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# SIGN-R1 and complement factors are involved in the systemic clearance of radiation-induced apoptotic cells in whole-body irradiated mice

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ABSTRACT

Although SIGN-R1-mediated complement activation pathway has been shown to enhance the systemic clearance of apoptotic cells, the role of SIGN-R1 in the clearance of radiation-induced apoptotic cells has not been characterized and was investigated in this study. Our data indicated that whole-body γ-irradiation of mice increased caspase-3$^+$ apoptotic lymphocyte numbers in secondary lymphoid organs. Following γ-irradiation, SIGN-R1 and complements (C4 and C3) were simultaneously increased only in the mice spleen tissue among the assessed tissues. In particular, C3 was exclusively activated in the spleen. The delayed clearance of apoptotic cells was markedly prevalent in the spleen and liver of SIGN-R1 KO mice, followed by a significant increase of CD11b$^+$ cells. These results indicate that SIGN-R1 and complement factors play an important role in the systemic clearance of radiation-induced apoptotic innate immune cells to maintain tissue homeostasis after γ-irradiation.



## 1. Introduction

Billions of cells undergo apoptosis as a part of physiological homeostasis (50 ~ 70 × $10^9$/each day) and are rapidly removed by professional phagocytes in higher organisms [1]. Delayed clearance of apoptotic cells have been linked with various inflammatory diseases and autoimmune diseases such as atherosclerosis, systemic lupus erythematosus, and rheumatoid arthritis [2–4]. Therefore, the rapid clearance of apoptotic cells by phagocytes is important for maintaining tissue homeostasis and for promoting an anti-inflammatory response in an effort to prevent an immune response against self-antigens [5].

In higher organisms, complements, such as C1q and mannose binding lectin, directly bind to apoptotic cells and mediate the opsonization of C3 on apoptotic cells [6]. In addition, professional phagocytes, such as macrophages and immature DCs, efficiently remove billions of apoptotic cells that are generated as a part of physiological homeostasis [7]. Transmembrane pattern-recognition receptors, including specific intercellular adhesion molecule-3-

*Abbreviations:* SIGN-R1, Specific intercellular adhesion molecule-3-grabbing nonintegrin-related gene 1; C3, complement component C3; C4, complement Component C4; CD11b, Cluster of differentiation 11b; FITC, Fluorescein isothiocyanate; CFSE, Carboxyfluorescein succinimidyl ester; CVF, Cobra venom factor.

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grabbing nonintegrin-related gene 1 (SIGN-R1), are involved in the clearance of apoptotic cells [8]. SIGN-R1, a murine homolog of human dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), has been shown to bind to C1q on splenic marginal zone (MZ) macrophages and also enhance C3 deposition on apoptotic cells for promoting systemic apoptotic cell clearance [8].

Over the last century, ionizing radiation has been used for treating a variety of human cancers [9]. Although the aim of radiotherapy is to induce apoptotic and non-apoptotic cell death in cancer cells, radiotherapy inadvertently also results in the damage of normal tissues [10]. Lymphoid organs, such as the lymph nodes, thymus, and spleen, are highly radiation-sensitive [11]. Thus, radiation treatment can functionally alter the immune system and break self-tolerance against apoptotic cells, causing various organ-specific autoimmune diseases such as gastritis, thyroiditis, and orchitis [12]. Although apoptotic lymphocytes are rapidly cleared from the radiation field under physiological conditions [11], the mechanisms involved in the clearance of radiation-induced apoptotic cells remain largely unknown.

Here, we investigated the role of SIGN-R1 and complements in the systemic clearance of radiation-induced apoptotic cells *in vivo* and assessed the detailed clearance mechanism of radiation-induced apoptotic cells.

## 2. Materials and methods

### 2.1. Mice, cells, and reagents

C57BL/6 mice were purchased from B&K Universal Limited (Hull, England). SIGN-R1 KO mice were kindly provided by The Consortium for Functional Glycomics (CFG, http://www.functionalglycomics.org). To obtain C3-depleted mice, control mice were injected intraperitoneally with 60 U/kg of cobra venom factor (CVF) 1 day prior to the experimental challenge. All protocols for the animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Konkuk University (Permit Number: KU11107). Mouse lymphocytic leukemia cells (L1210) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics. The following materials were purchased: Carboxyfluorescein succinimidyl ester (CFSE; Molecular Probes/LifeTechnologies), Tissue-Tek OCT compound (Sakura Finetek Japan Co., Japan), CVF (Quidel Corporation, USA), Annexin-V (Phoenix Flow System), and Apoptag® (S7110; Millipore, Billerica, MA).

### 2.2. Antibodies and microscopy

Rabbit polyclonal antibody (PAb-C13) and hamster monoclonal antibody against SIGN-R1 (22D1) were obtained as described previously [13,14] The following antibodies were purchased: Anti-human C1q (Abcam), anti-mouse C3 (ICN Pharmaceuticals), mouse C4 (ICN Pharmaceuticals), anti-activated caspase-3 (Cell Signaling), anti-SER-4, anti-CD45R (B220), anti-TCRβ, anti-F4/80 and anti-CD11b (BD Biosciences Pharmingen). As secondary reagents, we used HRP-, FITC-, or phycoerythrin-conjugated immunoglobulins (Jackson ImmunoResearch Laboratories). Following whole-body γ-irradiation or after intravenously injecting apoptotic cells in mice, mouse tissues were snap frozen in OCT compound. After fixing with cold acetone, 10 μm of the cryosections of the tissues were cut at −20 °C and immunolabeled as described below (see Section 2.4) using the various antibodies (antibodies enlisted in Section 2.2) and analyzed using a fluorescence deconvolution microscope (Olympus Corporation, USA).

### 2.3. Irradiation and generation of apoptotic cells

Mice or cells received γ-irradiation using a $^{137}$Cs γ-ray source (Atomic Energy of Canada, Ltd., Ontario, Canada) at a dose rate of 3.2 Gy/min. Mice were sacrificed at 6 or 24 h and cryosections of tissues were prepared and then immunostained. L1210 mouse lymphoblast cells were exposed to 10 Gy of γ-irradiation and incubated for 5 days to induce apoptosis, which induced apoptosis in 30% of the treated lymphoblast cells. A single-cell thymocyte suspension ($10^7$ cells/ml of RPMI-1640 medium), which was prepared with cells obtained from freshly isolated thymus, was incubated with 3 mM dexamethasone and incubated overnight to induce apoptosis, which induced apoptosis in 70% of the treated thymocytes. Apoptosis induction for cells was confirmed by conducting FACS analysis, which was performed by staining the cells with Annexin-V and PI by following manufacturer's protocols. Apoptosis induction for tissues was confirmed by TUNEL assay following the manufacturer's protocols.

### 2.4. Immunoblot analysis

Tissues were lysed in NP-40 buffer [10% Glycerol, 50 mM HEPES (pH 7.4), 150 mM NaCl, 2 mM $CaCl_2$, 1% NP-40, and 1 mM PMSF that was supplemented with protease inhibitor cocktail (Sigma–Aldrich, St. Louis, MO). Each lysed sample was mixed with an equal volume of 5× sodium dodecyl sulfate (SDS) sample buffer with 5% of mercaptoethanol and boiled at 100 °C for 10 min. The proteins in the lysates were separated using SDS/PAGE gel electrophoresis. The resolved proteins on each gel were transferred onto a PVDF membrane, which was then incubated with a primary antibody solution. The PVDF membranes were then washed three times using a wash buffer. Antibody-reactive bands on the blots were visualized by incubating the membrane with peroxidase-labeled secondary antibodies followed by treatment with West-ZOL plus (Intron). Signal was detected by using LAS-4200 (Fuji film). Signal quantification was performed with ImageJ 1.47f (NIH) on unprocessed image sections.

## 3. Results

### 3.1. γ-irradiation induces apoptosis in lymphocytes, but not in macrophages, in the secondary lymphoid organs

Twenty-four hours after administering whole-body γ-irradiation (6.0 Gy), the C57BL/6 mice were sacrificed and cryosections of spleen, lymph nodes and thymus were prepared and then immunostained for SIGN-R1 along with either B220 (a marker of B cells) or TCRβ (a marker of T cells). Administration of whole-body γ-irradiation decreased both T- and B lymphocytes in the examined tissues (Fig. 1A). To characterize the underlying mechanisms involved in lowering the lymphocyte numbers, spleen sections obtained 6 h or 24 h post-irradiation were immunostained for detecting caspase-3 (a marker of early apoptotic cells) and SIGN-R1 (Fig. 1B). Caspase-3+ cells were remarkably increased in the white pulp (WP) within 6 h after irradiation, indicating that apoptosis was initiated immediately after irradiation (Fig. 1B). To further characterize the caspase-3 positive cells, the spleen sections were immunostained by using antibodies against caspase-3 and SIGN-R1 along with either B220 or TCRβ (Fig. 1B). Most of the B cells and a small population of T cells in the WP were caspase-3 positive (Fig. 1C), indicating that apoptosis is the primary reason for the disappearance of lymphocytes in the WP.

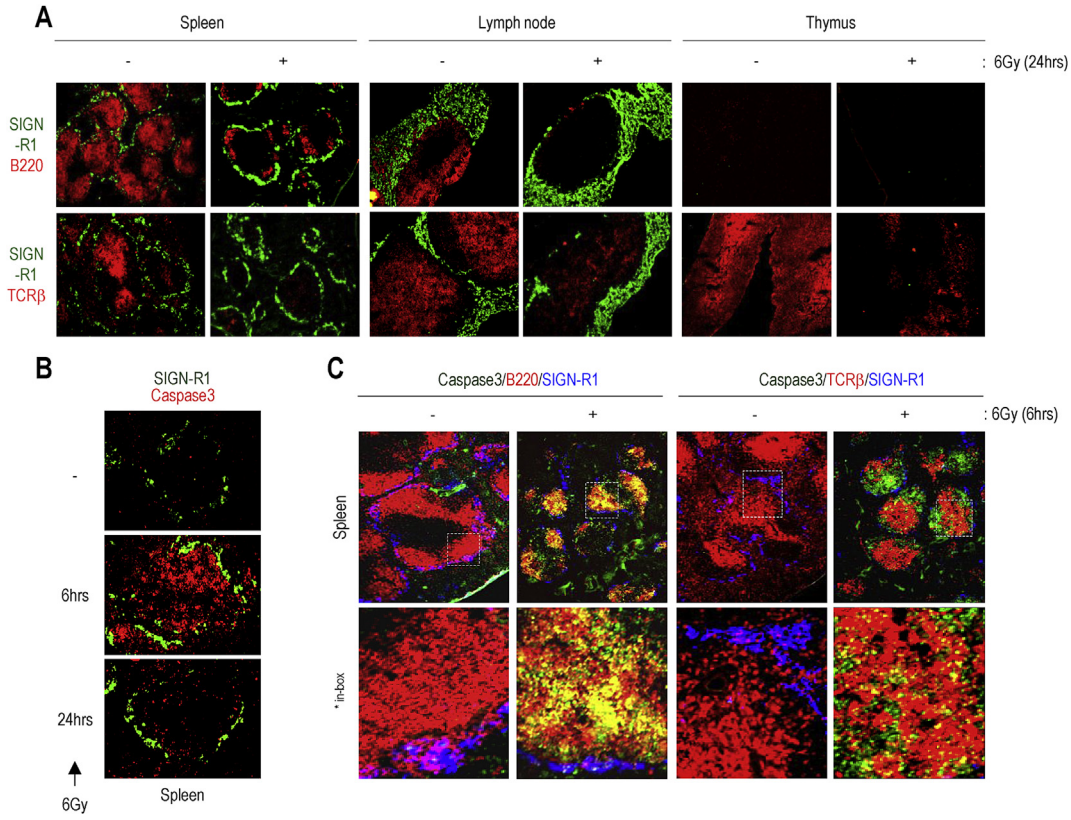


**Fig. 1.** The effects of whole-body γ-irradiation on lymphocytes and macrophages in secondary lymphoid organs. C57BL/6 mice were administered single whole-body γ-irradiation (6.0 Gy) at a dose rate of 3.2 Gy/min. Non-irradiated mice were used as control. (A) Twenty 4 h after γ-irradiation, the cryosections of spleen, lymph nodes, and thymus from control and γ-irradiated mice were immunostained for SIGN-R1 (green) along with either B220 (red, a marker of B cells) or TCRβ (red, a marker of T cells). (B) After 6 h or 24 h post-irradiation, the cryosections of spleens from control and γ-irradiated mice were immunostained for evaluating the expression of SIGN-R1 (green) and caspase-3 (red, a marker of early apoptotic cells). (C) After 6 h post-irradiation, the cryosections of spleens from control and γ-irradiated mice were immunostained for detecting SIGN-R1 (blue), caspase-3 (green), and B220 or TCRβ (red). The representative areas of the white pulp were enlarged in below in-boxes.

### 3.2. *Radiation-induced apoptotic cells increase and activate SIGN-R1 in the spleen*

To examine whether SIGN-R1 expression in the secondary lymphoid organ tissues is altered after administration of whole-body γ-irradiation, cryosections of spleen and lymph nodes of irradiated mice were obtained 6 h or 24 h post-irradiation and immunostained with antibodies against SIGN-R1. The increased expression of SIGN-R1 was distinctly apparent in splenic MZ after 6 h following irradiation and in the lymph node medullar region after 24 h following irradiation (Fig. 2A). To confirm these results quantitatively, spleen and lymph node tissues shown in Fig. 2A were used to perform immunoblotting for SIGN-R1. The data indicated that there was increased SIGN-R1 expression at 6 h or 24 h in spleen or lymph node tissues, respectively (Fig. 2B).

To examine the role of early increased expression of SIGN-R1 in the spleen after 6 h post-irradiation, spleen cryosections shown in Fig. 2A (panels in 1st and 2nd columns) were immunostained for evaluating the prevalence of apoptotic cells and for assessing SIGN-R1 expression. Apoptotic cell numbers were significantly increased in the entire tissue section of the spleen that was assessed (Fig 2C). Notably, the number of apoptotic cells in the WP was higher than that in the RP (Fig 2C). The SIGN-R1$^+$ macrophages in the splenic MZ were significantly enlarged and mediated the uptake of a large number of apoptotic cells (Fig. 2C). This finding was consistent with that of a previous study, which showed that macrophages from mice that were administered whole-body γ-irradiation had enlarged and developed membrane ruffling [15]. To confirm that SIGN-R1$^+$ macrophages were responsible for the uptake of the radiation-induced apoptotic cells, CFSE-labeled radiation-induced apoptotic L1210 cells were intravenously injected into mice for 1 h and the splenic cryosections were immunostained for detecting SIGN-R1 and SER-4. Although most of SER-4$^+$ macrophages showed the uptake of a few apoptotic cells, all of SIGN-R1$^+$ macrophages in the splenic MZ performed efficient uptake of apoptotic L1210 cells and primarily mediated uptake of the large aggregates of apoptotic cells (Fig. 2D).

### 3.3. *γ-irradiation simultaneously increases complement C4 and C3 levels only in the spleen, leading to C3 activation*

To assess whether γ-irradiation increases complement levels *in vivo*, the levels of C4 and C3 were evaluated by performing immunoblot staining of the secondary lymphoid organ tissues (Fig. 2B). Previous studies have demonstrated that C4 is cleaved into C4a and C4b during its activation [16] and C4b migrates as 3 major bands [sized 94–96 kDa (αC4), 72–77 kDa (βC4), and 32–35 kDa (γC4)] on western blots [17]. After 6 h post-irradiation, γC4 was increased only in the spleen and was not elevated in lymph node, thymus, and liver (Fig. 3A). The increase of C3 was monitored by performing immunoblot analysis of the same tissues by using polyclonal anti-C3 antibody (Fig. 3A). This antibody dominantly recognizes the two major components of native C3 (αC3 and βC3), as well as the fragments of αC3 (70 kDa iC3b fragment and 43 kDa

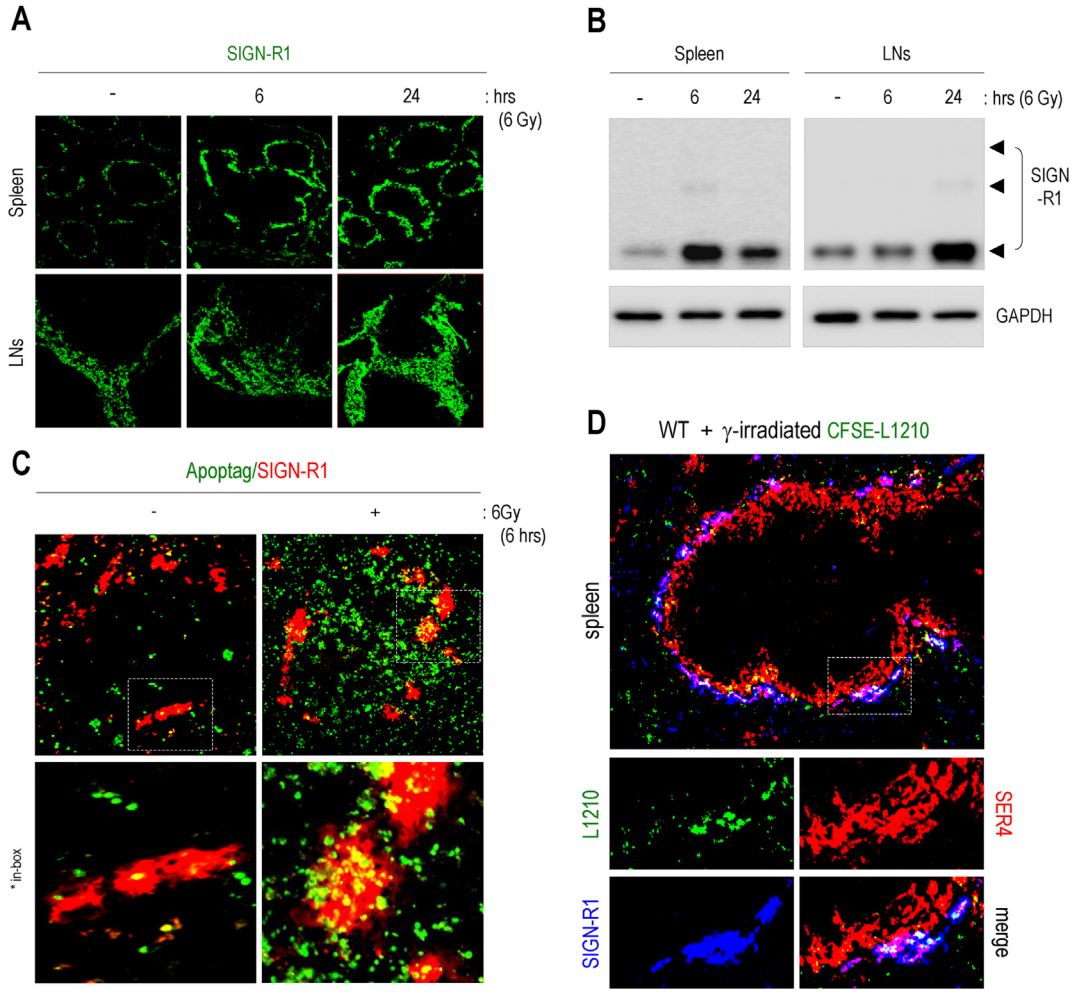


**Fig. 2.** The effect of whole-body γ-irradiation on the expression of SIGN-R1 and the activation of SIGN-R1$^+$ macrophages in the spleen. C57BL/6 mice were administered whole-body γ-irradiation (6.0 Gy) at a dose rate of 3.2 Gy/min. Non-irradiated mice were used as control. (A) After 6 h or 24 h post-irradiation, the cryosections of spleen from control or γ-irradiated mice were immunostained for SIGN-R1 (green). (B) After 6 h or 24 h post-irradiation, the tissue lysates of spleen and lymph nodes from control or γ-irradiated mice were immunoblotted for SIGN-R1. (C) After 6 h post-irradiation, the cryosections of spleens from control or γ-irradiated mice were immunostained for SIGN-R1 (red) and the apoptotic cells (green) were visualized with TUNEL assay. (D) After L1210 cells were exposed to 10 Gy of γ-irradiation and incubated for 5 days, radiation-induced apoptotic L1210 cells were labeled with 5 mM of CFSE (green) for 1 h at 37 °C and intravenously injected into mice. After 1 h, the spleen cryosections were obtained and immunostained for SIGN-R1 (blue) and SER-4 (red). The representative area of the splenic MZ was enlarged in the below in-boxes with separate colors.

iC3b fragment) [18]. C3 level was rapidly increased in the spleen than in lymph node, thymus, and liver after 6 h post-irradiation (Fig. 3B).

Next, the activation of C3 was monitored by performing immunoblot analysis of the tissues (Fig. 3A). The generation ratio of 43 kDa iC3b fragment, a marker of C3 activation, was also analyzed. Previous studies have shown that after complement system is activated, αC3 and the 70 kDa iC3b fragments are degraded and 43 kDa iC3b is then accumulated without βC3 accrual [18,19]. Our data showed that the generation of 43 kDa iC3b was most significant in the spleen, and was either present at low levels or absent in other tissues (Fig. 3C). Thus, C3 activation predominantly occurred in spleen following γ-irradiation.

### 3.4. SIGN-R1 may mediate the systemic clearance of radiation-induced apoptotic cells in vivo

SIGN-R1 first mediates the early recognition and uptake of apoptotic cells in the splenic MZ, followed by participation in systemic recognition and clearance of apoptotic cells in the SIGN-R1-deficient liver through the complement deposition pathway [18]. Therefore, in this study, the role of SIGN-R1 in the systemic clearance of radiation-induced apoptotic cells was examined in spleen and liver tissues. Twenty four hours after the control mice and SIGN-R1 KO mice were exposed to 6.0 Gy of γ-irradiation, the clearance of apoptotic cells in the spleen and liver of both mice was compared by performing immunostaining of spleen and liver cryosections for visualizing apoptotic cells and SER-4 positive cells to designate the splenic MZ. The remnant apoptotic cells were much higher in the WP and RP of the SIGN-R1 KO spleen than in both splenic regions of the control mice (Fig. 4A). Similarly, the remnant apoptotic cells in the liver were also much higher in the SIGN-R1 KO mice than in the control mice and these results were quantitatively confirmed using several liver sections of both control and SIGN-R1 KO mice (Fig. 4B). Thus, SIGN-R1 mediates the systemic clearance of radiation-induced apoptotic cells in the spleen and liver.

CD11b$^+$ cells are one of the major scavenger cells that remove apoptotic cells *in vivo* [20]. Therefore, we examined whether the delayed clearance of radiation-induced apoptotic cells resulted in the recruitment of CD11b$^+$ cells in both spleen and liver of SIGN-R1

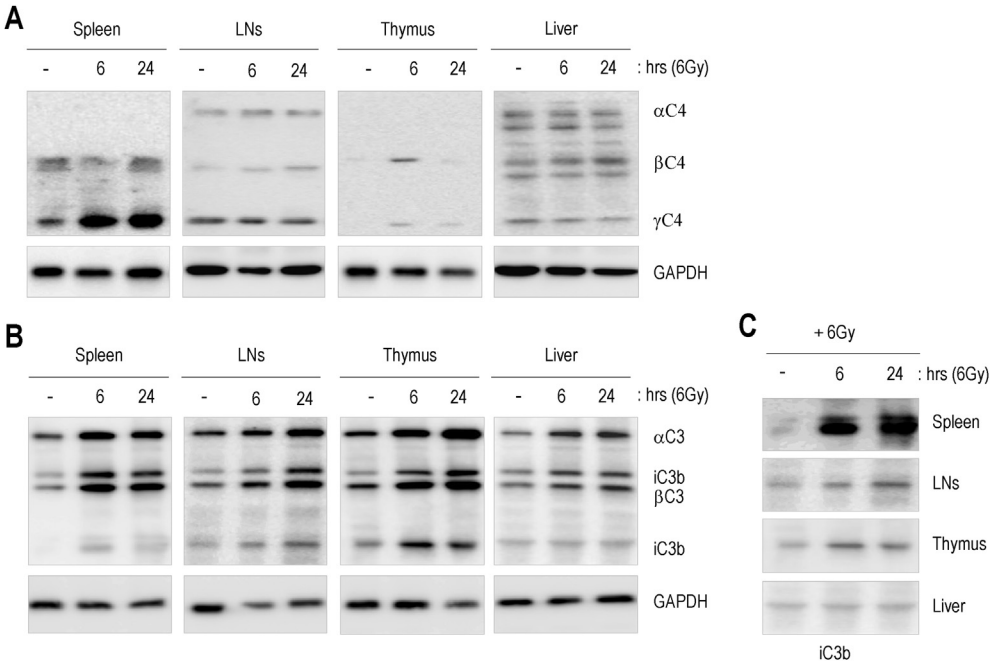


**Fig. 3.** The effects of whole-body γ-irradiation on the expression of complements and on the activation of complement in spleen. C57BL/6 mice were administered single whole-body γ-irradiation (6.0 Gy) at a dose rate of 3.2 Gy/min. Non-irradiated mice were used as control. (A) After 6 h or 24 h post-irradiation, the tissue lysates of spleen, lymph nodes, thymus, and liver from control and γ-irradiated mice were used for performing immunoblot analysis for evaluating the expression of complement C4. (B) As shown in Fig. 3A, but the tissue lysates were used to perform immunoblot analysis for detecting complement C3 expression. GAPDH expression was used as loading control for blots shown in both (3A) and (3B). (C) As shown in Fig. 3B, but the basal level of 43 kDa iC3b was normalized and the quantity of the generated 43 kDa iC3b was compared among tissues.

KO mice. The tissue cryosections that were immunostained for CD11b and SER-4 showed that considerably higher numbers of $CD11b^+$ cells were recruited in the RP and WP regions of the SIGN-R1 KO mice spleens than in the corresponding spleen regions of the control mice (Fig. 4C). In addition, the numbers of $CD11b^+$ cells were remarkably increased in the liver of SIGN-R1 KO mice as compared to the negligibly small numbers of the liver of control mice (Fig. 4D). This finding was also quantitatively confirmed by analyzing several liver sections of SIGN-R1 KO and control mice.

## 4. Discussion

In agreement with previous reports [15,21], our data also indicated that lymphocytes in the secondary lymphoid organs were the most vulnerable to irradiation and that the lymphocytes underwent apoptosis within 24 h after administration of whole-body γ-irradiation (Fig. 1). Recently, it was reported that SIGN-$R1^+$ macrophages in the splenic MZ mediate the systemic clearance of circulating apoptotic cells through the complement deposition pathway [18]. Therefore, it could be expected that SIGN-R1 also mediates a systemic clearance for radiation-induced apoptotic cells *in vivo*. Expectedly, our data showed that SIGN-R1 deficiency caused delayed clearance of apoptotic cells in the spleen and liver of SIGN-R1 KO mice that were assessed 24 h post-irradiation (Fig. 4A and B). In addition, 24 h post-irradiation, the numbers of $CD11b^+$ cells in both spleen and liver of SIGN-R1 KO mice were higher than those in the spleen and liver of control mice (Fig. 4C and D), especially showing the noticeable increase of $CD11b^+$ cells in the WP of SIGN-R1 KO mice (Fig. 4C). These results provide a strong indication that SIGN-$R1^+$ macrophages play an important role in the systemic clearance of radiation-induced apoptotic cells *in vivo* and the increased numbers of $CD11b^+$ cells into the white pulp of SIGN-R1 KO mice is likely to compensate the delayed clearance of apoptotic cells in the absence of SIGN-R1.

Previous studies have demonstrated that after 4–6 h post-irradiation, there was a transient and rapid increase in the synthesis of proteins, including complements such as C4, C3, and other complement factors [22,23]. In agreement with the previous findings, our data indicated that single-dose whole-body γ-irradiation (6 Gy) rapidly increased complements such as C4 and C3 (Fig. 3A and B). In particular, simultaneous increase of C3 and C4 was only observed in the spleen after γ-irradiation administration (Fig. 3). These data strongly indicate that C4-involved complement pathways are predominantly activated in the spleen. This finding was further supported by the data demonstrating the evident activation of C3 only in the spleen after γ-irradiation, but not in other tissues such as lymph nodes, thymus, and liver (Fig. 3C).

Furthermore, apoptotic cells are abruptly increased after γ-irradiation in the secondary lymphoid organs (Fig. 1). Previous studies have shown that complement activation has direct effects on the recognition and clearance of radiation-induced apoptotic cells [15] and that the opsonization of apoptotic cells followed by the activation of complement pathways enhances the clearance of apoptotic cells [18]. SIGN-R1 has also been shown to dominantly activate the classical complement pathway for the clearance of apoptotic cells [18]. Therefore, the primary increase of complement C4 and C3 (Fig. 3A and B) along with the increase of SIGN-R1 and the activation of SIGN-$R1^+$ macrophages, especially in the splenic MZ (Fig. 2), provides the optimal condition for SIGN-R1 to rapidly mediates the systemic clearance of a number of apoptotic cells in the spleen (Fig. 4A).

In conclusion, this study elucidated the integral role of SIGN-R1 and complements in the clearance of radiation-induced apoptotic cells. Furthermore, the findings of this study provided an insight into the role of homologous human C-type lectins, such as DC-SIGN and liver/lymph node-specific-SIGN (L-SIGN, DC-SIGN-R; CD209L), in radiation-induced apoptotic cell clearance.

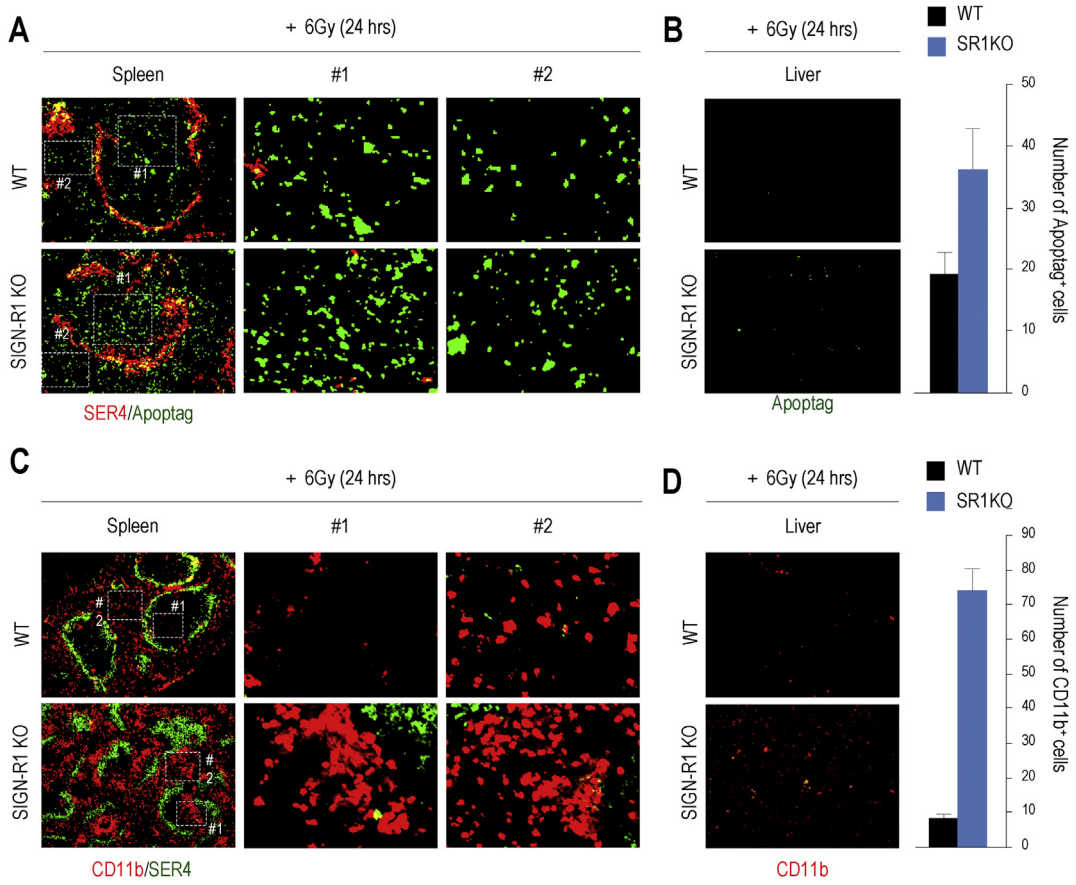


**Fig. 4.** The systemic clearance of radiation-induced apoptotic cells in the spleen and liver was dependent on SIGN-R1. CFSE-labeled radiation-induced apoptotic L1210 cells ($1 \times 10^8$ cells; green) were intravenously injected into the control mice and the SIGN-R1 KO mice. (A) After 24 h, splenic cryosections were obtained from both control and SIGN-R1 KO mice and the cryosections were immunostained for visualizing apoptotic cells (green) by using TUNEL assay and SER-4 (red) staining. The enlarged representative images of WP and RP of both splenic cryosections are shown in box 1 and box 2, respectively. (B) The liver cryosections were immunostained for visualizing apoptotic cells (green) by using TUNEL assay. The numbers of apoptotic cells were counted using 7 adjacent liver cryosections of control and SIGN-R1 KO mice and the average apoptotic cell numbers was also calculated (right panel). (C) The splenic cryosections obtained from control and SIGN-R1 KO mice were immunostained for CD11b (red). (D) Representative images of $CD11b^+$ cells (red) in liver cryosections of control and SIGN-R1 KO mice are shown. The numbers of recruited $CD11b^+$ cells were counted in 7 adjacent liver cryosections of control and SIGN-R1 KO mice and the average $CD11b^+$ was calculated (right panel).

## Conflict of interest statement

The authors have declared that no conflict of interest exists.

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## Transparency document

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## References

[1] K.S. Ravichandran, U. Lorenz, Engulfment of apoptotic cells: signals for a good meal, Nat. Rev. Immunol. 7 (2007) 964–974.
[2] I. Baumann, W. Kolowos, R.E. Voll, et al., Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus, Arthritis Rheum. 46 (2002) 191–201.
[3] G.S. Firestein, M. Yeo, N.J. Zvaifler, Apoptosis in rheumatoid arthritis synovium, J. Clin. Invest 96 (1995) 1631–1638.
[4] D.M. Schrijvers, G.R. De Meyer, M.M. Kockx, et al., Phagocytosis of apoptotic cells by macrophages is impaired in atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 1256–1261.
[5] I.K. Poon, C.D. Lucas, A.G. Rossi, K.S. Ravichandran, Apoptotic cell clearance: basic biology and therapeutic potential, Nat. Rev. Immunol. 14 (2014) 166–180.
[6] A.J. Nauta, N. Raaschou-Jensen, A. Roos, et al., Mannose-binding lectin engagement with late apoptotic and necrotic cells, Eur. J. Immunol. 33 (2003) 2853–2863.
[7] A. Hochreiter-Hufford, K.S. Ravichandran, Clearing the dead: apoptotic cell sensing, recognition, engulfment, and digestion, Cold Spring Harb. Perspect. Biol. 5 (2013) a008748.
[8] M.G. Prabagar, Y. Do, S. Ryu, et al., SIGN-R1, a C-type lectin, enhances apoptotic cell clearance through the complement deposition pathway by interacting with C1q in the spleen, Cell Death Differ. 20 (2013) 535–545.
[9] J. Bernier, E.J. Hall, A. Giaccia, Radiation oncology: a century of achievements, Nat. Rev. Cancer 4 (2004) 737–747.
[10] B. Pinar, L.A. Henriquez-Hernandez, P.C. Lara, et al., Radiation induced apoptosis and initial DNA damage are inversely related in locally advanced breast cancer patients, Radiat. Oncol. 5 (2010) 85.
[11] S.A. Lorimore, P.J. Coates, E.G. Wright, Radiation-induced genomic instability and bystander effects: inter-related nontargeted effects of exposure to ionizing radiation, Oncogene 22 (2003) 7058–7069.
[12] N. Sakaguchi, K. Miyai, S. Sakaguchi, Ionizing radiation and autoimmunity. Induction of autoimmune disease in mice by high dose fractionated total lymphoid irradiation and its prevention by inoculating normal T cells, J. Immunol. 152 (1994) 2586–2595.

[13] Y.S. Kang, J.Y. Kim, S.A. Bruening, et al., The C-type lectin SIGN-R1 mediates uptake of the capsular polysaccharide of Streptococcus pneumoniae in the marginal zone of mouse spleen, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 215–220.
[14] Y.S. Kang, S. Yamazaki, T. Iyoda, et al., SIGN-R1, a novel C-type lectin expressed by marginal zone macrophages in spleen, mediates uptake of the polysaccharide dextran, Int. Immunol. 15 (2003) 177–186.
[15] S.A. Lorimore, P.J. Coates, G.E. Scobie, et al., Inflammatory-type responses after exposure to ionizing radiation in vivo: a mechanism for radiation-induced bystander effects? Oncogene 20 (2001) 7085–7095.
[16] H. Milgrom, J.G. Curd, R.A. Kaplan, et al., Activation of the fourth component of complement (C4): assessment by rocket immunoelectrophoresis and correlation with the metabolism of C4, J. Immunol. 124 (1980) 2780–2785.
[17] K. Yazdanbakhsh, S. Kang, D. Tamasauskas, et al., Complement receptor 1 inhibitors for prevention of immune-mediated red cell destruction: potential use in transfusion therapy, Blood 101 (2003) 5046–5052.
[18] M.G. Prabagar, Y. Do, S. Ryu, et al., SIGN-R1, a C-type lectin, enhances apoptotic cell clearance through the complement deposition pathway by interacting with C1q in the spleen, Cell Death Differ. 20 (2013) 535–545.
[19] Y.S. Kang, Y. Do, H.K. Lee, et al., A dominant complement fixation pathway for pneumococcal polysaccharides initiated by SIGN-R1 interacting with C1q, Cell 125 (2006) 47–58.
[20] E. Esashi, T. Sekiguchi, H. Ito, et al., Cutting Edge: a possible role for CD4+ thymic macrophages as professional scavengers of apoptotic thymocytes, J. Immunol. 171 (2003) 2773–2777.
[21] R.D. Neff, B. Cassen, Relative radiation sensitivity of circulating small and large lymphocytes, J. Nucl. Med. 9 (1968) 402–405.
[22] S. Braunstein, M.L. Badura, Q. Xi, et al., Regulation of protein synthesis by ionizing radiation, Mol. Cell Biol. 29 (2009) 5645–5656.
[23] M. Chen, J. Zhao, C. Luo, et al., Para-inflammation-mediated retinal recruitment of bone marrow-derived myeloid cells following whole-body irradiation is CCL2 dependent, Glia 60 (2012) 833–842.