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EFFECTS OF ATOMIC BOMB RADIATION ON THE DIFFERENTIATION OF HUMAN PERIPHERAL BLOOD B LYMPHOCYTES AND ON THE FUNCTION OF CONCANAVALIN A-INDUCED SUPPRESSOR T LYMPHOCYTES

原爆放射線のヒト末梢血Bリンパ球分化能及びコンカナバリンA誘導サプレッサーTリンパ球機能に与える影響

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SUMMARY

The differentiation of human peripheral blood B lymphocytes into immunoglobulin-producing cells (Ig–PC) by pokeweed mitogen (PWM) and the function of concanavalin A (ConA)-induced suppressor T lymphocytes were examined in order to elucidate the late effects of atomic bomb radiation. A total of 140 individuals, 70 with exposure doses of 100 rad or more and an equal number of controls with exposure dose of 0 rad matched by age and sex were selected from the Nagasaki Adult Health Study sample who were examined between October 1980 and January 1982.

The differentiation of peripheral blood B lymphocytes into Ig-PC by PWM and the function of ConA-induced suppressor T lymphocytes both tended to be more depressed among those in the exposed group than in the control group, but no statistically significant difference was observed between the two groups. The function of ConA-induced suppressor T lymphocytes

要 約

原爆放射線の後影響を明らかにするためにヒト末梢血Bリンパ球の pokeweed mitogen (PWM) による免疫グロブリン産生細胞 (Ig-PC) への分化能，及びコンカナバリンA (ConA) 誘導サプレッサーTリンパ球の機能について検討した．対象者は1980年10月から1982年1月までに検診を受けた長崎の成人健康調査集団中100 rad 又はそれ以上の線量に被曝した70名，及び0 rad に被曝し性・年齢を一致させた同数の対照者の計140名とした．

末梢血Bリンパ球の PWM による Ig-PC への分化能及び ConA 誘導サプレッサーTリンパ球の機能はいずれも対照者に比し被曝群が低下している傾向にあったが，統計学的な有意差はなかった．ConA 誘導サプレッサーTリンパ球の機能は年齢とともに低下する

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tended to decrease with age, but a statistical significance was only detected for percent suppression against IgM-PC.

INTRODUCTION

Lymphocytes are highly sensitive to radiation. It has been found that in cancer patients radiation therapy results in a remarkable decrease in lymphocyte count in the peripheral blood, and the effects are sustained for several years.[1-3] It has also been clarified in many animal experiments that among the lymphocytes in the lymph nodes and spleen, B lymphocytes are more sensitive to radiation than T lymphocytes.[4,5] Among T lymphocytes, however, suppressor T lymphocytes seem to be more sensitive than other T lymphocytes.[6]

B lymphocytes in the human peripheral blood can be stimulated by PWM to differentiate into Ig-PC. It has been shown that this differentiation is dependent on helper T lymphocytes[7] and is suppressed by suppressor T lymphocytes induced by ConA.[8] A study was made on the function of radiosensitive B lymphocytes and suppressor T lymphocytes of A-bomb survivors in an attempt to clarify whether radiation effects still persist more than 35 years after exposure to A-bomb radiation. Further, Ichimaru et al[9] have recently reported that multiple myeloma tends to be more frequent among A-bomb survivors. Multiple myeloma cells are a neoplastic form of plasma cells which are differentiated from B lymphocytes. This suggests that B lymphocytes in A-bomb survivors may possibly have some abnormalities. This was an additional reason for studying the antibody production system in A-bomb survivors.

MATERIALS AND METHODS

The subjects were 70 individuals with a T65 dose[10] of 100 rad or more and an equal number of controls with 0 rad dose, matched by age and sex and randomly selected from those in the Nagasaki Adult Health Study sample who were examined from October 1980 to January 1982. The distribution of the exposed and control groups in this study is shown in Table 1 by age and sex. The present study subjects consist of matched pairs of 14 males and of 56 females. The number of females far exceeds the number of males. The age was over 50 years in most

傾向にあったが，IgM-PC に対する% suppression についてのみ統計学的に有意差が認められた．

緒　言

リンパ球は放射線感受性の高い細胞であり，癌患者に放射線治療照射を行った場合末梢血中のリンパ球数は著減し，その影響は数年以上に及ぶことが知られている．[1-3] また多くの動物実験において，リンパ節や脾臓のリンパ球では，Tリンパ球よりもBリンパ球の方が高い放射線感受性を示すとされている．[4,5] しかしTリンパ球の中ではサプレッサーTリンパ球の感受性が一層高いようである．[6]

ヒト末梢血Bリンパ球は PWM の刺激により Ig-PC へと分化するが，この分化能はヘルパーTリンパ球に依存性であり，[7] ConA によって誘導されるサプレッサーTリンパ球によって抑制されることが知られている．[8] このような観点から，放射線感受性の高いBリンパ球やサプレッサーTリンパ球の機能を原爆被爆者において測定し，被爆後35年以上を経過した今日でも放射線による影響が残っているか否かを明らかにしようと試みた．また市丸ら[9]により近年多発性骨髄腫患者が被爆者に多発する傾向のあることが明らかにされており，骨髄腫細胞はBリンパ球から分化した形質細胞の腫瘍化したものであり，被爆者におけるBリンパ球の質的異常が考慮される．このような意味からも被爆者の抗体産生系について検討を加えてみた．

材料及び方法

対象は1980年10月から1982年1月までの間に診察された長崎の成人健康調査集団中，T65D 線量測定法[10]で100 rad 又はそれ以上の線量に被曝した者70名と，対照者として性・年齢を一致させた被曝線量が0 rad の者70名を用いた．表1にこの研究の被曝群及び対照群の性及び年齢別分布を示した．今回の調査対象者は男性14名，女性56名がそれぞれ一対であり，女性の数が男性より圧倒的に多い．被爆後

TABLE 1 DISTRIBUTION OF EXPOSED AND CONTROL SUBJECTS BY AGE AND SEX
表1　被爆者及び対照者の分布，年齢及び性別

Control	Age	Exposed						
		30-39	40-49	50-59	60-69	70-79	Total	
Total	30-39	1					1	(1.4)
	40-49		10	4			14	(20.0)
	50-59		1	39	2		42	(60.0)
	60-69			1	6		7	(10.0)
	70-79					6	6	(8.6)
	Total	1	11	44	8	6	70	(100.0)
		(1.4)	(15.7)	(62.9)	(11.4)	(8.6)	(100.0)	
Male	30-39	1					1	(7.1)
	40-49		4	1			5	(35.7)
	50-59			4	1		5	(35.7)
	60-69				2		2	(14.3)
	70-79					1	1	(7.1)
	Total	1	4	5	3	1	14	(100.0)
		(7.1)	(28.6)	(35.7)	(21.4)	(7.1)	(100.0)	
Female	30-39							
	40-49		6	3			9	(16.1)
	50-59		1	35	1		37	(66.1)
	60-69			1	4		5	(8.9)
	70-79					5	5	(8.9)
	Total		7	39	5	5	56	(100.0)
			(12.5)	(69.6)	(8.9)	(8.9)	(100.0)	

Percentage in parentheses　括弧内は%

cases, since more than 35 years had elapsed since A-bomb exposure. The mean T65 dose in the exposed group was 263 rad.

35年以上を経過しているため多くは50歳以上であった．被曝群の T65D 平均線量は263 rad であった．

Culture Conditions. From each subject, 6 ml of heparinized venous blood was drawn and mononuclear cells (MNC) were separated by Böyum's method[11] and washed three times with balanced salt solution (BSS). The MNC were resuspended to 4×10^5 cells/ml concentration in RPMI 1640 culture solution containing 20% heat-inactivated fetal calf serum, 5×10^{-5} M 2-mercaptoethanol, 50 μl/ml penicillin G, and 50 μg/ml streptomycin. All subsequent cultures were conducted in plastic culture tubes with V-shaped bottom (Eiken, Tokyo).

培養条件． 各被検者より静脈血6 ml をヘパリン添加採血し，Böyum の方法[11]に準じて単核細胞（MNC）を分離し，平衡化食塩水（BSS）で3回洗浄した．MNC は20%非動化ウシ胎児血清（FCS），5×10^{-5} M 2-mercaptoethanol, 50 μl/ ml penicillin G 及び 50 μg/ ml streptomycin 添加 RPMI 1640 培養液に 4×10^5 細胞/ ml になるように浮遊させた．以下の培養はすべてV底のプラスチック製培養試験管（栄研，東京）で行った．

Induction of B Lymphocytes to Ig-PC by PWM Stimulation. A volume of 0.5 ml of MNC suspention (2×10^5 cells) from study subjects was cultured at 37°C in a 5% CO_2 incubator supplemented with 5 μl PWM (GIBCO, New York).

Bリンパ球のPWM 刺激による Ig-PC への誘導． 調査対象者の MNC 浮遊液 0.5 ml（2×10^5 細胞）に PWM（GIBCO 社，ニューヨーク）5 μl を添加し，37℃，5 % CO_2 恒温器で培養した．7 日間の培養後

After seven days of cultivation, the number of Ig-PC was counted.

Determination of ConA-induced Suppressor T Lymphocyte Activity. ConA-induced suppressor T lymphocytes were prepared by the method of Haynes and Fauci.[8] A 0.5 ml MNC suspension (2×10^5 cells) was cultured for 48 hours at 37°C in a 5% CO_2 incubator supplemented with 5μg ConA (Sigma Chemical Co., St. Louis). Control cells treated likewise without the addition of ConA were also prepared. The cultured cells were washed three times with 0.3 M α-methyl-D-mannopyranoside (αMM, Sigma Chemical Co.) to remove ConA and then rinsed once with culture solution. Thus, ConA-induced suppressor T lymphocytes and control cells were obtained.

Each of the following cell populations was cultured at 37°C in a 5% CO_2 incubator in 0.5 ml culture solution supplemented with 5μl PWM. After seven days of cultivation, the number of Ig-PC was counted:

(1) Preserved viable MNC 2×10^5 cells (MNC were preserved at 4°C for 48 hours until ConA-induced suppressor T lymphocytes were obtained and tested for viability by Trypan blue) + ConA-induced suppressor T lymphocytes
(2) Preserved viable MNC 2×10^5 cells + control cells of ConA-induced suppressor T lymphocytes
(3) ConA-induced suppressor T lymphocytes alone
(4) Control cells of ConA-induced suppressor T lymphocytes alone

The function of ConA-induced suppressor T lymphocytes was calculated from the following formula:

$$\text{Percent suppression} = \left(1 - \frac{\text{Ig-PC number in(1)} - \text{Ig-PC number in(3)}}{\text{Ig-PC number in(2)} - \text{Ig-PC number in(4)}}\right) \times 100$$

The value of percent suppression less than 0% was set at 0% and those greater than 100% were set at 100% and statistical analysis was performed.

Assay for the Enumeration of Ig-PC. The number of Ig-PC (IgPoly-PC: total number of immunoglobulin-producing cells, IgG-PC: IgG-

Ig-PC を数えた.

ConA 誘導サプレッサーTリンパ球活性の測定. Haynes 及び Fauci[8] の方法に準じて ConA 誘導サプレッサーTリンパ球を作製した. つまり, 培養液に浮遊させた MNC 0.5 ml (2×10^5 個) に ConA (Sigma Chemical 社, セントルイス) 5 μg を添加し, 37℃, 5% CO_2 恒温器で48時間培養した. またコントロール細胞として, ConA 無添加で同様に培養した. ConA を除くために培養細胞を 0.3 M α-methyl-D-mannopyranoside (αMM, Sigma Chemical 社) で3回洗浄し, 更に培養液で1回洗浄した. 以上により ConA 誘導サプレッサーTリンパ球及びそのコントロール細胞を得た.

次の各々の細胞群は 5 μl PWM 添加の 0.5 ml 培養液中で, 37℃, 5% CO_2 恒温器で培養した. 培養7日後 Ig-PC を数えた:

(1) 保存した viable な MNC 2×10^5 個 (ConA 誘導サプレッサーTリンパ球ができ, トリパンブルーで viability を算定するまで MNC を4℃で48時間保存した) に ConA 誘導サプレッサーTリンパ球を加えて培養.
(2) 保存した viable な MNC 2×10^5 個に ConA 誘導サプレッサーTリンパ球のコントロール細胞を加えて培養.
(3) ConA 誘導サプレッサーTリンパ球のみ.
(4) ConA 誘導サプレッサーTリンパ球のコントロール細胞のみ.

次の計算式で ConA 誘導サプレッサーTリンパ球の機能を算出した:

% Suppression 値が0%以下の場合は0%とし, 100%以上のものは100%として解析した.

Ig-PC 数の算定. Hammarström ら[12] の protein A 結合赤血球によるプラーク法で免疫グロブリン産生細胞数 (IgPoly-PC: 免疫グロブリン産生細胞の総数, IgG-

producing cells, IgM-PC:IgM-producing cells) was counted by protein A hemolytic plaque assay as described by Hammarström et al.[12] The cultured cells were washed once with RPMI 1640 culture solution and resuspended in 4 ml of the same culture solution. A 100 μl aliquot was mixed with 50 μl of protein A (Pharmacia Fine Chemicals, Uppsala, Sweden)-bound suspension of sheep red blood cells (SRBC), 25 μl of rabbit antihuman immunoglobulin (Poly, IgG, and IgM) antibody (MBL, Tokyo) of 20× dilution, and 25 μl of complement derived from guinea pigs and absorbed twice by SRBC. A volume of 100 μl of the mixture was placed in a Cunningham chamber and incubated at 37°C for three hours. The number of plaques formed was counted macroscopically. The assay was performed without the knowledge of exposure status.

Statistical Procedure. The values of each examined item of the two exposure groups in the matched pairs were compared using Wilcoxon matched-pairs signed-ranks test[13] to examine the dose effect. Age effect of each examined item was tested by common regression analysis and Kruskal-Wallis one-way analysis of variance.[13]

RESULTS

Effects of A-bomb Radiation on the Differentiation of Peripheral Blood B Lymphocytes. The median numbers of IgPoly-PC, IgG-PC, and IgM-PC by group are shown in Table 2. IgPoly-PC, IgG-PC, and IgM-PC in the exposed group were 212.0, 140.0, and 29.5, respectively, which were all lower than the corresponding values of 232.5, 163.5, and 40.0 in the control group. However, no statistically significant differences were observed between the two groups.

Effects of A-bomb Radiation on the Function of ConA-induced Suppressor T Lymphocytes. The median values of percent suppression against IgPoly-PC, IgG-PC, and IgM-PC on the function of ConA-induced suppressor T lymphocytes by exposure group are shown in Table 3. The values against IgPoly-PC, IgG-PC, and IgM-PC in the exposed group were 80.5%, 77.2%, and 75.0%, respectively, which were all lower than the corresponding values of 82.3%, 87.5%, and 82.1% in the control group. However, no significant difference was demonstrated between the two groups.

PC:IgG 産生細胞数，IgM-PC:IgM 産生細胞数）を算定した．つまり培養細胞を RPMI 1640培養液で1回洗浄した後，同培養液4 ml に再浮遊させ，その一部（100 μl）とprotein A（Pharmacia Fine Chemicals 社，ウプサラ，スウェーデン）結合ヒツジ赤血球（SRBC）浮遊液50 μl，20倍に希釈したウサギ抗ヒト免疫グロブリン（Poly，IgG 及び IgM）抗体（MBL，東京）25 μl 及び SRBC で2回吸収したモルモット補体25 μl を混合した．その混合液100 μl を Cunningham chamber に流入し，37℃で3時間培養後出現したプラークを肉眼で数えた．実験は被曝状態の知見なしで行われた．

統計解析． 線量効果を調べるために，一対中の二つの被曝群の検査項目ごとの測定値を Wilcoxon matched-pairs signed-ranks test[13] を用いて比較した．検査項目ごとの加齢の影響は通常の回帰解析及び Kruskal-Wallis の分散片側検定[13] を用いて調べた．

結　果

原爆放射線の末梢血Bリンパ球分化能に与える影響． 表2 に対象集団の IgPoly-PC，IgG-PC 及び IgM-PC の中央値を示した．被曝群の IgPoly-PC，IgG-PC 及び IgM-PC はそれぞれ212，140及び29.5と対照群の232.5，163.5及び40.0に比しいずれも低値であった．しかし2群間に統計的有意差は認められなかった．

原爆放射線の ConA 誘導サプレッサーTリンパ球機能に与える影響． 表3 に対象集団の IgPoly-PC，IgG-PC 及び IgM-PC に対する ConA 誘導サプレッサーTリンパ球機能における% suppression の中央値を示した．被曝群の IgPoly-PC，IgG-PC 及び IgM-PC に対する% suppression はそれぞれ80.5%，77.2% 及び75.0% と対照群の82.3%，87.5% 及び82.1% に比しいずれも低値であった．しかし2群間に統計的有意差は認められなかった．

After seven days of cultivation, the number of Ig-PC was counted.

Determination of ConA-induced Suppressor T Lymphocyte Activity. ConA-induced suppressor T lymphocytes were prepared by the method of Haynes and Fauci.[8] A 0.5 ml MNC suspension (2×10^5 cells) was cultured for 48 hours at 37°C in a 5% CO_2 incubator supplemented with 5 μg ConA (Sigma Chemical Co., St. Louis). Control cells treated likewise without the addition of ConA were also prepared. The cultured cells were washed three times with 0.3 M α-methyl-D-mannopyranoside (αMM, Sigma Chemical Co.) to remove ConA and then rinsed once with culture solution. Thus, ConA-induced suppressor T lymphocytes and control cells were obtained.

Each of the following cell populations was cultured at 37°C in a 5% CO_2 incubator in 0.5 ml culture solution supplemented with 5 μl PWM. After seven days of cultivation, the number of Ig-PC was counted:

(1) Preserved viable MNC 2×10^5 cells (MNC were preserved at 4°C for 48 hours until ConA-induced suppressor T lymphocytes were obtained and tested for viability by Trypan blue) + ConA-induced suppressor T lymphocytes
(2) Preserved viable MNC 2×10^5 cells + control cells of ConA-induced suppressor T lymphocytes
(3) ConA-induced suppressor T lymphocytes alone
(4) Control cells of ConA-induced suppressor T lymphocytes alone

The function of ConA-induced suppressor T lymphocytes was calculated from the following formula:

$$\text{Percent suppression} = \left(1 - \frac{\text{Ig-PC number in(1)} - \text{Ig-PC number in(3)}}{\text{Ig-PC number in(2)} - \text{Ig-PC number in(4)}}\right) \times 100$$

The value of percent suppression less than 0% was set at 0% and those greater than 100% were set at 100% and statistical analysis was performed.

Assay for the Enumeration of Ig-PC. The number of Ig-PC (IgPoly-PC: total number of immunoglobulin-producing cells, IgG-PC: IgG-

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(2) 保存した viable な MNC 2×10^5 個に ConA 誘導サプレッサーTリンパ球のコントロール細胞を加えて培養.
(3) ConA 誘導サプレッサーTリンパ球のみ.
(4) ConA 誘導サプレッサーTリンパ球のコントロール細胞のみ.

次の計算式で ConA 誘導サプレッサーTリンパ球の機能を算出した:

% Suppression 値が0%以下の場合は0%とし, 100%以上のものは100%として解析した.

Ig-PC 数の算定. Hammarström ら[12] の protein A結合赤血球によるプラーク法で免疫グロブリン産生細胞数 (IgPoly-PC: 免疫グロブリン産生細胞の総数, IgG-

producing cells, IgM-PC:IgM-producing cells) was counted by protein A hemolytic plaque assay as described by Hammarström et al.[12] The cultured cells were washed once with RPMI 1640 culture solution and resuspended in 4 ml of the same culture solution. A 100 μl aliquot was mixed with 50 μl of protein A (Pharmacia Fine Chemicals, Uppsala, Sweden)-bound suspension of sheep red blood cells (SRBC), 25 μl of rabbit antihuman immunoglobulin (Poly, IgG, and IgM) antibody (MBL, Tokyo) of 20× dilution, and 25 μl of complement derived from guinea pigs and absorbed twice by SRBC. A volume of 100 μl of the mixture was placed in a Cunningham chamber and incubated at 37°C for three hours. The number of plaques formed was counted macroscopically. The assay was performed without the knowledge of exposure status.

Statistical Procedure. The values of each examined item of the two exposure groups in the matched pairs were compared using Wilcoxon matched-pairs signed-ranks test[13] to examine the dose effect. Age effect of each examined item was tested by common regression analysis and Kruskal-Wallis one-way analysis of variance.[13]

RESULTS

Effects of A-bomb Radiation on the Differentiation of Peripheral Blood B Lymphocytes. The median numbers of IgPoly-PC, IgG-PC, and IgM-PC by group are shown in Table 2. IgPoly-PC, IgG-PC, and IgM-PC in the exposed group were 212.0, 140.0, and 29.5, respectively, which were all lower than the corresponding values of 232.5, 163.5, and 40.0 in the control group. However, no statistically significant differences were observed between the two groups.

Effects of A-bomb Radiation on the Function of ConA-induced Suppressor T Lymphocytes. The median values of percent suppression against IgPoly-PC, IgG-PC, and IgM-PC on the function of ConA-induced suppressor T lymphocytes by exposure group are shown in Table 3. The values against IgPoly-PC, IgG-PC, and IgM-PC in the exposed group were 80.5%, 77.2%, and 75.0%, respectively, which were all lower than the corresponding values of 82.3%, 87.5%, and 82.1% in the control group. However, no significant difference was demonstrated between the two groups.

PC:IgG 産生細胞数，IgM-PC:IgM 産生細胞数）を算定した．つまり培養細胞を RPMI 1640培養液で1回洗浄した後，同培養液4 ml に再浮遊させ，その一部（100 μl）とprotein A（Pharmacia Fine Chemicals 社，ウプサラ，スウェーデン）結合ヒツジ赤血球（SRBC）浮遊液50 μl，20倍に希釈したウサギ抗ヒト免疫グロブリン（Poly，IgG 及び IgM）抗体（MBL，東京）25 μl 及び SRBC で2回吸収したモルモット補体25 μl を混合した．その混合液100 μl を Cunningham chamber に流入し，37℃で3時間培養後出現したプラークを肉眼で数えた．実験は被曝状態の知見なしで行われた．

統計解析． 線量効果を調べるために，一対中の二つの被曝群の検査項目ごとの測定値を Wilcoxon matched-pairs signed-ranks test[13] を用いて比較した．検査項目ごとの加齢の影響は通常の回帰解析及び Kruskal-Wallis の分散片側検定[13] を用いて調べた．

結　果

原爆放射線の末梢血Bリンパ球分化能に与える影響． 表2に対象集団の IgPoly-PC，IgG-PC 及び IgM-PC の中央値を示した．被曝群の IgPoly-PC，IgG-PC 及び IgM-PC はそれぞれ212，140及び29.5と対照群の232.5，163.5及び40.0に比しいずれも低値であった．しかし2群間に統計的有意差は認められなかった．

原爆放射線の ConA 誘導サプレッサーTリンパ球機能に与える影響． 表3に対象集団の IgPoly-PC，IgG-PC 及び IgM-PC に対する ConA 誘導サプレッサーTリンパ球機能における% suppression の中央値を示した．被曝群の IgPoly-PC，IgG-PC 及び IgM-PC に対する% suppression はそれぞれ80.5%，77.2% 及び75.0% と対照群の82.3%，87.5% 及び82.1% に比しいずれも低値であった．しかし2群間に統計的有意差は認められなかった．

TABLE 2 COMPARISON OF THE DIFFERENTIATION OF B LYMPHOCYTES BY EXPOSURE GROUP

表2　Bリンパ球分化能の比較，被曝群別

No. of pairs	Exposed		Control		Statistical test Wilcoxon matched-pairs signed-ranks test
	Median	Max Min	Median	Max Min	
			IgPoly-PC		
70	212.0	764 21	232.5	615 36	z = −0.442 p = 0.66
			IgG-PC		
70	140.0	550 11	163.5	417 11	z = −0.702 p = 0.48
			IgM-PC		
70	29.5	208 5	40.0	197 2	z = −0.111 p = 0.91

The number of Ig-PC was counted by the plaque-forming method using protein A-bound SRBC after incubating MNC mixed with PWM for seven days.

Ig-PC 数は PWM 添加で7日間培養した MNC について protein A 結合 SRBC を用いたプラーク法によって数えた．

IgPoly-PC: Total number of immunoglobulin-producing cells
免疫グロブリン産生細胞の総数

IgG-PC: Number of IgG-producing cells
IgG 産生細胞数

IgM-PC: Number of IgM-producing cells
IgM 産生細胞数

TABLE 3 COMPARISON OF THE FUNCTION OF CONA-INDUCED SUPPRESSOR T LYMPHOCYTES BY EXPOSURE GROUP

表3　ConA 誘導サプレッサーTリンパ球機能の比較，被曝群別

No. of pairs	Exposed		Control		Statistical test Wilcoxon matched-pairs signed-ranks test
	Median	Max Min	Median	Max Min	
			% suppression against IgPoly-PC		
67	80.5	100 0	82.3	100 0	z = −1.016 p = 0.31
			% suppression against IgG-PC		
69	77.2	100 0	87.5	100 0	z = −1.253 p = 0.21
			% suppression against IgM-PC		
63	75.0	100 0	82.1	100 0	z = −0.471 p = 0.64

The extent of suppression of the differentiation of B lymphocytes to Ig-PC by incubation with ConA-induced suppressor T lymphocytes was compared between the exposed and control groups.

ConA 誘導サプレッサーTリンパ球による B リンパ球の Ig-PC への分化能に与える抑制程度を被曝群間で比較した．

TABLE 4 SUMMARY OF COMMON REGRESSION ANALYSIS FOR RELATIONSHIP BETWEEN AGE, DIFFERENTIATION OF B LYMPHOCYTES, AND FUNCTION OF CONA-INDUCED SUPPRESSOR T LYMPHOCYTES

表4　年齢，Bリンパ球分化能及び ConA 誘導サプレッサーTリンパ球機能間の関連についての通常の回帰解析の総括

Variable		Number	Regression Analysis		
Y	X		Constant (SD)	Coefficients (SD)	Test of significance for coefficients P
IgPoly-PC	Age	140	213.8 (8432.9)	0.611 (1.663)	>.05 NS
IgG-PC	Age	140	146.2 (3979.0)	0.385 (1.142)	>.05 NS
IgM-PC	Age	140	69.5 (457.9)	−0.478 (0.387)	>.05 NS
% suppression against IgPoly-PC	Age	137	87.1 (239.7)	−0.228 (0.280)	>.05 NS
% suppression against IgG-PC	Age	139	91.6 (277.2)	−0.327 (0.301)	>.05 NS
% suppression against IgM-PC	Age	133	110.5 (345.5)	−0.714 (0.335)	<.05 *

NS Not significant　*Significant at the 5% level
顕著でない　5%の水準で有意

Effects of Aging on the Differentiation of Peripheral Blood B Lymphocytes and on the Function of ConA-induced Suppressor T Lymphocytes. Table 4 shows the regression analysis on the relationship between age, differentiation of B lymphocytes, and function of ConA-induced suppressor T lymphocytes. Regression coefficient was only significant for percent suppression against IgM-PC and age. The value of percent suppression against IgM-PC decreased significantly with increasing age.

Table 5 compares the median value of the differentiation of B lymphocytes and the function of ConA-induced suppressor T lymphocytes by three age-groups (< 50, 50-60, and 60+). The statistical difference of these examined items by age-group was tested by Kruskal-Wallis one-way analysis of variance.[13] There were no statistically significant differences between the three age-groups.

DISCUSSION

It has been known that the incidence of leukemia is high among A-bomb survivors, especially

末梢血Bリンパ球分化能及び ConA 誘導サプレッサーTリンパ球機能の加齢による影響．　表4は年齢，Bリンパ球の分化能及び ConA 誘導サプレッサーTリンパ球の機能間の回帰解析を示す．回帰係数は年齢及び IgM-PC に対する% suppression についてのみ有意であった．つまり IgM-PC に対する% suppression は加齢とともに有意に低下した．

表5は三つの年齢群（<50，50-60及び60+）別のBリンパ球分化能及び ConA 誘導サプレッサーTリンパ球機能の中央値を比較する．年齢群別検査項目の統計的差異は Kruskal-Wallis の分散片側検定によって調べたが，[13] 三つの年齢群間には統計的有意差はなかった．

考　察

原爆被爆者に白血病が多発することが明らかにされており，特に100 rad 以上の線量に被曝した者の発病

TABLE 5 EFFECTS OF AGING ON THE DIFFERENTIATION OF B LYMPHOCYTES AND THE FUNCTION OF CONA-INDUCED SUPPRESSOR T LYMPHOCYTES

表5　Bリンパ球分化能及びConA誘導サプレッサーTリンパ球機能の加齢による影響

Age									Kruskal-Wallis one-way analysis of variance (df = 2)
<50			50-60			60+			
No.	Median	Max Min	No.	Median	Max Min	No.	Median	Max Min	
					IgPoly-PC				
27	274	764 65	86	208.5	602 34	27	243	627 21	H=2.23 P>.05
					IgG-PC				
27	165	550 29	86	145.5	461 32	27	145	417 11	H=0.978 P>.05
					IgM-PC				
27	40	208 4	86	36.5	197 2	27	29	164 3	H=2.69 P>.05
					% suppression against IgPoly-PC				
26	84.5	100 0	84	81.9	100 0	27	78.2	100 0	H=0.86 P>.05
					% suppression against IgG-PC				
26	90.0	100 28.6	86	80.9	100 0	27	79.5	100 0	H=2.91 P>.05
					% suppression against IgM-PC				
24	88.0	100 0	82	81.0	100 0	27	76.5	100 0	H=1.43 P>.05

among those exposed to 100 rad or more.[14,15] It has also been elucidated by chromosome analysis (direct bone marrow method) that hemopoietic cells of proximally exposed survivors have chromosome aberrations.[15] Thus, the development of leukemia in A-bomb survivors is a direct deleterious effect of A-bomb radiation on the DNA of hemopoietic cells. It is also possible to speculate that immunologically competent cells were destroyed by A-bomb radiation, resulting in disorder of the immunologic surveillance system, and thus the abnormal clones produced ultimately led to the induction of clinical leukemia. Decrease of phytohemagglutinin (PHA)-induced transformation of lymphocytes in heavily exposed survivors has been reported.[16] However, few studies on the antibody production system of A-bomb survivors have been conducted. Although more than 35 years have lapsed since A-bomb exposure, survivors exposed to 100 rad

率が高いことが知られている.[14,15] また近距離被爆者には，骨髄直接法による染色体分析の結果，造血細胞に染色体異常のあることが明らかにされている.[15] このため，被爆者における白血病の発症は，原爆放射線の造血細胞DNAに対する直接的な障害作用によるものと考えることができる．また，原爆放射線により免疫担当細胞が破壊され，そのために免疫監視機構の乱れが生じ，このことが発生した異常クローンを許容し，白血病の発症につながったとも考えられる．重度被爆者におけるPHAによるリンパ球幼若化反応の低下が報告されている.[16] しかし，原爆被爆者における抗体産生システムについての研究は少ない．そこで，我々は被爆後35年以上を経過した今日ではあるが，原爆放射線被曝による影響が残っているか否かを100 rad以上の線量に被曝した者を対象に検討を加え

or more were examined in order to determine whether A-bomb radiation effects still remain at this time. In humans, previous studies of this kind have usually dealt with lymphocytes of cancer patients treated by radiation therapy. It has been reported that a decrease of lymphocytes after therapeutic irradiation persists for several years,[1-3] and that the mitogenic responses to PHA and ConA are reduced.[17,18] It has also been shown that suppressor T lymphocytes are more sensitive to radiation than helper T lymphocytes in in vitro experiments on the functional aspects of lymphocytes.[6] Another report has indicated that the ratio of suppressor T lymphocytes to helper T lymphocytes defined by monoclonal antibodies does not change after radiation therapy.[19]

The present analysis demonstrated no statistically significant decrease between the exposed and control groups in the differentiation of B lymphocytes into Ig-PC by PWM stimulation and in the function of ConA-induced suppressor T lymphocytes. However, the differentiation of B lymphocytes into IgPoly-PC, IgG-PC, and IgM-PC as well as the function of ConA-induced suppressor T lymphocytes showed lower values for every examination in the exposed group than in the control group. This may indicate that some effects of A-bomb radiation still remain in lymphocytes of the survivors. To our knowledge there has been no report which demonstrates difference in social life or dietary condition between A-bomb survivors and nonexposed individuals which could induce such differences in immunologic function.

When the effects of aging on the differentiation of B lymphocytes and on the function of ConA-induced suppressor T lymphocytes were examined, a significant decrease of percent suppression against IgM-PC was observed with increase of age. This suggests that certain immunologic function of ConA-induced suppressor T lymphocytes decreases with age. Although there may have been a difference in response of immunologic function in irradiated subjects by age no attempts were made to examine this in the present study due to the small sample size when classified by dose and age.

Although the present analysis has certainly not significantly demonstrated the existence of a radiation effect, the statistical procedures used

てみた．これまでのヒトにおける研究の多くは，放射線治療を受けた癌患者のリンパ球についてなされており，治療照射後のリンパ球減少は数年以上に及ぶことや，[1-3] PHA や ConA などのマイトーゲンに対する反応性が低下すること[17,18] などが報告されている．また in vitro の実験において，リンパ球の機能的側面からみた場合サプレッサーTリンパ球は，ヘルパーTリンパ球に対して放射線感受性が高いことが明らかにされている．[6] しかし，単クローン性抗体によって測定したサプレッサーTリンパ球とヘルパーTリンパ球の比率は放射線治療後も不変であったとする報告がある．[19]

本調査では被曝群Bリンパ球の PWM による Ig-PC への分化能及び ConA 誘導サプレッサーTリンパ球の機能は，対照群に比し統計的に有意に低下しているという結果は得られなかった．しかし，Bリンパ球の IgPoly-PC，IgG-PC 及び IgM-PC へのいずれへの分化能及び ConA 誘導サプレッサーTリンパ球の機能も，すべての測定値で被曝群が対照群に比べて低下していたことは，わずかながらも原爆放射線の影響が被爆者リンパ球に残っていることを示しているのかもしれない．著者らの知りうる限りでは，被爆者，非被爆者間にこのような免疫能の差を招くような社会生活や栄養状態の違いは報告されていない．

Bリンパ球分化能及び ConA 誘導Tリンパ球機能の加齢による影響を調べたところ，加齢とともに IgM-PC に対する% suppression の有意な低下を認めた．これは ConA 誘導サプレッサーTリンパ球の機能が加齢とともに低下することを示唆する．被曝者間で年齢により免疫反応に差異があるかもしれないが，本調査においては，調査集団が小さいので線量及び年齢別の解析は試みなかった．

今回の解析では有意な放射線の影響は示されなかったが，ここで使用した統計解析法はBリンパ球の分化

here may have rather poor power to detect small shifts in B lymphocyte differentiation or suppressor T lymphocyte function.

能及びサプレッサーTリンパ球機能の小さな変動を探知する力に欠けていたかもしれない.

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