Original Article

The effects of residence duration in high background radiation areas on immune surveillance

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Abstract

Purpose: The effective dose received by humans from natural sources is about 2.4 mSv y^{-1}, but this is 10.2 mSv y^{-1} for inhabitants of Ramsar, a city in northern Iran. Carcinogenesis is one of the most studied effects of radiation, especially in high doses. Nonetheless carcinogenesis of low doses is uncertain. A recent epidemiological study in high background radiation areas of Ramsar showed that the cancer incidence in this era is lower than neighbors. The reason of this different behavior is under study yet. NK cells, helper, and Cytotoxic T cells are most important components of the tumor immune surveillance. The counts and activities of these cells and also leukocytes, lymphocyte, neutrophil cells, and other important parameters were studied in the residents of Ramsar with different duration of exposure to chronic low dose radiation. **Materials and Methods:** Fifty residents of high background radiation areas, who were between 25 and 35 years and fully healthy, were selected randomly and their consent was obtained. Then, 2 cc fresh peripheral bloods were taken in sterile conditions. Complete blood cell counts were performed by an automatic hematology analyzer and CD4+, CD8+, NK, and CD107a+ cell counts were determined by monoclonal antibodies and flowcytometry. CD4+ and CD8+ percentages and the CD4/CD8 ratio were determined and the data were analyzed using SPSS 16. **Results:** The percentages of CD4+ cells increase, but the counts of CD107a+ cells decline in higher exposure durations. The other parameters did not have significant regression with exposure duration. **Conclusions:** These confirm that living in high background radiation areas may induce changes in the immune system gradually and address more investigations.

Key words: Cytotoxic T cells, high background radiation areas, helper T cell, natural killer cell, Ramsar

INTRODUCTION

Natural radioactivity and cosmic rays are the main sources of radiation exposures. Effective dose of the public varies substantially depending on where they live, occupation, personal habits, diet, building type, and house utilization patterns.[1] The effective dose of humans from natural sources is about 2.4 mSv y^{-1} while for all artificial sources is 0.8 mSv y^{-1}.[2] A few geographical hotspots receive high natural background radiation.[3] Ramsar, located in Mazandaran province in northern Iran, is one example. The average and maximum annual effective doses of the inhabitants of Ramsar are 10.2 and 260 mSv, respectively.[4] The radiation effective doses of Ramsar inhabitants are mainly due to Ra-226 and its decay products.[4] In addition, the concentrations of natural radionuclides in food and drinking water of the residents are higher than the world average, and are correlated with the high concentration of these radionuclides in soil and water.[2] Studies of inhabitants of high background radiation areas (HBRAs) are very useful in predicting disease risks from chronic radiation exposures in nuclear and medical workers and in other exposed populations.[3]

In the last 10 years, there has been a significant increase in studies about the function of the immune system due in part to the importance of immune function in the maintenance of a disease-free homeostasis.[5] T cells can home into antigen-expressing tumor even in deep tissue

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beds and can continue to proliferate in response to immunogenic proteins expressed in cancer until eradication of the tumor cells. In addition, immunological memory can be generated.[6] The immune system has been reported to suppress the development and progression of neoplastic lesions by not well understood mechanisms. Nevertheless, tumors may suppress the immune system, especially by the formation of exosomes. Uptake of the exosomes by immune cells may suppress of the number and activity of natural killer (NK) cells and also suppressing the activity of T cells.[7] T lymphocytes can be broadly classified as cytotoxic CD8+ and CD4+ T cells. The CD4+ T-cell response can elicit both immune stimulatory or immune inhibitory effects. Specific CD4+ T-helper (Th) cell phenotypes are crucial for the expansion and persistence of tissue-destructive CD8+ T cells. CD8+ T cells can directly kill an antigen-expressing cell.[6]

Natural killers are an important component of the innate immune response to tumors. They are able to lyse tumor cells or virally infected cells without prior antigen sensitization.[8] Cytotoxic T cells (CTLs), also known as CD8+ T cells, recognize their targets by binding to antigen associated with MHC class I, which is present on the surface of nearly every cell of the body.[9] Through IL-10, adenosine, and other molecules secreted by regulatory T cells, the CD8+ cells can be inactivated to an anergic state, which prevents autoimmune diseases such as experimental autoimmune encephalomyelitis.[9]

In this study, the effective radiation dose received by inhabitants of HBRAs of Ramsar was estimated by external radiation measurements, occupancy factor, and period of residence of individuals, and then, the changes on CD4+, CD8+, NK cells, leukocytes, red blood cells, platelets (PLT), and their percentages were investigated. The regression between each parameter and period of residence was examined. The purpose of the study was to investigate the association between exposure time and changes in immune cells.

MATERIALS AND METHODS

Fifty residents of HBRAs, whose ages were between 25 and 35 years and who were fully healthy, were selected randomly. The mean age was 30.2 ± 3.72. Then, the aim of the project was explained and their consent was obtained.

A questionnaire was filled by interview and people with smoking, pregnancy, medications, surgery, cancer, and immune-related diseases were excluded. The mean age of participants was 30.2 ± 3.72 and their maximum and minimum was 25 and 35, respectively. Lifestyles, age, and sex were matched.

Complete blood cell counts

Two cubic centimeters of fresh peripheral blood were taken in sterile conditions and cell counts were performed using an automatic hematology analyzer (sysmex kx-21, Japan).

Helper T cells

Twenty microliters of each blood sample were stained for 15 min at room temperature in dark with 20 μL CD4/CD45 antihuman monoclonal antibody (Partec, Germany), and 400 μL of buffer 1 and then 400 μL of buffer 2 were added to each sample and were analyzed with a flowcytometer (Partec PAS, Germany).

Cytotoxic T cells

According to the CD8 easy count kit (Partec, Germany) instruction, 20 μL of each sample were stained for 15 min at room temperature in dark with 10 μL CD8 antihuman monoclonal antibody (Partec, Germany).

In addition, 400 μL of buffer 1 and then 400 μL of buffer 2 were added to each sample and the samples were analyzed with a flowcytometer (Partec PAS, Germany).

NK cells and CD107a+ cells

Twenty microliters of each blood sample were stained with 10 μL of CD107a-PE (Exbio, Czech Republic) and 10 μL CD45-PE (Partec, Germany) monoclonal antibody, and the other 20 μL of blood samples were stained with 10 μL CD56-PE (Exbio, Czech Republic) and 10 μL CD45-PE (Partec, Germany) for 15 minutes in the dark conditions. Then, buffer 1 and buffer 2 were added (Partec, Germany). The samples were shacked, and the numbers of *in vivo* stimulated CD107a+ cells and CD56+ cells were counted using a calibrated flowcytometer (Partec PAS, Germany). This study was approved by the accredited Ethics Committee of Babol University of Medical Sciences, Babol, Iran.

Statistical analysis

The statistical analysis of results was done using SPSS 16. For regression analysis, we used log transformation of cell counts and cumulative doses since their distributions are skewed and have long tails.

RESULTS

The mean exposure duration was 22.28 ± 8.71 and its minimum was 10 and its maximum was 35 years in studied individuals.

CD4+ and CD8+ percentages, CD4/CD8 ratio, CD4+, CD8+, NK and CD107a+ cell counts, and complete blood cell counts of inhabitants of HBRA are shown in Tables 1 and 2, respectively.

The regressions between exposure duration and CD4% ($P = 0.045$), also with CD107a+ counts ($P = 0.048$), CD8% are shown in Figure 1.

There is not significant regression between exposure duration and the other studied parameters including Log CD4, Log CD8, Log NK, Log LYM, Log NEUT, Log WBC, Log MXD counts and CD4/CD8 ratio, CD8%, LYM%, NEUT%, and MXD%.

Table 1: CD4+ and CD8+ percentages, CD4/CD8 ratio, CD4+, CD8+, NK, and CD107a+cell counts of inhabitants of high background radiation area

Parameter	Mean
CD4%	59.0220±9.07
CD4+cells/μL	656.14±212.84
CD8%	22.4012±9.63
CD8+cells/μL	219.43±106.20
CD4/CD8 ratio	3.5755±2.08
NK cells/μL	244.35±226.13
CD107a+cells/μL	6.93±5.20

Table 2: The complete blood counts results

Parameter	Mean	Parameter	Mean
WBC	7022±1512	NEUT%	57.27±7.45
RBC	4537796±374966	LYM	2430.55±606.07
PLT	222593±54451	MXD	451.48±171.30
HGB	12.94±1.32	NEUT	4108.00±866.94
HCT	38.51±3.71	RDWsd	41.73±3.48
MCV	85.17±8.03	RDWcv	12.88±0.98
MCH	28.61±2.74	PDW	13.99±2.11
MCHC	33.60±1.33	MPV	10.65±0.99
LYM%	35.44±6.83	PLCR	31.05±7.79
MXD%	8.41±6.66		

DISCUSSIONS

These results showed that living in HBRAs may induce some changes in the immune system gradually. The percentages of CD4+ cells have increased over years that inhabitants lived there. CD4+ or T-helper (Th) cell phenotypes are crucial for the expansion and persistence of tissue-destructive CD8+ T cells.[6] CD107a+ cells that are the marker of CD8 and NK cell activity[8] decrease gradually in the individuals living in HBRAs. This can be due to higher cumulative doses.

Lacoste-Collin *et al.* studied the biological effects of 10 cGy year^{-1} gamma rays on the life span of 560 4-week-old SJL/J female mice and on various parameters of the cell-mediated immune response and they reported that life span was slightly prolonged there. In lymph nodes and spleen, lower percentages of CD4+ and CD8+ T cells were observed in irradiated mice before 32 weeks. However, the percentages of CD49+ NK cells were increased in the spleens of irradiated mice at 28 weeks and at 32 weeks, while NK cell activity remained unchanged in exposed mice.[10]

Farooque *et al.* focused on the immunostimulatory effects of low dose radiation at *in vivo* models and its clinical efficacy. They focused effects that supporting the use of low dose radiation regimens as an anticancer treatment.[11]

Matsubara *et al.* have emphasized on the existence of a lag time between the time of low dose irradiation *in vivo* and the appearance of radioresistance.[12] They showed that low-dose irradiation induces thymocyte apoptosis with a peak at 6 h postirradiation, but returned to background levels after 24 h. At the same time, no morphological alteration of splenocytes and no early modification of

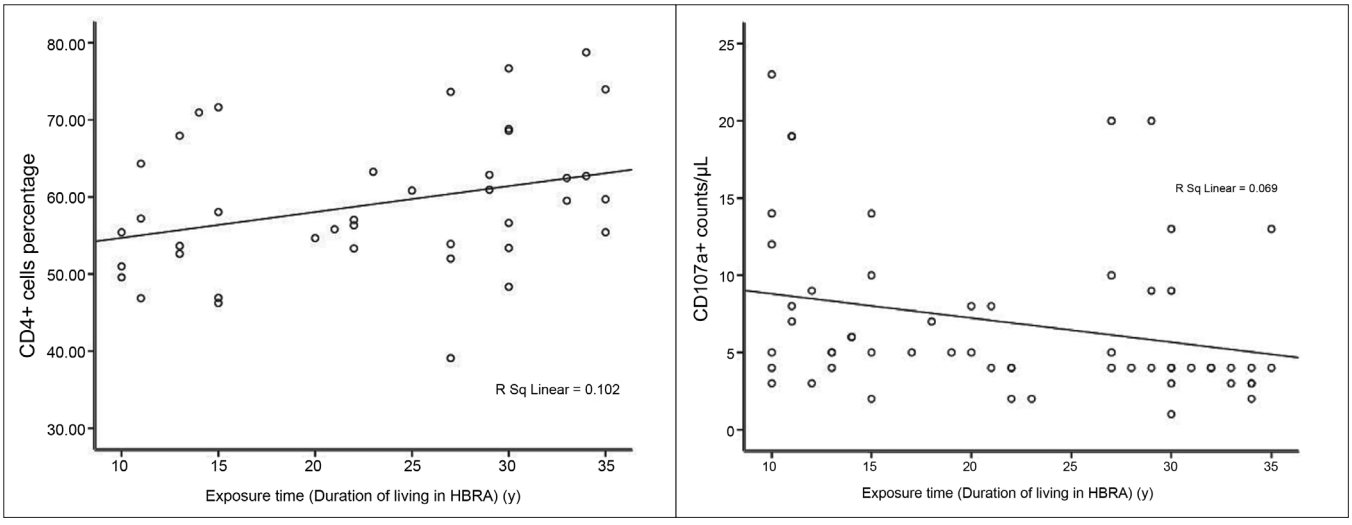

Figure 1: Regression between exposure duration and CD4 and also with CD107a counts. The CD4+ cells percentage increases, but the count of CD107a+ cells decreases with duration of living in HBRA of Ramsar

the intensity of T-cell-dependent immune responses have been seen.[12]

Shankar *et al.* reported that alteration in the immune response following the low dose irradiation depends on antigen, type of response as well as the strain of mice used. Furthermore, the alterations in the expression of pro-apoptosis gene p53 and activation-induced apoptosis in the effector or regulatory cells seem to contribute to the end result.[13]

Concerning *in vivo* radiation exposure, no changes in NK cell activity were found in 1341 atomic bombing survivors residing in Hiroshima.[14] Koike *et al.* evaluated the immune status in children with goiter living in highly contaminated area of Gomel. They found increased serum levels of IgG, IgM and IgE, and depressed NK cell activity.[15] Attar *et al.* showed that the total serum antioxidant level in the exposed inhabitants of HBRAs of Ramsar was significantly lower than control and they also had higher lymphocyte-induced IL-4 and IL-10 production, and lower IL-2 and IFN-γ production. However, lymphocyte proliferation in response to PHA was unaffected.[16] The authors conclude that the immune system of individuals exposed to high dose ionizing radiation has adapted to its environment by shifting from a Type 1 to a Type 2 response to promote anti-inflammation. This may be because inflammatory Type 1 responses generate more free radicals than Type 2 responses, in addition to the free radicals generated as a result of high environmental radiation. Thus, the serum total antioxidant level in the exposed residents was lower than the unexposed group.[16]

Evaluated hormone levels associated with immune responses of inhabitants in HLNRAs of Ramsar was seen and their study demonstrated a decreased level of DHEA and increased level of cortisol in the elderly HLNRA group, which might be possibly associated with higher cumulative doses.[17] There was a higher incidence of hypothyroidism in the HLNRA group, mostly in the elder group. A significant increase of CD69 expression on CD4+ T lymphocytes was found in the young HLNRA group. Low levels of DHEA in the elder group were correlated to their increased IgE levels.[17] The comparison of CD4+ and CD8+ cell counts, their percentages, and the CD4+/CD8+ ratio in 2011 by Borzoueisileh *et al.* showed that the increase in CD4+% and CD8+% in the HBRA group and other measurements were not significantly different between HBRA and ordinary background radiation area (OBRA) residents of Ramsar.[4]

Our results showed that the percentage of CD4+ cells increases but the count of CD107a+ cells decreases with duration of living in HBRA of Ramsar. Other measured parameters, CD8+, NK cells, leukocytes (WBC) and content of the mixture (MXD), neutrophil, lymphocyte counts, and percentages did not show significant regression by exposure time.

CONCLUSION

We concluded that alterations have seen in the immune systems of HBRA inhabitants, but the study of other T-cell subsets including Th1 (T helper), Th2, Th17, and regulatory T cells to determine other aspects of the immune involvement could be important. The ratios of Th1/Th2 and Th17/Tregs that could reflect altered states of immune responses against foreign antigens or tumor antigens and the proportions or counts of peripheral leucocytes including monocytes and polymorph nuclear leucocytes, which also modify or even suppress antitumor immunity, are important.

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