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ABSTRACT

Measurements of mononuclear cells in peripheral blood of persons with long term internal depositions of plutonium indicate a preferential reduction in suppressor T-lymphocytes (T_s) in some individuals. The decrease in T_s cells is apparently due to altered radiosensitivity, which is demonstrated in cultured cells subjected to in vitro x-ray radiation. The increase in ratios correlates with the quantity of plutonium deposition in these subjects, but there are wide individual differences. Confirmatory studies are needed in other persons with long term alpha or chronic gamma radiation exposure. If confirmed, the implications would include recognition of a potential mechanism for an enhanced immune system reactivity in some individuals exposed to chronic low level radiation.

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Lymphoid cells perform the unique functions of the immunological defense system in man and animals. Immunological competence requires recognition of foreign antigens introduced by bacterial or viral infections or abnormal cells, production of specific antibodies, and regulation of the overall immune response. The vital function of this system can be no better demonstrated than to witness the lethal consequences of infection or increased probability of cancer induction for persons afflicted with acquired immunodeficiency syndrome (AIDS).

Central to these immune functions are lymphocyte cells. These white blood cells can be identified in subsets such as B or T lymphocytes (B or T cells) and monocytes or macrophages. In addition, the T-cells can be identified as either helper T-cells (T_H) or suppressor T-cells (T_s). The T_H cells assist the B-lymphocytes in identifying antigens. The B-cells are precursors of antibody-secreting plasma cells, which produce the necessary immunoglobulin (I_g) molecules. The T_s cells inhibit the response when not needed and prevent excess reactions. Normally, there are about twice as many T_H cells as T_s cells in the blood.

Several aspects of lymphocyte function make these cells important in the study of radiation effects. First, lymphocytes are the most radiosensitive cells in the blood; the decrease of lymphocytes after acute high doses is a useful biological dosimeter [1,2]. Second, although the radiosensitivity of lymphocytes to high doses is easily recognized, knowledge of cellular function after low dose radiation is not well known. Doria [3] points out our limited knowledge to understand changes in the immune system after radiation exposures. Third, changes in immunological function after repeated low level radiation doses may affect long term cancer risk, the major health effect driving the radiation protection guidelines.

The purpose of this study is to enumerate the various types of lymphocytes in persons exposed to chronic ionizing radiation from internal plutonium deposition compared with nonexposed controls. The cultured lymphocytes are also exposed to in vitro radiation to examine potential differences in radiosensitivity of these cells as a result of prior radiation exposure history.

METHODS

The study subjects were eighteen persons from a group of male workers who were exposed to plutonium (Pu) at Los Alamos in 1944-1945. The average age of this irradiated group is 66 ±5 years. This group has had periodic medical and radiological measurement studies since the 1950s[4,5,6]. The unexposed

control subjects were 15 age matched male employees (age 64 ±4 years) without occupational radiation exposure history.

Peripheral blood samples were taken from each of the plutonium exposed persons and nonexposed controls. The mononuclear cells were isolated and then divided into two portions. The first portion was used to determine the lymphoid subsets at the time of blood collection (Day 0). The cells from the second portion were subdivided into three parts for x-ray irradiation of 0 (control), 0.5, or 2 Gy. These cells were then cultured both with and without the mitogen, phytohemagglutinin (PHA). After 6 days in culture, the subsets were again measured for comparison of cell survival under the several experimental conditions. A detailed methodology for these studies has been previously described[7].

Classification of subsets was accomplished by adding monoclonal antibodies (MAbs) which are directed against specific cell surface markers. Separated mononuclear cells were labeled simultaneously with fluoresceinated B1 (B cells), phycoerythrin Leu2a (T_s cells), LeuM3 with Texas-red goat-anti-mouse Ig (monocytes) and biotinylated Leu3a with allophycocyanin-avidin (T_H). In addition, propidium iodide was used to identify and exclude dead cells from the analysis.

Flow cytometric analysis using 2-color excitation (488 nm and 605 nm) was used to identify and to count the above types of mononuclear cells. Five colors of emission were detected, corresponding to the four MAbs and propidium iodide color tags.

RESULTS

The number of white cells and mononuclear subsets from peripheral blood was determined first. The mean total white blood count was the same for the Pu workers (6500 cells/μl) and the controls (6200 cells/μl). The mean of the differential counts of lymphocytes and monocytes was also nearly identical for Pu workers and controls.

Figure 1 shows the T_H/T_s ratios at the time of blood collection for each plutonium exposed subject plotted as a function of the estimated becquerels of internal Pu deposition and for the controls. This figure shows a scattering of values for some persons with the larger internal depositions of Pu. A weak positive dose/response relationship (linear correlation R = .48) is present. Five of the 18 exposed individuals have ratios from 5.5 to 19.6, which are above the highest ratio (5.1) observed in the controls. The median values of the ratios on Day 0, however, are the same for Pu exposed persons and controls as shown on Table 1.

The observed increased ratios are present in about half of the individuals with long term Pu depositions of about 500 Bq or more. This observation could result from individuals exhibiting different lymphocyte responses to alpha radiation, from variation in alpha doses due to different distributions of plutonium deposition in individuals, or from other unknown factors of human heterogeneity. Additional data are necessary to confirm these suggestions.

Peripheral blood mononuclear cells were cultured six days to determine the in vitro radiosensitivity of lymphocyte subsets after 0, 0.5, and 2 Gy of 250 Kvp x-rays. In the unirradiated cultures, seven of 18 Pu exposed workers (39%) had T_H/T_s ratios above 6, which was higher than ratios recorded in all but one control subject. The ratio increases were due to a decrease in the number of T_s cells. T_H cell counts were at normal levels in all individuals.

Irradiation of the cultures caused the T_H/T_s ratios of the plutonium workers to be at increasingly higher values compared with the controls. The cultures after 0.5 Gy showed 9 out of 17 Pu workers (53%) had ratios above 8.5 compared with only 2 of 13 controls (15%). Similarly, after 2 Gy, 10 out of 16 (62%) Pu workers had ratios above 20 compared with only 3 of 13 controls (23%).

In addition, the higher ratios observed were always in Pu exposed individuals although the lower ratios in the group overlapped the frequency distribution of normal ratios. The number of subjects in each group was small and the numerical values of the higher ratios were widely scattered; however, the median values listed in Table 1 reveal the relative increase in T_H/T_s ratios of the plutonium subjects after <u>in vitro</u> radiation of lymphocytes. The increased ratios occur because of greater radiosensitivity of T_s compared to that of T_H cells.

Mononuclear cells were also irradiated with 0, 0.5, and 2 Gy in vitro and cultured for six days with PHA to determine the

radiosensitivity of stimulated lymphocyte subsets. The response of lymphocytes from Pu workers following in vitro irradiation in the presence of PHA was compared with that of control subjects. After 0.5 Gy, 78% of T_H cells from Pu workers survived whether PHA was present or not. For controls, T_H survival was less (66%) than for Pu workers, but survival was also unaffected by PHA stimulation. In contrast, survival of T_s was greatly increased for Pu workers and controls when stimulated with PHA. The increase for Pu workers from 51% ±5 without PHA to 97% ±8 with PHA was significantly greater than for controls, 37% ±5 without PHA to 53 ±9 with PHA.

In the presence of PHA stimulation, the radiosensitivity of T_H cells did not change. The T_s cells, however, became much more radioresistant; this occurred to a greater extent in Pu exposed persons than in the controls.

DISCUSSION

These results suggest that there are measurable differences in the several subsets of lymphocytes taken from Pu exposed persons compared with the controls. Although both T_H and T_s cells are both radiosensitive, the T_s are about 3 times as sensitive as T_H cells. The most striking observation in this study is that the T_s cells have increased radiosensitivity in blood from some persons with long term exposure to the alpha irradiation from Pu. This change is best demonstrated by increases in T_H/T_s ratios in cultured lymphocytes from Pu workers compared with the ratios in control persons. This

finding is apparently due to an altered radiosensitivity of T_s cells in the exposed persons.

Although the increased ratios are more apparent after in vitro irradiation of the cultured cells, about 30 percent of the Pu exposed persons had ratios in fresh blood samples significantly above those of age-matched controls . Are increased ratios of this magnitude important in the functioning of the immune defense? Although this study by itself cannot answer the question, the presence of a high T_H / T_s ratio may indicate reduced T_s cell suppressible activity. A heightened T_H action is then possible, which may produce an enhanced immune defense mechanism for these persons. Such enhancement might be significant because immunoreactivity is known to decrease with age.

The Pu exposures of these workers were primarily by inhalation of relatively insoluble particles. Radiation exposure of lymphocytes by Pu alpha particles occurs primarily in tracheobronchial lymph nodes and bone marrow, although peripheral lymphocytes may be exposed also in liver and lung. Tissue studies have shown that after inhalation exposure, the highest concentrations of Pu in the body are found in the tracheobronchial lymph nodes[8]. The next highest concentrations are found in the lung, liver, and bone. Two deceased Manhattan District Pu workers have had measurements of Pu made in post mortem tissue samples. The Pu distribution was found to be in good agreement generally with the above description. McInroy [9]

has also found that the red bone marrow contains about 2 to 4% of the total activity found within bone.

The estimated whole body deposition of plutonium in 1987 for the individuals in this study ranges from 50 to 3150 Bq (1.4 to 85 nCi) with a median of 520 Bq (14 nCi). Although the actual radiation doses to lymphocytes in these individuals is unknown, the Pu distribution in the body and length of exposure (42 years) suggest a chronic exposure by alpha particles.

A logical question is "What has been the health experience of this group of plutonium workers?". Mortality is the principal endpoint for which some comparison data are available. The standardized mortality ratio (SMR) of the 26 Manhattan District plutonium workers for all causes of death is 0.44 (90% confidence interval = 0.12 to 1.12) using expected deaths calculated from mortality rates of white males in the U.S. general population. For all cancer deaths, the SMR is about the same (0.49). In a similar analysis, mortality from all causes for all male coworkers at Los Alamos employed in the 1945-6 period is 0.70 (90% CI = 0.62 to 0.79) compared with white males in the U.S. general population. From these data it appears there is no excess mortality of these plutonium workers. The data are too sparse to determine if it is indeed less than expected.

Data on plutonium workers at the Rocky Flats facility showed increased rate ratios for combined lymphopoietic and hematopoietic cancers when compared with unexposed coworkers[15]. Workers were deemed exposed if Pu deposition exceeded 74 Bq.

The highest rate ratio of about 10 (90% CI = 1.3 to 94) was found when a five-year induction time was used. Somewhat lesser rate ratios were found at longer and shorter induction times. This finding suggests the hematopoietic/lymphopoietic cells may be at increased risk of cancer induction in Pu exposed individuals.

Other investigators have used functional tests of lymphocytes to study persons exposed to external gamma radiation. Liu et al [11,12] found stimulation of immunologic parameters, such as, increased plaque-forming cell reaction, in irradiated mice and in persons living in the high natural background area in China. Bloom et al. [13] studied atomic bomb survivors now residing in the United States. All four parameters of cellular immune function tested showed greater response in the radiation exposed group compared with the controls, but only one test, natural cell-mediated cytotoxicity, was statistically significant. These data are fragmentary, but suggest that in some circumstances the immune system might be stimulated by low-level ionizing radiation.

CONCLUSION

This study suggests that exposure to alpha particles from internally deposited Pu may preferentially reduce T_s cell levels. This reduced number of T_s cells may improve immunoreactivity in aging humans. This observation needs to be confirmed with a larger group of plutonium exposed individuals than reported here and also individuals exposed chronically to low doses of external

gamma radiation. If such follow-up studies confirm reduced T_s cell levels after long term low level radiation doses, a simple determination of T_H/T_s ratio may provide useful information on prospective long term biological response. Such a conclusion will be dependent on correlations made with on-going human epidemiologic studies of radiation workers.

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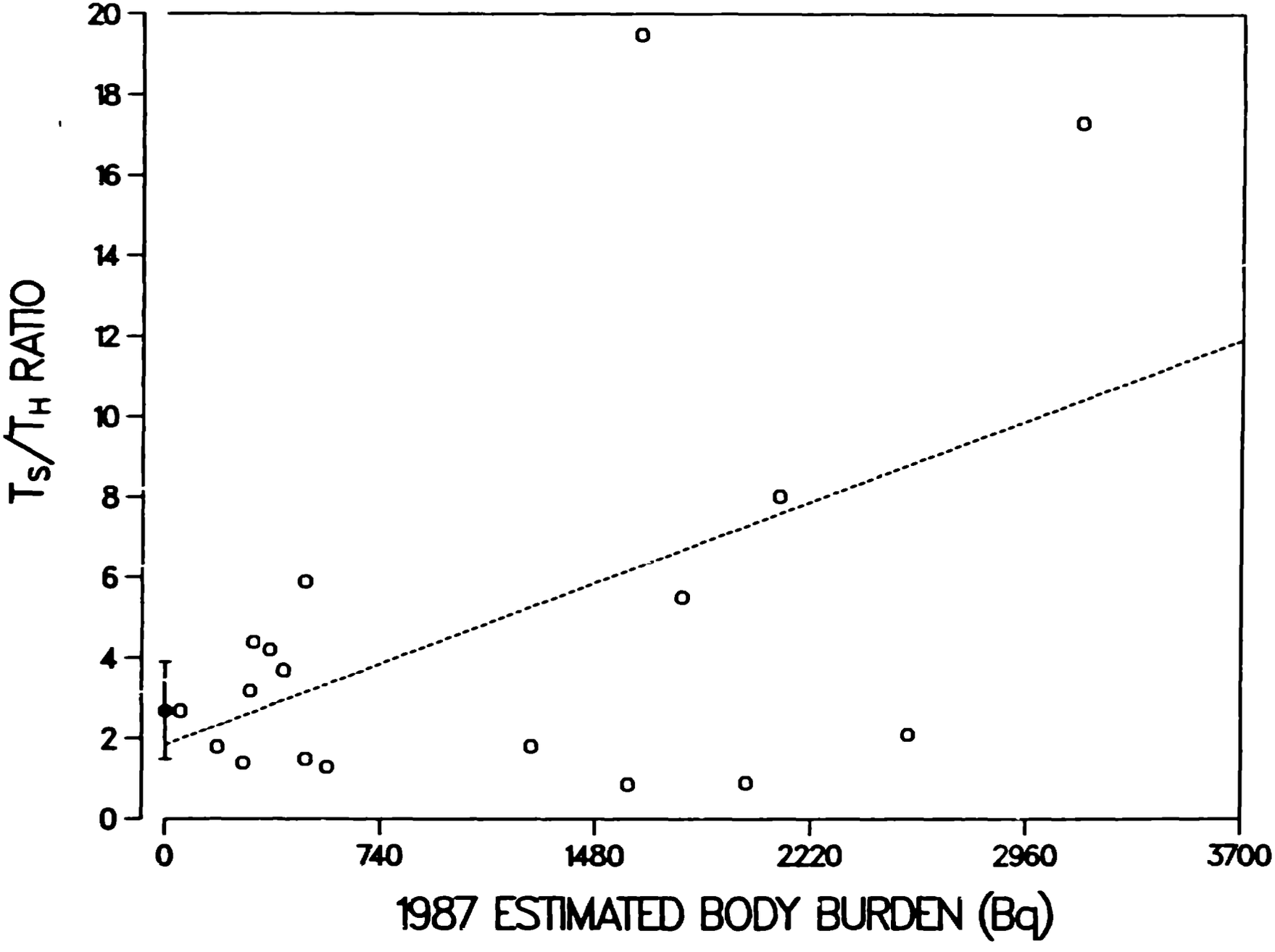
20
18
16
14
12
10
8
6
4
2
0
T_S/T_H RATIO
0
740
1480
2220
2960
3700
1987 ESTIMATED BODY BURDEN (Bq)

FIGURE LEGEND

Figure 1. Helper/suppressor T-cell ratios in fresh blood plotted as a function of 1987 estimated Pu depositions. Solid circle at 0 Bq shows mean $\pm$ S.D. for controls.

Table 1

MEDIAN VALUES OF T_H/T_S RATIOS

	Fresh Blood	Six-day cultured cells after radiation (grays)		
		0	0.5	2.0
Control Subjects	2.9	3.3	4.8	10.3
Pu Exposed	2.9	5.4	8.8	22.9