Repopulated antigen presenting cells induced an imbalanced differentiation of the helper T cells in whole body gamma irradiated mice

Hae-Ran Park[a], Sung-Kee Jo[a*], Sang-Kee Paik[b]
[a]*Radiation Food and Biotechnology Team, Korea Atomic Energy Research Institute (KAERI), P.O. Box 105, Yusong, Daejon, 305-600, South Korea, skjo@kaeri.re.kr*
[b]*Department of Biology, Chungnam National University, Yusong, Daejeon, 305-764, South Korea*

1. Introduction

Therapeutic irradiation of cancer patients, although it may be protected by several antioxidant agents against free radicals, often induces chronic sequelae such as inflammation (allergic inflammation) [1]. This is a limiting factor for radiotherapy. Following radiotherapy, the inflammation or injury can occur in any organ with a high radiosensitivity such as the lung, bladder, kidney, liver, stomach and intestine. The mechanism by which ionizing radiation initiates inflammation is, however, poorly understood. In recent studies, it was suggested that a factor for irradiation-induced inflammation might be the over production of IL-4 that enhances fibroblast proliferation and collagen synthesis [2]. During the early stages after irradiation, type 2 of the helper T cells might be the major source of IL-4 [3], and later on there seems to be an activation of the other IL-4 producing cell types, *e.g.* macrophages or mast cells [4]. This is interesting because inflammation is classically seen to be dominated by Th1 cells secreting IFN-γ.

In the previous study, we were interested in the enhancement of the IL-4 and the IgE production during the development of immune cells after γ-irradiation. We were able to deduce that IL-4 production was increased because of the shifted differentiation of the naive Th cells by the repopulated antigen presenting cells after irradiation.
The aim of the present study was to precisely define whether antigen-presenting cells (APCs) of whole body irradiation-treated mice could influence the shifted differentiation of the Th cells. This view can be demonstrated by confirming that the shifted functional status of the Th cells is induced by the altered function of the repopulated macrophages after whole body irradiation (WBI).

2. Methods and Results

2.1. Preparation of cells

The isolation of T cells from spleen was isolated by MACS and peritoneal adherent macrophages were isolated from TG-stimulated mice. Antigen (OVA)-specific Th cells were established as described previously by Kimoto [5]. The cells were confirmed as $CD4^+$ Th cells in flow cytometry (above 97%; data not shown).

3.2. APCs of WBI mice stimulate Th cells to secrete Th2-type cytokine rather than Th1-type cytokine in vitro

Because the IL-4 level in the supernatant of primary spleen cell culture was very low in previous study, we made up OVA-specific Th cells to test the difference between normal and WBI mice. In a co-culture of the OVA-specific Th cells with MMC-treated spleen cells obtained from normal or WBI (4 Gy) mice as antigen presenting cells in the presence of the antigen, the secreted cytokines were determined. When the OVA-specific Th cells were co-cultured with the spleen cells of the WBI mice, IFN-γ secretion was markedly diminished compared to the co-culture with those of normal mice. By contrast, the IL-4 level was markedly augmented (Figure 1). At this time, there was no difference in the viable OVA-specific Th cells proliferated by the APCs of the normal and WBI mice (data not shown).

Also, we obtained the similar data when OVA-specific Th cells were co-cultured with macrophages known the best-defined APCs (data not shown). These results suggest that the APCs from the WBI mice preferentially sensitize the Th2 cells rather than the Th1 cells.

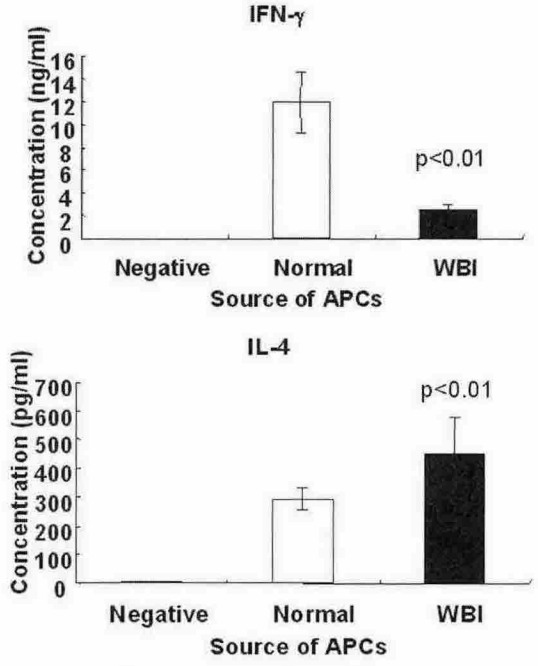

Figure 1. Comparison of cytokine secretion in co-culture of OVA-specific Th cells with spleen cells from either normal or WBI mice. OVA-specific Th cells were

cultured with MMC treated splenic lymphocytes from either normal or WBI mice. These cells were stimulated with OVA. Three spleens of mice were pooled. After 2 days, the levels of the cytokines in the culture supernatants were measured by indirect ELISA. The IFN-g secreted by MMC-treated normal or WBI spleen cells was 746 pg/ml or 413 pg/ml. The IL-4 level was <4.1 pg/ml.

3.3. APCs from irradiated mice orchestrate shifted differentiation of naive T cells.

To study the ability of the APCs from WBI mice to induce a differentiation of the naive T cells, PACs and primary T cells obtained from normal or WBI (4Gy) mice were co-cultured in the presence of OVA. The cytokine concentration in the culture supernatant was shown in Figure 2. The IFN-γ level was lower, but the IL-4 level was higher in the culture of the T cells and PACs from the WBI mice compared with those from the normal mice ($p<0.01$). In this condition, PACs from the WBI mice induced the T cells from the normal mice to produce a lower level of IFN-γ and a higher level of IL-4 than the PACs from the normal mice ($p<0.01$). On the other hand, PACs from the normal mice induced the T cells from the WBI mice to produce a higher level of IFN-γ and a lower level of IL-4 compared with the PACs from the WBI mice ($p<0.01$).

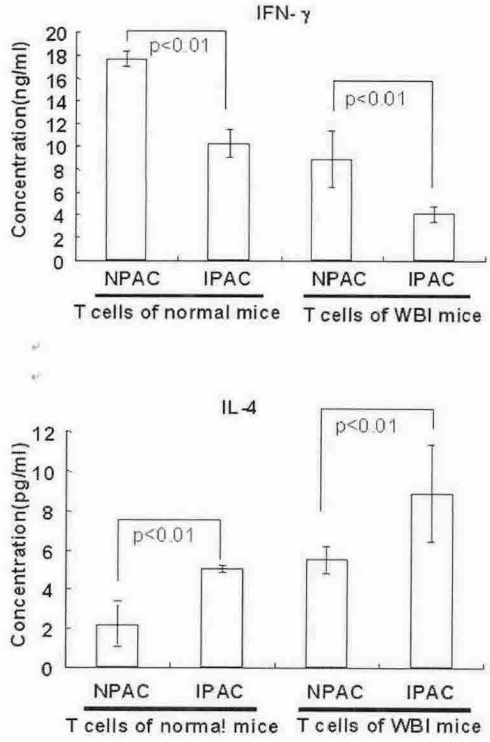

Figure 2. Comparison of cytokine secretion in co-culture of T cells of spleen cells and peritoneal adherent cells from either normal or WBI mice. T cells separated by MACS were co-cultured with the MMC treated peritoneal adherent cells from either normal or WBI mice. These cells were stimulated with OVA. The levels of the cytokines in the culture supernatants were measured by indirect ELISA. Spleen cells or PACs of three mice were pooled NPAC, Peritoneal adherent cells from normal mouse; IPAC, Peritoneal adherent cells from irradiated mouse

3. Conclusion

The results suggest that the antigen-presenting cells of the WBI mice contribute to the inducing of the shifted differentiation of the naïve helper T cells into the dominant Th2 cells.

The prevalence of the Th2 cells found in the irradiated mice agrees with the view that this regulatory cell population is involved in repairing inflammatory tissue damage. Under yet undefined circumstances, further investigation is needed to establish the exact difference of the antigen presenting cells between the normal and WBI mice.

REFERENCES

[1] T. Hayashi, Y. Kusunoki, M. Hakoda, Y. Morishita, Y. Kubo, M. Maki, F. Kasagi, K. Kodama, D.G. Macphee, and S. Kyoizumi, Radiation dose-dependent increases in inflammatory response markers in A-bomb survivors. *International Journal of Radiation Biology*, **79,** 129-136, 2003.

[2] Sempowski, G.R., Beckmann, M.P., Derdak, S. and Phipps, R.P., 1994, Subsets of murine lung fibroblasts express membrane-bound and soluble IL-4 receptors. *Journal of Immunology*, **152,** 3606-3614

[3] Westermann, W., Schobi, R., Rieber, E.P. and Frank, K.H., 1999, Th2 cells as effectors in postirradiation pulmonary damage preceding fibrosis in the rat. *International Journal of Radiation Biology*, **75,** 629-638.

[4] Buttner, C., Skupin, A., Reimann, T., Rieber, E.P., Unteregger, G., Geyer, P. and Frank, K.H., 1997, Local production of interleukin-4 during radiation-induced pneumonitis and pulmonary fibrosis in rats. Macrophages as predominant source of interleukin-4. *American Journal of Respiratory Cell and Molecular Biology*, **17,** 315-325

[5] Kimoto, M. and Fathman, C.G., 1980, Antigen reactive T cell clones. 1. Transcomplementary hybrid IA-reagion gene product function effectively in antigen presentation. *Journal of Experimental Medicine*, **152,** 759-770