with K 19 *versus e* (arrows) ; g. 8 days after WBI: Just a small number of TEC in the M is positive; h. 8 days after WR-638 and WBI: Increased number of labeled TEC in the M *versus* g. C=cortex, (Magnification, e, f, g, h = x 25; a, b, c, d = x 10).

Primary regenerative phase

During the primary regeneration of the thymus, parallel to the reduction of the medulla, is the recovery of the cortical lymphatic structure (Figure 1). Staining with mAbs, reactive with CK 8 and 18, shows a pattern similar to the control one. However, it is interesting that in the second week after irradiation large areas without epithelium in the cortex are frequently seen (Figure 2). In WR-638-protected rats these zones are less prominent, especially at day 14 after irradiation (Table 2). On the other hand, from day 4 after irradiation a small subset of cortical TEC expresses CK 19, what is not the case in the control (Table 1, Figure 5). This finding is even more prominent in WR-638 protected rats (Figure 5).

Compared to primary involution the phenotypic characteristics of subcapsular/subtrabecular TEC are also changed in irradiated rats in this phase. Namely, the intensity of subcapsular/subtrabecular cell layer staining is reduced (Figure 5), or even totally absent on the majority of the observed thymic sections, especially when reactivity with K7 mAb was examined. At the same time, reduction in number and antigen expression on medullary TEC revealed by K7, K19 and KL1 mAbs in irradiated rats compared with the previous phase is prominent (Figure 5). It is maximally expressed at day 8 after irradiation (Figure 5). Further increase of number and magnitude of HC is also observed in irradiated animals compared with primary involution. It was evident when staining with mAb KL1 was performed (Figure 4). On the other hand, in the early phase of primary regeneration (from days 2 until 4) staining of subcapsular and medullar TEC, as well as HC with K7, K19 and KL1 mAbs is more reduced in WR-638-protected rats than in only-irradiated ones (Figure 5). However, later in this phase (from days 4 until 14) the number of medullar TEC which expressed CK 3, 7, 10 and 19 is constantly increasing (Figure 5). Therefore, decreased progression of medullar atrophy with prominent changes in antigen characteristics of subcapsular/ medullar TEC were seen in protected animals in this phase compared to only-irradiated ones (Figure1, Table 2).

During the experiment the radioprotector WR-638 itself did not cause changes in antigen expression of thymic epithelial compartment in the control animals.

DISCUSSION

In our previous experiments it was shown that X–ray irradiation (3.5 Gy) caused cyclic changes in the rat thymus manifested as: primary involution (until day 2), primary regeneration (from days 2 until 14), secondary involution (from days 14 until 21) and secondary regeneration (from days 21 until 30) (Dragojević-Simić *et al*., 1994). These manifestation the consequence of destruction and regeneration of thymic lymphoid tissue. It was generally accepted that the elements of thymic microenvironment were more resistant to the noxious effects of X-ray irradiation. However, there are also few reports that sublethal whole-body X-ray irradiation can affect its microenvironment, including TEC (Huiskamp *et al*., 1985; Adkins *et al*., 1988; Čolić *et al*., 1988; Stojanović *et al*., 1995). We have shown that aminothiol radioprotector WR-638 (aminoethylphosphorotioate) reduced the magnitude of thymocyte depletion in the primary involutive phase primarily as a result of cortical thymocyte protection, especially $CD4^+CD8^+$ subpopulation. WR-638 accelerated the regeneration of $CD4^-CD8^-$ and $CD4^-CD8^+$ thymocyte subsets, followed by subsequent increase of $CD4^+CD8^+$ and $CD4^+CD8^-$ thymocyte subsets (Dragojević-Simić *et al.*, 1994). However, the effects of this protector on irradiated rat thymi epithelial compartment was not investigated at all. In the present work most of the observed phenotypic changes of TEC during the primary involution phase are the result of a massive depletion of lymphoid cells and rapid shrinkage of cortical regions in irradiated rats. In WR-638-protected rats changes in antigen expression on cortical TEC were not observed either, although staining with K8 and K18 mAbs was less confluent due to prominent preservation of cortical thymocytes. However, phenotypic alterations in subcapsullar flat epithelial cells defined by anti-CK 7, anti-CK 19 and anti-CK 3/10 mAbs can only partially be explained by changes in cortical lymphoid compartment of both irradiated and protected rats. Namely, it is well known that CK are the major components of TEC intermediate filaments and very heterogeneous proteins which belong to a family of at least 20 different polypeptides (Boyd *et al*., 1993; Schuurman *et al*., 1997). TEC contain a wide range of keratin subunits from low to high molecular mass. Extreme differences in CK expression in particular TEC subsets have been observed not only in different mammalian species, but is also variable during ontogeny (Čolić *et al*., 1990; Čolić *et al*., 1990a; Boyd *et al*., 1993; Pavlović *et al*., 1993; Stojanović *et al*., 1995; Schuurman *et al*, 1997) and after application of some agents which also affect thymic microenvironment such as X-ray irradiation and corticosteroid treatment (Stojanović *et al*., 1995). In our experiments, not only subcapsular flat epithelial cell layer was more intensively stained with K7 and K19 mAbs in whole-body X-ray irradiated rats, but also KL1 showed reactivity with it, in irradiated as well as in WR-638-protected animals in the primary involution phase, contrary to the control ones. Staining with first two mentioned mAbs were less prominent in WR-638-protected rats. On the other hand, an increase in number of TEC expressing CK 19, larger and more numerous HC stained with K8 and KL1 mAbs, expression of CK 19 on them, rarely seen in HC of non-irradaited rats, as well as HC stained with K 7, not present in control animals at all, were observed in the medulla of irradiated

rats compared to the control. In our previous work we identified 6 TEC subsets and subcapsular/perivascular TEC (TEC-CK type 1) shared CK 7, 8, and 19 with a subset of medullary TEC (Čolić *et al*., 1989). Furthermore, KL 1 mAb recognizing CK pair 3/10 binds to a subset of medullary TEC and HC in rats, HC in guinea pig thymus and the suprabasal layer of human skin, and it is a marker of terminal epithelial cell differentiation (Lobach and Haynes, 1987; Čolić *et al*., 1990). Since, high molecular mass keratins are restricted only to HC it is supposed that they represent dense aggregates of terminally differentiated medullary epithelium such as TEC-CK type 4 (CK 7^- 8^+ 10^+ 18^+ 19^+) and TEC-CK type 5 (CK 7^- 8^+ 10^+ 18^- 19^-) defined in our previous experiments (Čolić *et al*., 1989).

Concerning this and our present findings it is obvious that WR-638 and/or irradiation procedure cause prominent changes in phenotypic characteristics of subcapsular/medullary TEC, but it is difficult to determine their real nature. In our previous paper we also demonstrated a decreased number and distribution of TEC reactive with R-MC 18, 19 and 20 mAbs in the medulla of the thymus of AO rats subjected to sublethal whole-body X-ray irradiation (5 Gy), in the primary involution phase in comparison to the control (Čolić *et al*., 1988; Čolić *et al*., 1988b). Larger and more numerous HC were also present, as well as increased expression of antigens detected by these mAbs (Čolić *et al*., 1988). In this experiment, in the primary regeneration phase, prominent reduction in number and antigen expression on medullary TEC detected by K7, K19 and KL1 mAbs was observed, maximally at day 8 after irradiation, as well as a further increase of number and magnitude of HC. Adkins *et al*. (1988) showed that mice given multiple doses of sublethal total lymphoid irradiation had a greatly reduced number and an abnormaly organized subset of medullary epithelial cells for a long time after irradiation. All these data, especially the one concerning HC, suggest direct damage of medullar epithelial cells after the irradiation procedure. However, in WR-638-protected rats from day 2 until day 4 after the irradiation procedure, staining of subcapsullar and medullar TEC, as well as HC was reduced comparing to only-irradiated ones. Later in this phase (from day 4 until day 14) in the same group of animals the number of medullar TEC which expressed CK 3, 7, 10 and 19 was constantly increasing. Data concerning the nature of WR-638 influence on TEC of irradiated rat thymus is completely missing at the moment. On the other hand the radioprotector itself did not cause any changes in antigen expression of thymic epithelium in the control animals. Furthermore, during the second week after irradiation the absence of reactivity of mAbs which detect TEC in large zones in the cortex was frequently seen, while this was less prominent in protected rats, especially at day 14 after this procedure. A more detailed analysis showed that this areactivity corresponds to zones without epithelium. These epithelial-free areas have been described so far in humans, mice and rats, and are localized just beneath the covering subcapsular epithelial layer in the outer cortex, although it can expand to deep cortex and reach the medulla (Kendall *et al*, 1990; Schuurman *et al*, 1997). They are filled with small lymphocytes as in the thymic cortex and contain various macrophage subtypes, which are strongly major histocompatibility complex (MHC) II positive (Bruintjes *et al*., 1993; Schuurman *et al*., 1997). The significance of epithelial-free

areas is not yet known, but it is supposed that they are not solely storage places for lymphocytes as these can undergo cell division. Therefore, we consider that our finding of large zones in the cortex without epithelium is directly connected with the intensity of thymocyte proliferation during the primary regeneration phase after irradiation. They are probably regenerating by enlargement of described small subcapsullar epithelial-free areas because TEC of the cortex are displaced peripherally as a result of more intensive thymocyte proliferation. Actually, it was shown that in some pathological conditions like pre-leukemic phase in the thymus of AKR mice and in the rat thymus after cyclosporine administration these zones without TEC can be quite prominent (Bruintjes et al, 1993; Schuurman *et al*., 1997). By decreasing the number of thymocytes the normal cytoarchitecture restores. However, there were some other data showing that during recovery after irradiation (6 Gy), changes of the epithelial tissues itself in the thymic cortex contribute to the abrupt proliferation, and possibly to the abrupt maturation of thymocytes, while medullar epithelial tissue remained sparse and appeared inactive for a long period (Wang *et al*., 1999). Arudchelvan *et al*. (2000) have found changes in different epithelial cell subtypes in the cortex as well as in the medulla of the irradiated rat thymus. The precise nature of these TEC changes after irradiation is still unknown, and needs further investigation. Anyway, in our experiment the effect of WR-638 on this process, manifested by faster appearance and disappearance of large zones without TEC after irradiation, is probably also in correlation with proliferation of thymocytes intensity in the cortex. On the other hand, from day 4 after irradiation there was an interesting finding that a small subset of cortical TEC expressed CK 19, not previously described in the control, but even more prominent in WR-638 protected rats. We obtained similar results after hydrocortisone treatment and sublethal X-ray irradiation in AO rats (Stojanović *et al*., 1995). It was previously observed that during fetal ontogeny most cortical TEC were strongly CK 19^{+} and switching on the expression of this marker toward the adult phenotype occurred during the early postnatal period (Čolić *et al*., 1990a; Pavlović *et al*., 1993). It is also known that proliferation and differentiation pathways of newly generated thymocytes after X-ray irradiation occur in cycles (Anderson and Warner, 1976) like those in fetal life (Nikolić-Žugić, 1994). Therefore, one can suppose that the reappearance of fetal markers on adult TEC can be, hypothetically, a response to the absence of certain thymocyte subsets after irradiation at the appropriate stage of their maturation. On the other hand, the nature of the effect of WR-638 on this process is completely unknown. It is understandable, since we do not know yet the precise role of CK in TEC differentiation and function in the normal thymus, nor after the irradiation procedure. In addition, biochemical and functional characteristics of most TEC antigens are not known at the moment. However, concerning all these data it can be said that restoration of the thymus after irradiation obviously involve the synchronous development of both stromal and lymphocytic components (Randle-Barrett and Boyd, 1995; Wang *et al*., 1999; Arudchelvan *et al*., 2000; Mizutani *et al*., 2002). In our previous work we have shown that WR-638 not only protected thymocytes, but also caused prominent changes in the number and phenotypic characteristics of macrophages and interdigitating cells in the

irradiated rat thymus (Dragojević-Simić *et al*., 1993a; Dragojević-Simić *et al*., 1994; Dragojević-Simić *et al*., 2001). The radioprotector also reduced alterations caused by radiation in its vascular structures. Therefore, WR-638 attenuated thymic inflammatory responses which appear after irradiation documented in our investigation as well as by other authors (Huiskamp *et al*., 1985; Dragojević-Simić *et al*., 2001; Mizutani *et al*., 2002). Furthermore, cytokines are considered to be an important factor regulating recovery after irradiation and intrathymic ones may control thymocyte proliferation and maturation (Dragojević-Simić *et al*., 1993a; Barcellos-Hoff, 1998; Hashimoto *et al*., 1999; Uchimura *et al*., 2000; Chung *et al*., 2001; Mizutani *et al*., 2002; Min *et al*., 2002; Toki *et al*., 2003). TEC, among others, are intrathymic sources of cytokines, including IL-1, IL-6, and IL-7 required for normal thymocyte differentiation and proliferation (Le *et al*, 1987; Le *et al*., 1990; Čolić *et al*., 1991; Chung *et al*., 2001; Toki *et al*., 2003). Anyway, our previous results implicate that the beneficial effects of WR-638 in enhancing the regeneration of irradiated thymi, influence not only the lymphoid compartment but also macrophages, interdigitating cells and mesenchymal stroma. This is in relation to IL-2 role in the proliferation and differentiation of thymocytes (Dragojević-Simić *et al*., 1993a; Dragojević-Simić *et al*., 1994; Dragojević-Simić *et al*., 2001). Whether this leads to some changes in intrathymic content and function of cytokines other than IL-2 needs further investigation.

In conclusion, we consider that this investigation of WR-638-induced alterations of the epithelial compartment of irradiated rat thymus probably contributes to its beneficial radioprotective effect. Furthermore, it has not only shed more light on the role and significance of stromal cells in T cell development, but also open further perspectives for investigation of protective and immunomodulatory properties of the aminothiol radioprotectors.

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FENOTIPSKE KARAKTERISTIKE MIKROSREDINE TIMUSA OZRAČENIH PACOVA ŠTIĆENIH RADIOPROTEKTOROM WR-638: EPITELNE ĆELIJE

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SADRŽAJ

Fenotipske promene epitelnih ćelija timusa (TEĆ) mužjaka Wistar pacova ispitivane su tokom 15 dana od primene radioprotektora WR-638 (358 mg/kg telesne mase *ip*) i/ili izlaganja celog tela X-zracima u dozi od 3,5 Gy. One su bile identifikovane *in situ* na kriostatskim presecima timusa primenom imunohistohemijskih metoda pomoću anti-citokeratinskih monoklonskih antitela. Dokazano je da je WR-638 značajno smanjio promene u strukturi timusa izazvane X-zračenjem. U fazi primarne involucije promene u fenotipskim karakteristikama TEĆ u najvećoj meri mogu da se dovedu u vezu sa oštećenjima limfoidnog odeljka, iako faktori vezani za direkno delovanje protektora na njih ne mogu biti isključeni. Međutim, u fazi primarne regeneracije, WR-638 je doveo do značajnih promena u antigenskoj ekspresiji pojedinih subpopulacija TEĆ i to prvenstveno u subkapsuli i meduli timusa ozračenih pacova. Prikazani efekti WR-638 na epitelni odeljak mikrosredine timusa ozračenih pacova najverovatnije doprinose njegovom odličnom radioprotektivnom delovanju na ovaj limfatički organ.