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Functional Modifications of Macrophage Activity after Sublethal Whole-Body Irradiation (41118)

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Abstract. The effect of whole body x-irradiation on macrophage activity was evaluated by *in vitro* C3b-receptor-mediated ingestion and *in vivo* carbon clearance. Peritoneal macrophages from $B_6D_2F_1$ mice were tested from 20 min to 24 hr postirradiation and at doses up to 1000 rad x-irradiation. When compared to peritoneal macrophages from normal unstimulated mice, whole-body irradiation produced an increase in macrophage activity in both C3b-mediated ingestion and carbon clearance. The enhancement of macrophage activity was optimal at an exposure of 200 rad and waned as the exposure dose increased. Enhanced activity appeared as early as 20 min postirradiation and peaked by 3 hr. Although the mechanism for this observation is unknown, lymphocytes from irradiated mice were able to stimulate resident macrophages (from nonirradiated mice) *in vitro* to ingest C3b-coated erythrocytes.

The viability and functions of macrophages (Mϕ) are generally regarded as radioresistant (1, 2), although their precursors are radiosensitive (3, 4). Mϕ in irradiated tissues are capable of ingesting cellular debris (5) as are peritoneal Mϕ from irradiated animals (6). The radioresistance of phagocytic activity is also reflected in the ability of irradiated animals to clear bacteria (7–9) and colloidal carbon (10) from the circulation.

Several recent reports have indicated that whole-body irradiation (WBI) may actually result in augmented Mϕ activity. Cheers and Waller showed that Mϕ from lethally irradiated mice had enhanced bactericidal activity against *Brucella abortus* and *Listeria monocytogenes* (11). Furthermore, the tumoricidal activity of Mϕ obtained from irradiated mice has been recently reported (12). In this study, Mϕ obtained 3 hr postirradiation were lytic to a tumor cell line *in vitro*.

In view of these observations, experiments were performed to verify, by additional means, the augmentation of Mϕ function following WBI and to extend these observations to probe the mechanism(s) by which the Mϕ become modified. The parameter of C3b-receptor-mediated ingestion was chosen since Bianco and co-workers have shown this to be one manifestation of Mϕ activation (13).

The results show that peritoneal Mϕ obtained from sublethally irradiated mice exhibit C3b-receptor-mediated ingestion within 20 min of WBI. The enhancement of Mϕ activity is not limited to peritoneal Mϕ in that a parallel increase in colloidal carbon clearance *in vivo* is evident. Furthermore, one mechanism by which the Mϕ may become activated is via a signal derived from nonadherent cells.

Materials and Methods. $B_6D_2F_1$ mice, 10–12 weeks old (The Trudeau Institute, Saranac Lake, N.Y.), were used in all experiments. The mice were kept in autoclavable cages with air-filter bonnets, fed commercial Purina laboratory chow, and given acidified (pH 3.5), chlorinated (48.2 mg/liter) water.

During irradiation, the mice were confined in plastic 50-ml conical centrifuge tubes (Falcon Plastics, Oxnard, Calif.) and irradiated with a Picker Vanguard unit (280 kVp, 20 mA, 1.5 mm Cu HVL, 40 cm SSD). The irradiation field was 15 × 15 cm with 5 in. of plexiglas for backscatter. The dose rate was 64.47 rad/min mid Phantom Dose measured by a Victoreen Model 131, 100r thimble chamber. All sham-treated and irradiated mice were confined for identical times in the conical tubes.

Peritoneal cells (PC) were collected at various times post irradiation. Five milliliters of ice-cold Hanks' balanced salt solu-

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tion (HBSS), pH 7.4, and containing 10 U/ml sodium heparin, was injected into the peritoneum. After a gentle massage of the abdomen the fluid containing the PC was extracted with a syringe. The PC were washed twice in cold HBSS without heparin and resuspended in RPMI 1640 containing 10% heat-inactivated fetal bovine serum (RPMI/FBS), 100 U/ml, penicillin and 100 μg/ml streptomycin. The PC were placed into 35 × 10-mm petri dishes containing four 12-mm-diameter circular coverslips to yield a final number of 1.8×10^4 adherent Mϕ per coverslip (determined by direct counting using a gridded ocular). These Mϕ were adhered by incubating the PC for 1 hr at 37° in a humidified CO_2 environment and then removing the nonadherent cells by vigorous washing of the coverslips in warm HBSS. The coverslips were then placed in petri dishes containing RPMI/FBS with antibiotics.

The nonadherent cells used to cocultivate with resident Mϕ were obtained by two successive platings of PC in 37°, 5% CO_2 for 1 hr each time on plastic petri dishes. The nonadherent cells were resuspended in RPMI/FBS with antibiotics and added to resident Mϕ (from unstimulated syngeneic mice) in the same concentration as found in the original PC populations. The cultures were then incubated for 24 hr, and assayed for E(IgM)C3b ingestion.

The evaluation of Mϕ activation was achieved using the assay of Bianco *et al.* (14) in which sheep erythrocytes (E) were coated with rabbit IgM anti-sheep E and incubated with a complement source (C5-deficient DBA/2J serum) to deposit C3b on the E surface. The IgM- and C3b-coated E (referred to as E(IgM)C3b) attach to both normal and activated Mϕ, but are significantly ingested only by activated Mϕ (13). The coverslips with only adherent Mϕ were, after 24 hr in culture at 37° in a 5% CO_2 humidified atmosphere, incubated with E(IgM)C3b for 1 hr and then washed for 5–10 sec in warm hypotonic HBSS to lyse adherent extracellular E(IgM)C3b. The Mϕ were fixed in methanol and stained with Giemsa (Harleco, Philadelphia, Pa.). The percentage of ingesting Mϕ as well as the average number of E(IgM)C3b ingested were obtained by visual assessment. The ingestion index, the product of the percentage of ingesting Mϕ × the number of ingested E(IgM)C3b was recorded.

The *in vivo* assessment of systemic Mϕ activity was made using a carbon clearance assay (15). At various time intervals after the iv injection of colloidal carbon (Gunther–Wagner, Germany), blood was collected by retroorbital bleeding and assessed for carbon content using a Bausch and Lomb Spectronic 70 at 675 nm.

In all assays, the response of irradiated Mϕ was compared to the response of sham-treated (negative control) Mϕ and lipopolysaccharide (LPS)-stimulated (positive control) Mϕ. LPS-stimulated Mϕ were obtained by injecting 20 μg *Salmonella typhimurium* phenol-extracted LPS (Sigma, St. Louis, Mo.) intraperitoneally 2 days prior to harvest by the previously described method.

Results. The data of Fig. 1 indicate that, at 3 hr post-WBI, the C3b-mediated ingestion of E(IgM)C3b is evident at exposures of 100–800 rad, whereas at exposure greater than 800 rad there is not a significant enhancement of Mϕ function with respect to the Mϕ obtained from the peritoneum of sham-treated mice. The peak activity occurs at an exposure of 200 rad.

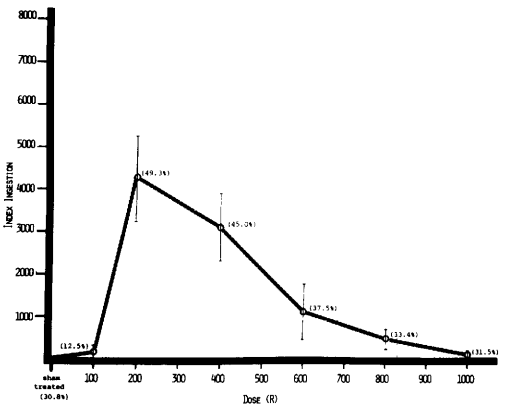

FIG. 1. Dose response of peritoneal macrophages explanted 3 hr post-WBI. Each point is the mean value ± SD of five coverslips. Ingestion index is the product of the percentage of ingesting Mϕ and the number of ingested E(IgM)C3b/100 Mϕ. Numbers in parentheses are the actual percentage of Mϕ ingesting E(IgM)C3b. LPS-stimulated Mϕ index = 10,400 ± 3000.

At all doses the viability of the PC from WBI mice was between 90 and 95%, whereas the viability of the PC from sham-treated mice was consistently 95%. The cellular composition, based on Giemsa-stained differentials, of the PC from both WBI mice and sham-treated mice was identical (60% lymphocytes, 25% Mϕ, 15% polymorphonuclear leukocytes).

The stimulation of Mϕ C3b-mediated ingestion following WBI (exposure of 200 rad) can be seen as early as 20 min postirradiation (Fig. 2), with peak activity occurring at 3 hr. The augmented Mϕ function begins to wane about 6–9 hr postirradiation, drops below normal at 12 hr postirradiation, then returns to normal level by 24 hr.

For the data presented in Fig. 2, the mice were irradiated so that the PC were collected simultaneously and evaluated for ingestion at the same time.

In order to determine if systemic Mϕ activation occurred following WBI, the carbon clearance assay was performed. Figure 3 shows that the ability to clear carbon following 200 rad WBI is evident at 6 hr following a short period of depressed clearance. The activity peaks at 12 hr postirradiation, and wanes by 24 hr.

In our earlier studies it was observed that if the nonadherent cells from WBI mice were allowed to remain in culture with the

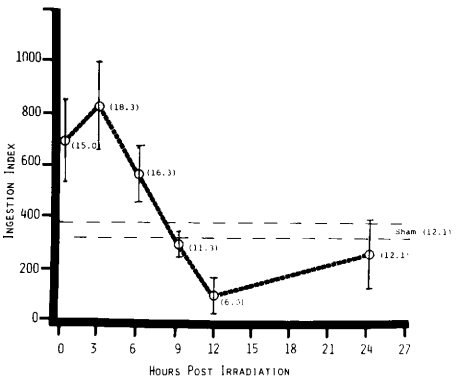

FIG. 2. Kinetics curve of Mϕ activity following WBI with 200 rad. Each point represents the mean value ± SD of five coverslips. Numbers in parentheses are the actual percentages of Mϕ ingesting E(IgM)C3b. Dotted lines represent sham-treated mice range. LPS-stimulated Mϕ index = 1642 ± 372.1.

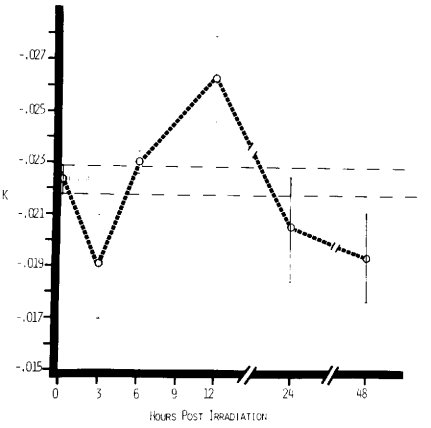

FIG. 3. Kinetics curve of *in vivo* carbon clearance following WBI with 200 rad. Each point represents the mean value ± SD of five mice. Dotted lines represent the range for sham treated mice. LPS stimulated Mϕ value = −0.256 ± 0.056.

Mϕ for the 24-hr incubation period, then these Mϕ compared to the same Mϕ incubated for 24 hr in the absence of the nonadherent cells, ingested E(IgM)C3b to a greater extent. This suggested the possibility that nonadherent peritoneal cells from WBI mice could induce Mϕ to augmented activity. Thus, nonadherent cells from mice exposed to 200 rad were cocultivated with resident Mϕ (syngeneic) obtained from untreated mice. As controls, resident Mϕ alone and resident Mϕ cocultivated with nonadherent cells from sham-treated mice were included. Figure 4 shows that the Mϕ exposed for 24 hr to the nonadherent PC from mice exposed to 200 rad WBI were significantly more activated according to the criterion of E(IgM)C3b ingestion.

Discussion. The present data substantiate the notion that Mϕ functions can be augmented following WBI by quantifying the increase in the ingestion of E(IgM)C3b. Furthermore, the Mϕ obtained from mice following WBI also exhibited enhanced spreading when compared to resident Mϕ (data not shown). Recently, Schultz and co-workers (12) demonstrated the Mϕ obtained from WBI mice were activated by the criterion of lysis of the MBL-2 lymphoblastic leukemia cells. However, the extent of Mϕ-mediated tumoricidal activity increased directly with the increase in dose of

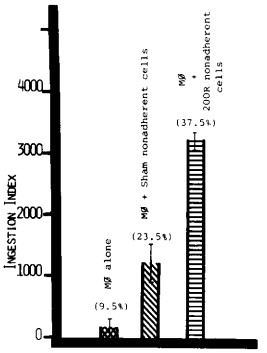

FIG. 4. Effect of coculture of resident Mϕ with nonadherent cells from mice WBI with 200 rad. Each point represents the mean value ± SD of five coverslips. Numbers in parentheses are the actual percentages of Mϕ ingesting E(IgM)C3b. LPS-stimulated Mϕ index = 4080.0 ± 132.0.

irradiation in their studies whereas, by contrast, the current study revealed that augmented ingestion via the C3b receptor was maximal at sublethal doses and absent at lethal doses. The mechanism for this observation is unknown.

The capacity of Mϕ from sublethally irradiated mice to express augmented C3b-mediated ingestion occurred rapidly within 20 min after WBI. This rapid occurrence coupled with the fact that maximal ingestion occurred at lower exposure doses precludes a role for the release of endotoxins due to intestinal epithelium damage.

The time post-WBI at which activated Mϕ are found is short, by 24 hr no Mϕ were found with augmented ingestion activity in the peritoneum. This was also seen *in vivo* during carbon clearance. The carbon clearance kinetics were similar to the ingestion of E(IgM)C3b in that both occurred within hours post-irradiation and were extinguished by 24 hr.

Such a signal may be derived by an unknown mechanism from lymphocytes stimulated either directly or indirectly, by the ionizing irradiation. Stimulation of Mϕ has been shown to occur via lymphocytes or their products (reviewed in (16)); however, Mϕ stimulation can occur through the direct action of certain natural products and fibroblast-derived interferon (17). The data of Fig. 4 support the former by the demonstration of the conversion of resident Mϕ from cells devoid of C3b-receptor-mediated ingestion to cells capable of ingestion of E(IgM)C3b following coculture with the nonadherent peritoneal cells obtained from mice exposed to 200 rad. It should be noted that a limited amount of Mϕ ingestion occurs upon cocultivation of resident Mϕ with normal washout lymphocytes. This may reflect the stimulating effects of ubiquitous, but small amounts, of endotoxin. Doses as little as 0.01 μg of endotoxin in culture of normal lymphocytes and resident Mϕ result in enhanced spreading and ingestion of E(IgM)C3b by the Mϕ (D. M. Wrigley and P. H. Saluk, in preparation). Although it is presently unknown if Mϕ can be stimulated to enhanced ingestion by the direct action of irradiation, these data imply that one mechanism is through the inductive process supplied by stimulated lymphocytes. Recently, a unique T-cell-derived lymphokine has been described which stimulates C3b-receptor-mediated ingestion *in vitro* (18). Preliminary *in vitro* irradiation experiments suggest that a direct effect of irradiation is ineffective. The fact that activated Mϕ are not found in the peritoneum 24 hr following WBI may reflect the transient nature of the signal supplied by the irradiation-stimulated lymphocyte. This is very speculative and current studies are aimed at exploring these issues by total *in vitro* irradiation of lymphocytes and resident Mϕ.

The current study confirms the observation of Schultz and co-workers (12) which show that Mϕ activation occurs following WBI and extends their study to include the parameter of augmented ingestion of E(IgM)C3b. In contrast to their observations, however, the present study using C3b-mediated ingestion, indicates that sublethal rather than high doses of irradiation leads to Mϕ activation. In addition, evidence was presented which showed that irradiation-induced Mϕ activation was not limited to the peritoneal Mϕ but was expressed systemically. Furthermore the data showed that one mechanism by which irradiation-induced Mϕ activation occurs is mediated through stimulated lymphocytes.

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