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Recovery of Peripheral Blood Cells in Irradiated Mice Pretreated With Bacterial Extract IRS-19

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Summary

The effect of antigenic bacterial lysate IRS-19 on the recovery of blood cells was studied in mice injured by a single dose of 7 Gy irradiation. The preirradiation administration of IRS-19 accelerated the recovery of leukocytes, reticulocytes and platelets in peripheral blood. The recovery of leukocytes 9-14 days after irradiation in protected animals was accompanied by a higher level of band forms of granulocytes as well as activated lymphoid and monocytoid cells.

Key words

IRS-19 • Irradiation • Hematopoiesis • Radioprotection

Introduction

Radiation as chemotherapy destroys hematopoietic stem and progenitor cells resulting in rapid loss of peripheral blood cells, especially leukocytes needed for host defense against microbial invasion. There have been numerous strategies, both experimentally and clinically designed, to enhance hematopoiesis following therapy and reduce the degree of myelosuppression.

Extensive research has demonstrated the importance of microorganisms and their products in the regulation of granulopoiesis. So far, the most potent stimulation of hematopoiesis and the corresponding increase in the survival of irradiated animals was achieved by administration of bacterial endotoxins (Meffered *et al.* 1953, Ainsworth and Mitchell 1968, Ainsworth 1988). Radioprotection in mice has also been observed with glucans (Pospíšil *et al.* 1982, Patchen and MacVitie 1986, Patchen *et al.* 1987), bacterial products such as lipopolysaccharides (LPS) (Behling *et al.* 1980) or Ivastimul-aqueous extract from Chlorococcal algae (Vacek *et al.* 1990). A common feature of all these experiments was that the compounds were administered prior to irradiation.

Our previous study showed a considerable radioprotective effect of Broncho-Vaxom^{R} (BV) which is the lyophilized extract of the eight most common bacteria which are free of endotoxins (Fedoročko *et al.* 1992, Macková and Fedoročko 1993, Fedoročko *et al.* 1994). Like BV, preirradiation administration of IRS-19, an antigenic bacterial lysate, increases the survival of irradiated mice and accelerates the recovery process of hematopoiesis in bone marrow and spleen (Fedoročko and Macková, in press).

The aim of this study was to assess the effect of preirradiation administration of IRS-19 on the recovery of

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peripheral blood elements in animals irradiated with a single dose of 7 Gy irradiation.

Material and Methods

Mice

Female C57Bl/6 mice, 8-10 weeks old (weighing ~20 g), were obtained from TOP Velaz, s.r.o. (Praha, Czech Republic). Animals were quarantined for a period of 2 weeks and were housed in rodent cages with five to seven animals per cage at about 23 °C. They were given Velaz/Altromin 1320 ST lab chow and tap water acidified to pH 2.4 *ad libitum*. Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals", prepared by the State Veterinary Office of the Slovak Republic, Bratislava.

IRS-19 administration

IRS-19 (Laboratoires de Thérapeutique Moderne, L.T.M.-Sarbach, Suresnes Cedex, France) is a lysate of eight bacteria (*Diplococcus pneumoniae, Streptococcus, Micrococcus pyogenes (Staphylococcus), Gafkya tetragena, Neiseria, Klebsiella pneumoniae Moraxella* and *Hemophilus influenzae*). The lysate was administered i.p. 24 h before irradiation in a volume 0.6 ml per mouse. Control animals received saline in the same volume and at the same time as the treated group.

Irradiation

Mice were placed in plexiglass containers and whole-body (unilaterally) exposed to 7 Gy of gamma rays (0.5 Gy/min). A Chisostat ^{60}Co source (Chirana, Czech Republic) was used for all irradiations.

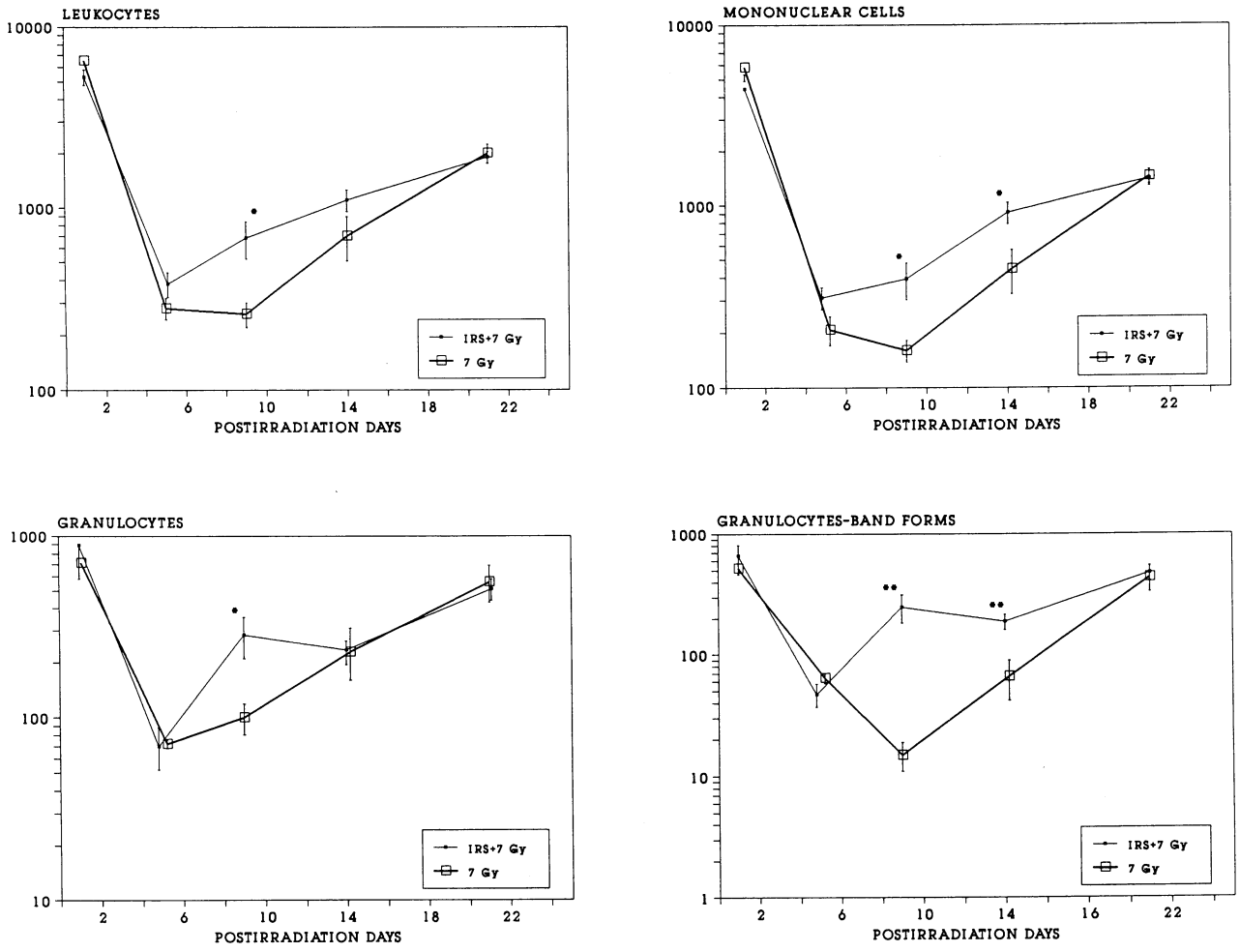

Fig 1. *The effect of preirradiation injection of IRS-19 on peripheral blood cells following total body irradiation with 7 Gy. Mice received an i.p. injection of 0.6 ml of IRS-19/mouse 24 h before irradiation. Cell numbers were measured in the peripheral blood from the mice on days 5-21 after irradiation. Values represent the mean ± S.E.M. of numbers from 7-8 mice. * P<0.05, ** P<0.01, *** P<0.001.*

Collection and analysis of peripheral blood cells

Animals (5-10 mice per group) were examined at various time intervals after the injection of IRS-19 and after irradiation. Mice were anesthetized by a Thiopental injection (VUAB, Roztoky near Prague, Czech Republic) immediately before blood was obtained by cardiac puncture. Leukocytes were counted using an automatic Coulter Counter Model ZF and for platelet counts a Bürker chamber was used. Reticulocytes were evaluated after staining blood smears with brilliant cresyl blue. White cell differentials were performed by counting 100 white blood cells on May-Grünwald-Giemsa (M-G) stained smears. The hematopoietic response was evaluated 24 h after IRS-19 or saline injection (i.e. at the time of presumed irradiation) and on day 5, 9, 14 and 21 after irradiation.

Statistics

The values given in the figures represent the mean ± S.E.M. The statistical significance of the differences was evaluated using Peritz's F-test. $P<0.05$ value was considered a significant difference.

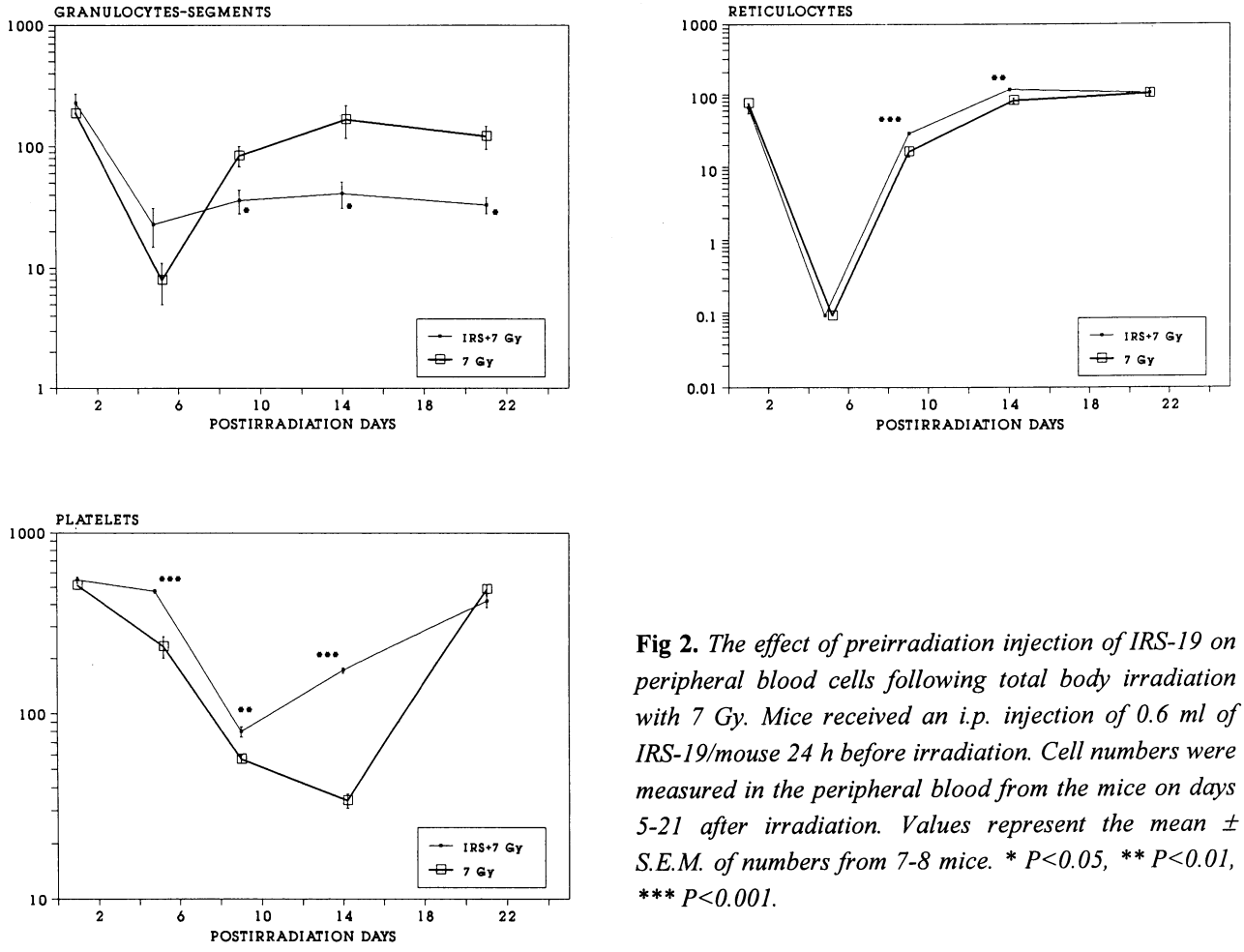

Fig 2. *The effect of preirradiation injection of IRS-19 on peripheral blood cells following total body irradiation with 7 Gy. Mice received an i.p. injection of 0.6 ml of IRS-19/mouse 24 h before irradiation. Cell numbers were measured in the peripheral blood from the mice on days 5-21 after irradiation. Values represent the mean ± S.E.M. of numbers from 7-8 mice. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.*

Results

Total body irradiation in both irradiated groups was followed by a marked depression of blood elements in peripheral blood. A relatively rapid decrease in the absolute number of leukocytes occurred on day 5 after irradiation in both irradiated groups, and was accompanied by a decrease in both mononuclear cells (MNC) and granulocytes (Fig. 1). Furthermore, peripheral reticulocyte counts declined during this time to negligible levels (Fig. 2). The platelet counts in mice, which had only been irradiated, decreased continuously up to day 14 after irradiation. IRS-19 administration 24 h before irradiation prevented a more profound platelet decrease within the first five postirradiation days, but then platelets also decreased until day 9 (Fig. 2).

Furthermore, IRS-19 pretreatment in irradiated mice accelerated the recovery of all peripheral blood cells. The most rapid recovery was seen in the reticulocytes in both irradiated groups, but the level of

reticulocyte counts was significantly higher in protected mice 9 and 14 days after irradiation ($P<0.001$ and $P<0.01$, respectively) (Fig. 2). Acceleration of recovery in protected animals was also seen in the recovery of platelets. The counts were found to be elevated by more than 300 % on day 14 compared with the level of platelets in animals which had been irradiated only ($P<0.001$) (Fig. 2).

The acceleration of recovery of absolute leukocyte number after IRS-19 application was accompanied by the recovery of granulocytes and mononuclear cells. The amount of MNC was increased by 144 % on day 9 ($P<0.05$), and the number granulocytes was increased by 184 % ($P<0.05$), i.e. higher than the values in the blood of nonprotected mice (Fig. 1). The higher rate of granulocyte recovery from days 5-9 after irradiation was primarily seen as a result of the extensive release of the band forms as well as the release of more immature myeloid cells, which evidently remained at significantly higher levels until day 14 ($P<0.01$) (Fig. 1). Microscopic evaluation of Giemsa-stained preparations also revealed that the mononuclear cells from IRS-19 treated mice on postirradiation days 5 and 9 predominantly consist of activated lymphoid cells and cells which presumably represent active or more immature monocytes. These cells may be seen singly or occasionally in clusters in the form of abundant ovoid cells with slightly textured pale blue cytoplasm (Fig. 3). Clusters of these cells are not normally present in peripheral blood smears. The typical small and large lymphocytes were seen in protected animals from day 14.

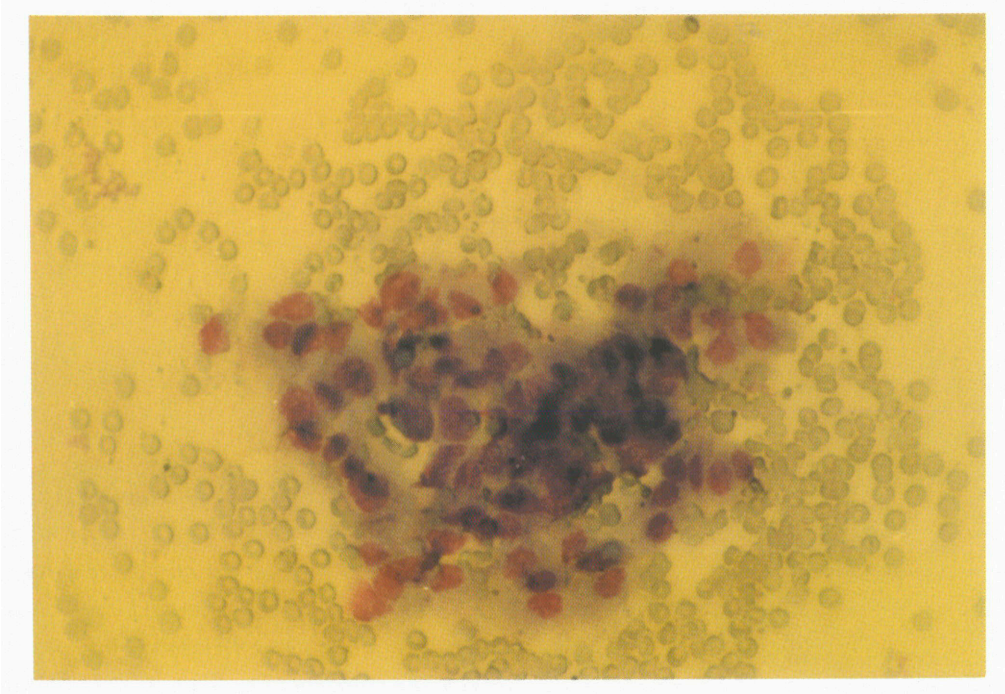

Fig. 3. *The clusters of activated mononuclear (monocytoid) cells in peripheral blood of mice pretreated with IRS-19 at postirradiation day 9. M-G , 1000 x.*

Discussion

As in the case of a variety of inflammatory and immunoenhancing agents administered to mice prior to irradiation, IRS-19 injection also increases the number of animals that survive after lethal irradiation (Fedoročko and Macková, in press). Previous studies demonstrated that IRS-19 in irradiated mice significantly accelerated the recovery of bone marrow and spleen cellularity and GM-CFC in these hematopoietic organs. The present study shows that the preirradiation application of IRS-19 evidently accelerates not only reparative processes of hematopoiesis in the bone marrow and spleen (Fedoročko and Macková, in press), but also the recovery of peripheral blood cells. The rapid rise in peripheral granulocytes on day 9 in protected animals was due to a significant increase in their band forms, which stayed at higher levels until postirradiation day 14, compared with non-protected mice. It is known that band forms of granulocytes as well as earlier myeloid cells are more easily increased during conditions of accelerated neutrophil release from the bone marrow into the blood,

for example after endotoxin (Chervenick and Boggs 1971, Kampschmidt and Upchurch 1980, Boggs *et al.* 1968, 1986) or some cytokines such as IL-1, TNF or G-CSF injection, which deplete the bone marrow reserve of neutrophils (Kampschmidt and Upchurch 1977, Fibbe *et al.* 1986, Ulich *et al.* 1987, 1988, Stork *et al.* 1988). On the basis of available *in vitro* and *in vivo* studies, one can assume that G-CSF, GM-CSF, IL-1 and IL-3 play crucial roles in the *in vivo* acceleration of granulocyte production.

It has further been shown by our results that the preirradiation application of IRS-19 also accelerated the recovery of mononuclear cells in peripheral blood, which was accompanied by an increase of evidently activated monocytoid and lymphoid cells, which were predominantly observed in blood smears during the first 10 postirradiation days. They not only play an important role in fighting infection but also in the regulation of hematopoiesis together with other microenvironmental cells by producing CSFs affecting the whole hematopoietic lineage (Gery *et al.* 1972, Zuckerman 1981, Dinarello 1984, Uckun *et al.* 1985, Rich 1986). It is the monocyte/macrophage (MM) that has also been shown to be involved in the production of factors affecting hematopoiesis. Because purified IL-1, which is a cytokine with multiple immunologic and inflammatory functions (Dinarello 1984), has been shown to be involved in the activation of T-lymphocytes (Matsushima *et al.* 1985) and B-lymphocytes (Howard *et al.* 1983), it appears that IL-1 may play an important role in the activation of the myeloid as well as the lymphoid lineages of the hematopoietic system in protected mice.

Recent data further indicate that the preirradiation administration of IRS-19 also accelerated the recovery of reticulocytes and platelets. In view of the fact that the level of platelets plays an important role in the seriousness of the course of the postirradiation syndrome and survival of the individual, the accelerated recovery of platelets is also a favorable sign. Platelets and reticulocytes are the products of megakaryocytopoiesis and erythropoiesis, which are profoundly influenced by LPS (Molendijk and Ploemacher 1984). Differentiation of multipotential stem cells along the megakaryocyte pathway and erythroid pathway is dependent on Meg-CSF, erythropoietin and a group of factor(s) known collectively as erythroid burst-promoting activity (BPA) which is derived from mononuclear cell types including lymphocytes and monocytes (Feldman *et al.* 1986). These are evidently activated in irradiated mice and in mice pretreated with IRS-19. Dose-dependent increase in platelet count have also been demonstrated in irradiated mice pretreated with a multifunctional peptide growth factor IL-6 (Patchen *et al.* 1991). IL-6 evidently also acts as an *in vivo* stimulus for erythropoiesis and myelopoiesis (Ulich *et al.* 1989).

It is not certain which growth factors might be optimal in promoting hematopoietic recovery, but often there are synergistic effects between CSFs and interleukins (Dinarello 1984, Donahue *et al.* 1988, Caracciolo *et al.* 1989), produced by multiple cell types, including the fibroblast population (Zucali *et al.* 1986, Kaushansky *et al.* 1988), the proliferation of which is stimulated also after treatment with bacterial LPS (Brocbank *et al.* 1983). We observed higher proliferation of fibroblasts as well as macrophages in the thymus of irradiated mice and in mice pretreated with immunomodulating agents (including IRS-19) (Macková and Fedoročko 1997). It is possible that some factors, such as cytokines, directly affect cells that constitute the hematopoietic organ microenvironment and may thereby influence cellular interactions between these and hematopoietic stem cells.

As after application of IRS-19, earlier recovery of circulating blood cells and other hematopoietic parameters in bone marrow and spleen was also observed in irradiated mice pretreated with other immuno-modulating agents such as endotoxins, LPS, glucans, Ivastimul, Broncho-Vaxom^{R} or muramyl dipeptide (Boggs *et al.* 1980, Pospíšil *et al.* 1982, Ainsworth 1988, Vacek *et al.* 1990, Patchen and MacVittie 1986, Patchen *et al.* 1987, 1990, Fedoročko *et al.* 1992, 1994, Macková and Fedoročko 1993, Vacek *et al.* 1999, Hofer *et al.* 2000). The exact mechanism of the effects exerted by preirradiation application of IRS-19 is not yet fully understood. However, because proliferation and differentiation of hematopoietic and lymphoid cells is mediated by endogenous growth and differentiation factors, it could be hypothesized that the radioprotective effect of IRS-19 as in the case of other immuno-modulating agents will depend on the induction of cytokines, the radioprotective effect of which has been reported by a number of investigators (Neta *et al.* 1986, Neta and Oppenheim 1988, Slordal *et al.* 1989, Schwartz *et al.* 1988, Oppenheim *et al.* 1989, Patchen *et al.* 1990, 1991, Zucali 1994).

In conclusion, these results like our previous results (Fedoročko and Macková, in press), suggest that the preirradiation application of immunomodulating agent IRS-19 evidently accelerates hematopoietic regeneration, which gives a better chance for animal survival.

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Reprint requests

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