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The Role of ROS in Ionizing Radiation-Induced VLA-4 Mediated Adhesion of RAW264.7 Cells to VCAM-1 Under Flow Conditions

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Abstract

Alteration of adhesion molecule expression on endothelial cells has a direct connection with ionizing radiation-induced atherosclerosis, which is an adverse effect observed after radiotherapy. However, minimal attention has been given to monocytes/macrophages role in atherosclerosis development, which are exposed to the radiation at the same time. Under flow conditions using a parallel plate flow chamber to mimic physiological shear stress, we demonstrate here that the avidity between very late antigen-4 (VLA-4) of RAW264.7 cells and its ligand vascular cell adhesion molecule-1 (VCAM-1), was increased after low dose (0.5 Gy) irradiation, but was reduced after higher dose (5 Gy) treatment of ionizing radiation despite the fact that the surface expression of VLA-4 was up-regulated at 5 Gy of ionizing radiation. Treating the cells with free radical scavenger N-acetylcysteine had no effect on VLA-4 expression, but did reduce the avidity between RAW264.7 cells and VCAM-1 to a similar level, independent of ionizing radiation dose. The effect of H₂O₂ treatment (from 1–100 μ M) on RAW264.7 cell adhesion to VCAM-1 generated a similar bell-shaped graph as ionizing radiation. These results suggest that ionizing radiation regulates adhesive interactions between VLA-4 and VCAM-1, and that reactive oxygen species might function as a regulator, for this increased adhesiveness but with altered expression of integrin not play a major role.

INTRODUCTION

Radiation therapy in combination with surgery is a powerful weapon against cancer. Unfortunately, some side effects following radiation therapy have been documented. Radiation therapy treatment has been linked closely to cardiovascular diseases, including pericarditis (1), cardiomyopathy (2), valvular disease (3), as well as thrombosis and atherosclerosis (4, 5). Research has demonstrated that chest irradiation is a major risk factor for cardiovascular disease: typical examples include radiation effects on Hodgkin lymphoma (6, 7) and breast cancer (8). Additionally, left-sided radiation therapy in breast cancer leads to a higher cardiovascular risk (9). Despite conflicting data, the comparison of older and newer radiation techniques demonstrate the benefits of reducing radiation dose to the chest (10).

Atherosclerosis has been shown to be as an inflammatory disease involving monocytes and macrophages (11). Low-density lipoprotein accumulates on the endothelium and acts as the initiator of atherosclerosis after being oxidized. Oxidized low-density lipoprotein (OxLDL) induces the expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) on different endothelial cells (12). Integrins, the

heterodimeric receptors composed of different α and β subunits (13) play major roles in the development of atherosclerosis (14). Inflammatory monocytes' adhesion involves very late antigen-4 (VLA-4, $\alpha 4\beta 1$ integrins) and $\beta 2$ integrins, although there are conflicting reports as to which molecule plays a more significant role during inflammation (15–18). After infiltration, monocytes can differentiate into macrophages and appear to be involved in development of atherogenesis. Macrophage homing to the plaque is also responsible for degradation and rupture (19). E-selectin, ICAM-1 and $\alpha 4\beta 1$ integrins, as well as $\alpha 4$ integrin and ICAM-1, also play important roles in macrophage homing (20). It has been shown that in IL-4 induced leukocytes recruitment, $\alpha 4$ integrins dominate the adhesion when endothelial selectins are blocked (21). Furthermore, blocking of VLA-4 effectively reduced the cell adhesion and therefore reduced plaque formation (22, 23).

Over the last two decades, extensive studies have confirmed that the endothelium is damaged by ionizing radiation (IR) and that it facilitates leukocytes, lymphocytes and platelet adhesion by up-regulation of ICAM-1, E-selectin, von Willebrand factor (vWF) and platelet endothelial cell adhesion molecule-1 (PECAM-1) (24–27). However, not enough attention has been given to how IR affects integrins in monocytes/macrophages, since during radiotherapy, not only the vessels, but also the blood cells are irradiated. In light of the importance of VLA-4 in the monocytes/macrophages adhesion, we investigated the role of VLA-4 mediated adhesion under flow conditions, our studies complement the current investigations that focus on the role of endothelial cells in radiation-associated atherosclerosis.

MATERIALS AND METHODS

Cell Culture

RAW264.7 mouse monocytes/macrophages obtained from the American Type Culture Collection (Manassas, VA) were cultured in Dulbecco's modified Eagle's medium (DMEM, Corning, Manassas, VA), containing 10% fetal bovine serum (Denville, Metuchen, NJ) and 1% penicillin and streptomycin (Invitrogen, Carlsbad, CA) at 37°C with 5% CO₂.

Flow Cytometry

Surface expression of integrins on RAW264.7 cells was evaluated by flow cytometry. The cells were pelleted by centrifugation, resuspended in phosphate-buffered saline (PBS) and fixed in 4% formaldehyde for 10 min. From each sample, 1×10^6 cells were rinsed, blocked with 5% bovine serum albumin (BSA) and incubated with FITC-conjugated anti- $\alpha 4$ integrin (CD49d, 56084, BD Biosciences, Franklin Lakes, NJ) or FITC-conjugated anti- $\beta 1$ integrin (CD29, sc-9970 FITC, Santa Cruz Biotechnology, Santa Cruz, CA) for 30 min in the dark. After washing with 5% BSA twice, cells were suspended in 0.5 ml PBS and analyzed on FACSsort flow cytometer (BD Biosciences).

Western Blotting

RAW264.7 cells were lysed in 2% NP-40 buffer (2% NP-40, 80 mM NaCl, 0.1% SDS) containing protease inhibitor cocktail set III (Millipore, Darmstadt, Germany). After 15 min incubation on ice, the lysates were centrifuged and the supernatants were collected. The proteins (100 μ g) were electrophoresed on an 8% SDS-PAGE, transferred to a nitrocellulose membrane and were blocked with 5% nonfat milk in tris-buffered saline with Tween (TBS-T) (USB, Cleveland, OH) for 1 h. Blots were incubated with anti-human $\alpha 4$ integrin (sc-14008, Santa Cruz Biotechnology) or anti-human $\beta 1$ integrin (sc-9970, Santa Cruz Biotechnology) at 4°C overnight. Membranes were washed with TBS-T three times and were probed with corresponding secondary antibodies for 0.5 h at room temperature. Blots were then washed with TBS-T three times and with tris-buffered saline (TBS) twice. The

signals were detected using West Pico Supersignal chemiluminescent substrate (Pierce, Rockford, IL).

Parallel Plate Flow Chamber Adhesion Assays

Dynamic flow adhesion assays were performed as previously described (28). Briefly, cell culture dishes were coated with 25 $\mu\text{g/mL}$ VCAM-1 (R&D Systems, Minneapolis, MN) at 4°C overnight. Coated dishes were blocked with 0.1% BSA in Dulbecco's phosphate-buffered saline (DPBS) (Invitrogen) for 2 h at room temperature and were then washed with DPBS. RAW264.7 cells ($1 \times 10^6/\text{mL}$) in DPBS were perfused through the chamber for 5 min at a shear stress of 1.2 dynes/cm². Cell motility was observed and recorded at a 4 $\times$ magnification using a Qimaging retiga 1300 videomicroscopy (Qimaging, BC, Canada). The numbers of adherent cells were quantified from the recorded video.

Statistical Analysis

Student's *t* test was performed to compare data in the different treatment groups. Data was considered statistically significant when $P < 0.05$.

RESULTS

IR Increases Surface Expression of $\alpha 4$ and $\beta 1$ Integrins in RAW264.7 Cells

Since VLA-4 ($\alpha 4\beta 1$ integrins) plays a major role in adhesion between monocytes/macrophages and endothelial cells (20, 22), we first determined the extent of the effect of IR on the cell surface expression of $\alpha 4$ and $\beta 1$ integrins. Flow cytometry analysis revealed that both $\alpha 4$ and $\beta 1$ integrins were increased on the cell surface after irradiation (5 Gy) (Fig. 1A and B). Despite the fact that there was only a slight difference at 8 h, both $\alpha 4$ and $\beta 1$ integrin peaked by 24 h after irradiation. Prolonged incubation time did not further increase the surface expression level of the proteins (Fig. 1A and B). Western blot analysis indicated that, while the expression of total $\alpha 4$ integrin was significantly increased, total $\beta 1$ integrin was not changed at 24 h postirradiation (Fig. 1C). These results suggest that the increased surface expression of an integrin is not always dependent on an induced expression of that specific integrin after irradiation.

IR-Altered Avidity of RAW264.7 Cells to VCAM-1 is Not Correlated to the Surface Expression of VLA-4 Integrins

The extent of the effect of IR-induced surface expression of VLA-4 on macrophage adhesion to VCAM-1, the receptor of VLA-4, was assessed using a parallel plate flow chamber assay. Physiological shear stress from 1 to 2 dynes/cm² was examined (29), and a shear stress of 1.2 dynes/cm² was adopted, under these condition there was a stable and countable amount of adhesion cells in each field of view. Our data indicated that the number of adherent cells to VCAM-1 was reduced by $19.7 \pm 2.7\%$ and $28.4 \pm 4.0\%$ at 8 and 24 h, respectively, after 5 Gy of ionizing radiation (Fig. 2A), and is not correlated to the increased VLA-4 expression after the same dose of ionizing radiation (Fig. 1A and B). Our data also revealed that while the affinity between RAW264.7 cells and VCAM-1 was decreased in a dose-dependent manner from 1–5 Gy at 24 h postirradiation, the affinity between the cells and VCAM-1 was increased by $17.3 \pm 4.5\%$ after being treated with 0.5 Gy at 24 h postirradiation (Fig. 2B).

To determine whether the reduced avidity of the macrophage after higher doses of IR treatment was due to the loss of viability of the cells, we analyzed the percentage of cell death after irradiation. Our data showed that the percentage of dead cells was only increased 11% 24 h after 5 Gy of IR and had no statistically significant change after lower doses (0.5 and 1 Gy) of IR (Fig. 2C). These results indicated that clinical doses of radiation do not

result in remarkable cell death at 24 h postirradiation (Fig. 2C), which agreed with a previous report indicating that macrophages are very resistant to IR (30). These results also suggest that the altered avidity of a macrophage to VCAM-1 is not correlated to the IR-induced surface expression of VLA-4 and cell death.

The IR-Altered Avidity of RAW264.7 Cells to VCAM-1 is ROS-Dependent

Since IR has been shown to generate free radicals, which play a significant role in regulating cell signaling after irradiation (31), we examined the extent of the effect of L-NAC, a free radical scavenger, on the adhesion of RAW264.7 cells perfused over a VCAM-1-coated chamber under the flow condition as described above. Our data showed that the avidities of L-NAC-treated RAW264.7 cells to VCAM-1 were reduced approximately 46% with no statistical differences among sham, 0.5 or 5.0 Gy of IR (Fig. 3A). Analysis of the surface expression of VLA-4 integrin indicated that $\alpha 4$ and $\beta 1$ integrin on the surface of RAW264.7 cells were not affected by the L-NAC treatment (Fig. 3B and C). These results suggested that the IR-altered avidity of RAW264.7 cells involves free radicals, but does not involve the cell surface expression of VLA-4 integrin.

To determine whether reactive oxygen species (ROS) exert a direct effect on VLA-4 mediated adhesion, we determined the extent of the effect of exogenous H_2O_2 on the avidity of RAW264.7 cells to VCAM-1. Our data showed that after exogenous H_2O_2 treatment, the avidity of RAW264.7 cells to VCAM-1 was increased by $15.3 \pm 6.0\%$ after treatment with $5 \mu M H_2O_2$ and was then reduced by $20.3 \pm 7.6\%$ or $46.0 \pm 11.4\%$ after treating with 50 or $100 \mu M H_2O_2$, respectively, at 0.5 h post-treatment (Fig. 4). These data further indicate that adhesion of RAW264.7 cells to VCAM-1 is ROS-dependent and follows a bell-shaped dose response.

DISCUSSION

While the biological effect of IR on the adhesion molecules of endothelial cells has been studied (32–35), little is known regarding the contributions of irradiated monocytes/macrophages to radiotherapy-associated side effects. Macrophages are known as radioresistant leukocytes that can tolerate high doses of IR (36). Previous work has shown that ICAM-1 and LFA-1 on alveolar macrophages increased one week after IR (37). However, the early response (within 24 h) of monocytes/macrophages to IR in contributing to their avidity to endothelial cells has not been well characterized. Therefore, in this study, we first determined the extent of the effects of IR on the expression and surface localization of VLA-4 ($\alpha 4\beta 1$) integrin, the major integrin that mediates the adhesion between monocytes/macrophages and endothelial cells (20, 22). Our data indicated that the surface levels of both $\alpha 4$ and $\beta 1$ integrins were increased. However, while the total expression of $\alpha 4$ integrin was increased, $\beta 1$ integrin was not changed at 24 h postirradiation (5 Gy) (Fig. 1). The results suggested that IR induces translocation of $\beta 1$ integrin to the surface of macrophages. However, while further investigation indicated that the increased surface VLA-4 was not correlated to an increased avidity of RAW264.7 cells to VCAM-1, the ligand of VLA-4 (Fig. 1 compared to Fig. 2). The results suggested that a conformation change of integrin, which has been shown to play an important role in cell adhesion (38–42), might be a major contributor for IR-induced alternation of cell adhesion. A dose-dependent analysis of the response of RAW264.7 cells to IR revealed that the avidity between RAW264.7 cells and VCAM-1 was increased with a relatively lower dose (0.5 Gy) and reduced with a relatively higher dose (1–5 Gy) of IR (Fig. 2B). The reduction of the avidity was not likely due to cell death after irradiation because the percentage of reduced avidity was much higher than the percentage of reduced viability of the cells (Fig. 2B compared to 2C). These results suggested that factor(s) other than VLA-4 were involved in the regulation of the avidity of RAW264.7 cells. While our data indicated that the avidity between

RAW264.7 cells and VCAM-1 was increased after 0.5 Gy IR and was decreased after 5 Gy IR, it does not suggest that a lower dose of radiation is worse than a higher dose for IR-induced atherosclerosis, since a high dose of IR has been shown to induce the expression of various adhesion molecules on endothelial cells (43).

It has been previously reported that sulfhydryl groups involved in VLA-4 mediated adhesion can be regulated by ROS (44). The VLA-4 mediated avidity of human leukocytes to VCAM-1 was increased and then decreased in response to a treatment of 5–100 μ M exogenous H_2O_2 (44). Considering that IR generates ROS in a dose dependent manner (45), we determined the effect of L-NAC (a free radical scavenger) on the IR-induced and VLA-4 mediated avidity of RAW264.7 cells. Our data demonstrated that, while the treatment of L-NAC did not alter the surface expression of VLA-4 in the presence or absence of IR (Fig. 3B and C), it reduced the avidity of RAW264.7 cells to VCAM-1 to a similar level, regardless of whether the cells were treated or not treated with IR (Fig. 3A). The L-NAC decreased avidity of the cells was likely due to the reduction of ROS, but not to reactive nitrogen species (RNS), since IR did not induce the expression of any one of the three nitric oxide synthases in RAW264.7 cells (data not shown). To further investigate the role of ROS in regulation of VLA-4 mediated adhesion, the avidity of RAW264.7 cells, which were treated with 1–100 μ M exogenous H_2O_2 , were determined by a parallel flow chamber assay. Consistent with our expectation, the avidity of RAW264.7 cells to VCAM-1 in responding to exogenous H_2O_2 was increased at lower (5 μ M) [H_2O_2] irradiation and then decreased at higher (50–100 μ M) [H_2O_2] irradiation (Fig. 4), which is similar to what has been documented in human leukocytes (44). In conclusion, we have demonstrated that VLA-4 mediated adhesion of RAW264.7 cells to VCAM-1 is altered by IR under flow conditions and ROS plays a key role in this process. Additionally, basal level of ROS appears to be required for maintaining normal affinity between VLA-4 and VCAM-1.

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References

1. Martin RG, Ruckdeschel JC, Chang P, Byhardt R, Bouchard RJ, Wiernik PH. Radiation-related pericarditis. *Am J Cardiol.* 1975; 35(2):216–20. [PubMed: 1119380]
2. Tolba KA, Deliargyris EN. Cardiotoxicity of cancer therapy. *Cancer Invest.* 1999; 17(6):408–22. [PubMed: 10434952]
3. Brand MD, Abadi CA, Aurigemma GP, Dauerman HL, Meyer TE. Radiation-associated valvular heart disease in Hodgkin's disease is associated with characteristic thickening and fibrosis of the aorticmitral curtain. *J Heart Valve Dis.* 2001; 10(5):681–5. [PubMed: 11603609]
4. Hoving S, Heeneman S, Gijbels MJ, Te Poele JA, Russell NS, Daemen MJ, et al. Single-dose and fractionated irradiation promote initiation and progression of atherosclerosis and induce an inflammatory plaque phenotype in ApoE(–/–) mice. *Int J Radiat Oncol Biol Phys.* 2008; 71(3):848–57. [PubMed: 18514779]
5. Stewart FA, Heeneman S, Te Poele J, Kruse J, Russell NS, Gijbels M, et al. Ionizing radiation accelerates the development of atherosclerotic lesions in ApoE–/– mice and predisposes to an inflammatory plaque phenotype prone to hemorrhage. *Am J Pathol.* 2006; 168(2):649–58. [PubMed: 16436678]
6. Ramyar A, Shafiei M, Moazzami K, Rezaei N. Severe valvular toxicity and pericarditis early after radiation therapy in a patient treated for Hodgkin's lymphoma. *Turk J Pediatr.* 2010; 52(4):423–5.

7. Castellino SM, Geiger AM, Mertens AC, Leisenring WM, Tooze JA, Goodman P, et al. Morbidity and mortality in long-term survivors of Hodgkin lymphoma: a report from the childhood cancer survivor study. *Blood*. Oct 29.
8. Gyenes G, Rutqvist LE, Liedberg A, Fornander T. Long-term cardiac morbidity and mortality in a randomized trial of pre- and postoperative radiation therapy versus surgery alone in primary breast cancer. *Radiother Oncol*. 1998; 48(2):185–90. [PubMed: 9783890]
9. Correa CR, Litt HI, Hwang WT, Ferrari VA, Solin LJ, Harris EE. Coronary artery findings after left-sided compared with right-sided radiation treatment for early-stage breast cancer. *J Clin Oncol*. 2007; 25(21):3031–7. [PubMed: 17634481]
10. Prosnitz RG, Chen YH, Marks LB. Cardiac toxicity following thoracic radiation. *Semin Oncol*. 2005; 32(2 Suppl 3):S71–80. [PubMed: 16015539]
11. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Eng J Med*. 2005; 352(16):1685–95.
12. Kume N, Cybulsky MI, Gimbrone MA Jr. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. *J Clin Invest*. 1992; 90(3):1138–44. [PubMed: 1381720]
13. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell*. 2002; 110(6):673–87. [PubMed: 12297042]
14. Lu X, Lu D, Scully MF, Kakkar VV. The role of integrin-mediated cell adhesion in atherosclerosis: pathophysiology and clinical opportunities. *Curr Pharm Des*. 2008; 14(22):2140–58. [PubMed: 18781968]
15. Meerschaert J, Furie MB. The adhesion molecules used by monocytes for migration across endothelium include CD11a/CD18, CD11b/CD18, and VLA-4 on monocytes and ICAM-1, VCAM-1, and other ligands on endothelium. *J Immunol*. 1995; 154(8):4099–112. [PubMed: 7535821]
16. Rosen H. Role of CR3 in induced myelomonocytic recruitment: insights from in vivo monoclonal antibody studies in the mouse. *J Leukoc Biol*. 1990; 48(5):465–9. [PubMed: 2230596]
17. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol*. 2007; 7(9):678–89. [PubMed: 17717539]
18. Cybulsky MI, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, et al. A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. *J Clin Invest*. 2001; 107(10):1255–62. [PubMed: 11375415]
19. Libby P. Changing concepts of atherogenesis. *J Intern Med*. 2000; 247(3):349–58. [PubMed: 10762452]
20. Patel SS, Thiagarajan R, Willerson JT, Yeh ET. Inhibition of alpha4 integrin and ICAM-1 markedly attenuate macrophage homing to atherosclerotic plaques in ApoE-deficient mice. *Circulation*. 1998; 97(1):75–81. [PubMed: 9443434]
21. Hickey MJ, Granger DN, Kubes P. Molecular mechanisms underlying IL-4-induced leukocyte recruitment in vivo: a critical role for the alpha 4 integrin. *J Immunol*. 1999; 163(6):3441–8. [PubMed: 10477616]
22. Shih PT, Brennan ML, Vora DK, Territo MC, Strahl D, Elices MJ, et al. Blocking very late antigen-4 integrin decreases leukocyte entry and fatty streak formation in mice fed an atherogenic diet. *Circ Res*. 1999; 84(3):345–51. [PubMed: 10024309]
23. Huo Y, Weber C, Forlow SB, Sperandio M, Thatte J, Mack M, et al. The chemokine KC, but not monocyte chemoattractant protein-1, triggers monocyte arrest on early atherosclerotic endothelium. *J Clin Invest*. 2001; 108(9):1307–14. [PubMed: 11696575]
24. Hallahan D, Kuchibhotla J, Wyble C. Cell adhesion molecules mediate radiation-induced leukocyte adhesion to the vascular endothelium. *Cancer Res*. 1996; 56(22):5150–5. [PubMed: 8912850]
25. Quarmby S, Hunter RD, Kumar S. Irradiation induced expression of CD31, ICAM-1 and VCAM-1 in human microvascular endothelial cells. *Anticancer Res*. 2000; 20(5B):3375–81. [PubMed: 11131637]
26. Heckmann M, Douwes K, Peter R, Degitz K. Vascular activation of adhesion molecule mRNA and cell surface expression by ionizing radiation. *Exp Cell Res*. 1998; 238(1):148–54. [PubMed: 9457067]

27. Jahroudi N, Ardekani AM, Greenberger JS. Ionizing irradiation increases transcription of the von Willebrand factor gene in endothelial cells. *Blood*. 1996; 10:3801–14. [PubMed: 8916944]
28. Burdick MM, Chu JT, Godar S, Sackstein R. HCELL is the major E- and L-selectin ligand expressed on LS174T colon carcinoma cells. *J Biol Chem*. 2006; 281(20):13899–905. [PubMed: 16565092]
29. Jones DA, Smith CW, McIntire LV. Leucocyte adhesion under flow conditions: principles important in tissue engineering. *Biomaterials*. 1996; 17(3):337–47. [PubMed: 8745331]
30. Gong Y, Hu L, Wu D. Effect of ⁶⁰Co gamma-irradiation on the nonspecific cytotoxicity of alveolar macrophages in vitro. *Environ Health Perspect*. 1992; 97:167–70. [PubMed: 1396453]
31. Riley PA. Free radicals in biology: oxidative stress and the effects of ionizing radiation. *Int J Radiat Biol*. 1994; 65(1):27–33. [PubMed: 7905906]
32. Stewart FA, Hoving S, Russell NS. Vascular damage as an underlying mechanism of cardiac and cerebral toxicity in irradiated cancer patients. *Radiat Res*. 2010; 174(6):865–869. [PubMed: 21128810]
33. Kiyohara H, Ishizaki Y, Suzuki Y, Katoh H, Hamada N, Ohno T, et al. Radiation-induced ICAM-1 expression via TGF-beta1 pathway on human umbilical vein endothelial cells; comparison between X-ray and carbon-ion beam irradiation. *J Radiat Res*. 2011; 52(3):287–92. [PubMed: 21343678]
34. Wu KL, Tu B, Li YQ, Wong CS. Role of intercellular adhesion molecule-1 in radiation-induced brain injury. *Int J Radiat Oncol Biol Phys*. 2012; 76(1):220–8. [PubMed: 20005455]
35. Ning S, Chen Z, Dirks A, Husbeck B, Hsu M, Bedogni B, et al. Targeting integrins and PI3K/Akt-mediated signal transduction pathways enhances radiation-induced anti-angiogenesis. *Radiat Res*. 2007; 168(1):125–33. [PubMed: 17722999]
36. Pons I, Gras G, Courberand S, Benveniste O, Dormont D. Consequences of gamma-irradiation on inflammatory cytokine regulation in human monocytes/macrophages. *Int J Radiat Biol*. 1997; 71(2):157–66. [PubMed: 9120351]
37. Kawana A, Shioya S, Katoh H, Tsuji C, Tsuda M, Ohta Y. Expression of intercellular adhesion molecule-1 and lymphocyte function-associated antigen-1 on alveolar macrophages in the acute stage of radiation-induced lung injury in rats. *Radiat Res*. 1997; 147(4):431–6. [PubMed: 9092922]
38. Chigaev A, Waller A, Zwartz GJ, Buranda T, Sklar LA. Regulation of cell adhesion by affinity and conformational unbending of alpha4beta1 integrin. *J Immunol*. 2007; 178(11):6828–39. [PubMed: 17513731]
39. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell*. 1992; 69(1):11–25. [PubMed: 1555235]
40. Guerrero-Esteo M, Ruiz-Velasco N, Munoz M, Teixido J. Role of two conserved glycine residues in the beta-propeller domain of the integrin alpha4 subunit in VLA-4 conformation and function. *FEBS Lett*. 1998; 429(1):123–8. [PubMed: 9657396]
41. Pujades C, Teixido J, Bazzoni G, Hemler ME. Integrin alpha 4 cysteines 278 and 717 modulate VLA-4 ligand binding and also contribute to alpha 4/180 formation. *Biochem J*. 1996; 313(Pt 3): 899–908. [PubMed: 8611173]
42. Munoz M, Serrador J, Nieto M, Luque A, Sanchez-Madrid F, Teixido J. A novel region of the alpha4 integrin subunit with a modulatory role in VLA-4-mediated cell adhesion to fibronectin. *Biochem J*. 1997; 327(Pt 3):727–33. [PubMed: 9581549]
43. Prabhakarpandian B, Goetz DJ, Swerlick RA, Chen X, Kiani MF. Expression and functional significance of adhesion molecules on cultured endothelial cells in response to ionizing radiation. *Microcirculation*. 2001; 8(5):355–64. [PubMed: 11687947]
44. Liu SY, Tsai MY, Chuang KP, Huang YF, Shieh CC. Ligand binding of leukocyte integrin very late antigen-4 involves exposure of sulfhydryl groups and is subject to redox modulation. *Eur J Immunol*. 2008; 38(2):410–23. [PubMed: 18196516]
45. Leach JK, Van Tuyle G, Lin PS, Schmidt-Ullrich R, Mikkelsen RB. Ionizing radiation-induced, mitochondria-dependent generation of reactive oxygen/nitrogen. *Cancer Res*. 2001; 61(10):3894–901. [PubMed: 11358802]

FIG. 1. Expression of $\alpha 4$ and $\beta 1$ integrins in RAW264.7 cells after irradiation with ionizing radiation. RAW264.7 cells were processed for flow cytometry at 8, 24 and 48 h after 5 Gy irradiation. Panel A: Surface expression of $\alpha 4$ integrin. Panel B: Surface expression of $\beta 1$ integrin. Panel C: Western blot analysis of $\alpha 4$ and $\beta 1$ integrin. The whole RAW264.7 cell lysate was prepared at 24 h postirradiation.

FIG. 2.

Adhesion of irradiated RAW264.7 cells to VCAM-1 under flow conditions. Cell numbers were counted after trypan blue staining and were adjusted to $5 \times 10^6/\text{mL}$. The cells were perfused over the chamber coated with VCAM-1 at a physiological stress of $1.2 \text{ dynes}/\text{cm}^2$. Relative amounts of adherent cells were determined after 5 min perfusion. Panel A: RAW264.7 cells were irradiated with 5 Gy and the cell adhesion was analyzed at 8 and 24 h postirradiation. Panel B: RAW264.7 cells were irradiated with 0.5, 1 or 5 Gy. The cell adhesion was analyzed at 24 h postirradiation. Panel C: Percentage of cell death was determined by trypan blue staining at 24 h postirradiation. Data shown are the means $\pm$ SD from three independent experiments. * $P < 0.05$.

FIG. 3.

Effect of L-NAC on VLA-4 mediated adhesion of RAW264.7 cells under flow conditions. RAW264.7 cells were pretreated with 20 mM L-NAC for 1 h prior to 0.5 and 5 Gy γ irradiation. At 24 h postirradiation, cells were counted after trypan blue staining to reach the same concentration ($5 \times 10^6/\text{mL}$) and perfused over the flow chamber at a physiological stress of $1.2 \text{ dynes}/\text{cm}^2$. Panel A: Relative amount of adherent cells after 5 min perfusion. Data shown are the means $\pm$ SD from three independent experiments. $*P < 0.05$. Panel B: Surface expression of $\alpha 4$ and (panel C) $\beta 1$ integrin was analyzed by flow cytometry at 24 h postirradiation (5 Gy) in the presence or absence of L-NAC (20 mM).

FIG. 4.

H₂O₂ regulates avidity of VLA-4 in RAW264.7 cells under flow conditions. Cells were treated with 1, 5, 50 and 100 μ M H₂O₂ and harvested after 30 min incubation. Cells were counted after trypan blue staining and adjusted to 5×10^6 /mL. Then the cells were perfused over the chamber coated with VCAM-1 at a shear stress of 1.2 dynes/cm². Cells that adhered to the chamber were counted after 5 min perfusion. Data shown are the means $\pm$ SD from three independent experiments. * $P < 0.05$.