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# ANALYSIS OF PERIPHERAL BLOOD LYMPHOCYTES OF ATOMIC BOMB SURVIVORS USING MONOCLONAL ANTIBODIES
# 原爆被爆者の末梢血リンパ球のモノクローナル抗体による解析

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*In the continued interest of accurately defining the late effects of the atomic bombs, the qualitative and quantitative characteristics of the A-bomb radiation exposure doses are periodically refined. If warranted by future dose assessments, the data reported here will be reanalyzed and subsequently reported.*
原爆の後影響を引き続いて正確に究明する目的をもって，原爆放射線被曝線量の質的・量的特質について定期的に改良を加えている．今後線量評価によって，その必要性が起これば，本報の資料を再解析の上，改めて報告する．

放影研 RADIATION EFFECTS RESEARCH FOUNDATION
RERF 財団法人 放射線影響研究所

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## SUMMARY

In order to study the effects of exposure to atomic bomb radiation on the immune competence of man, the proportions of peripheral blood lymphocyte subsets were determined by an indirect immunofluorescence antibody assay using monoclonal antibodies and fluorescence microscopy. The study was based on a total of 104 Adult Health Study participants in Hiroshima, including 29 individuals exposed to 100+ rad, 46 exposed to 1-99 rad, and 29 controls of 0 rad.

No change in the proportion of Leu-1 positive cells (total T cells) and Leu-2a positive cells (cytotoxic/suppressor T cells) among peripheral blood lymphocytes was observed with age, while Leu-3a positive cells (helper/inducer T cells) decreased with age and HLA-DR positive cells (B cells and monocytes) increased with age, being especially remarkable in the oldest age-group (more than 75). The proportion of HLA-DR positive cells was higher in males, but there was no significant sex difference in the proportions of other cell types. Radiation exposure did not significantly affect the proportions of Leu-1, Leu-2a, Leu-3a, and HLA-DR positive cells. No interaction between the effects of age and radiation exposure was demonstrated.

## 要 約

原爆放射線被曝の生体免疫能へ及ぼす影響を検討するため，モノクローナル抗体を用いた間接免疫蛍光抗体法を用い蛍光顕微鏡観察により末梢血リンパ球サブセットの割合を算定した．対象者は，広島の成人健康調査集団中 100rad 以上の線量に被曝した29人，1～99rad 46人，及び 0rad 群の対照者29人，合計104人とした．

末梢血リンパ球の Leu-1 陽性細胞（総T細胞）率，Leu-2a 陽性細胞（cytotoxic/suppressor T 細胞）率には加齢による変動はなかった．一方，加齢に伴って Leu-3a 陽性細胞（helper/inducer T細胞）の割合は減少し，HLA-DR 陽性細胞（B細胞及び単球）は増加したが，この所見は75歳以上に顕著であった．HLA-DR 陽性細胞は男性に高率であったが，その他の細胞には有意な性差は見られなかった．Leu-1，Leu-2a，Leu-3a，HLA-DR 陽性細胞の割合が放射線被曝によって有意な影響を受けることはなかった．また年齢と放射線被曝との相互効果も明らかでなかった．

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## INTRODUCTION

Human immunologic competence is maintained by complex intercellular interactions between a variety of lymphocyte subsets, macrophages, etc. Disturbances to such a regulatory mechanism can lead to autoimmune disease or immunodeficiency with the development of various clinical symptoms. Accordingly, the study of lymphocyte subsets is important to understand the mechanism of immunologic homeostasis.

Lymphocytes are highly sensitive to radiation relative to other mammalian tissues and cells,[1] and it has been reported that lymphocyte counts and lymphocyte subsets change following radiation therapy for malignant tumors of various organs.[2,3]

Evaluation of lymphocyte subsets of A-bomb survivors may be useful for the elucidation of the association of carcinogenesis, aging, and susceptibility to infection with radiation exposure. Until 1980, changes in peripheral blood lymphocyte subsets of A-bomb survivors were studied by using their ability to bind sheep red blood cells as well as complement receptors as markers. However, the use of monoclonal antibodies specific to lymphocyte subsets, recently introduced by cytotechnology in the area of immunology, has made it possible to make more specific and detailed analyses of the changes in each subset. Accordingly, using new techniques, including the immunofluorescence antibody method based on monoclonal antibodies and computer-assisted morphometric analysis (CAMA), the following items were studied in A-bomb survivors who received examinations at Hiroshima RERF between 24 August and 8 October 1981: 1) the changes in the proportions of peripheral blood lymphocytes such as T cells, cytotoxic/suppressor T cells, helper/inducer T cells, and B cells, and monocytes, 2) the abnormalities in the chromatin structure and texture of these cells, and 3) the changes induced in the chromatin texture after low dose irradiation of these cells in vitro. The results of analyses of 2) and 3) using CAMA are now being reviewed by G.B. Olson (University of Arizona). Here, as the first report are the results of fluorescence microscope analysis of the relationships between the proportions of lymphocyte subsets, aging, and exposure to A-bomb radiation.

## 緒　言

ヒト免疫能は，多様なリンパ球サブセット，マクロファージなどの複雑な細胞間相互作用によって維持されている．この調整機序の障害によって，自己免疫疾患又は免疫不全が起こり，種々の臨床症状を呈してくる．したがって，免疫恒常性の動態を理解するためには，リンパ球サブセットの検索が重要である．

リンパ球は，哺乳動物の組織や細胞の中で，放射線感受性が極めて高く，[1] また各臓器の悪性腫瘍の放射線治療によって，リンパ球数及びリンパ球サブセットが変動することも報告されている．[2,3]

発癌，加齢，及び易感染性と放射線被曝との関係の解明には，原爆被爆者のリンパ球サブセットに関する評価が有用であろう．1980年までに原爆被爆者の末梢血リンパ球サブセットにおける変化はヒツジ赤血球，並びに補体受容体に対する結合能をマーカーとして調査されたが，最近，細胞工学の免疫学の分野への導入によってもたらされたリンパ球サブセットに特異的なモノクローナル抗体の使用は，各サブセットの変化についてのより特異的かつ詳細な解析を可能にしている．そこで，モノクローナル抗体に基づく免疫蛍光抗体法やコンピューターによる形態測定解析(CAMA)などの新しい技法を用いて，1981年8月24日から10月8日までに広島の放影研に受診した原爆被爆者を対象に次の各項目を調べた：1) T細胞，cytotoxic/suppressor T細胞，helper/inducer T細胞，並びにB細胞などの末梢血リンパ球及び単球の割合の変動．2) これらの細胞の染色質構造及び構成の異常．3) これらの細胞の試験管内低線量放射線照射によって誘発された染色質構造内の変化．CAMA法を用いた2)及び3)の解析結果については，現在，G.B.Olson(Arizona 大学)が検討中である．今回は第1報として，蛍光顕微鏡下で観察したリンパ球サブセットの割合，加齢，及び原爆放射線被曝の関係に関する解析の結果を述べた．

## MATERIALS AND METHODS

### Samples

The study participants were selected from the Adult Health Study (AHS) population. In the AHS, medical examinations have been offered biennially since 1958 to a fixed population which initially numbered approximately 20,000.

Age at examination and radiation dose (T65DR total dose)[4] were classified into three and four categories, respectively (age: 36-55, 56-75, 76+; dose: 0, 1-49, 50-99, 100+). Persons not in Hiroshima at the time of A-bomb (NIC) or for whom T65DR dose estimates cannot be calculated were excluded from this study. Recently the accuracy of T65DR is being questioned,[5,6] however, it is anticipated that the general ordering of subjects into unexposed, and low, medium, and high doses will be preserved under any new dosimetry system.

An attempt was made to select a stratified random sample of approximately 120 persons (10 per age-dose category) from among those who visited Hiroshima RERF for AHS examinations between 24 August and 8 October 1981. Due to the time constraint and to the age-dose distribution of the AHS cohort, those aged over 56 years and in the 50-99 rad dose category were limited, although a total of 129 persons were selected to form the study group. Six persons cancelled their visits, and four refused to participate in this study. Thus 119 blood samples were actually available for testing. Complete test results were obtained for 108 individuals, since some tests failed (6 cases) and we could not obtain the necessary information for 5 cases. These 21 omitted cases were scattered throughout the age-dose categories apparently at random. Therefore, it would seem that the chance that our inferences are distorted by elimination of these cases is small.

Furthermore, since some diseases are known to affect the proportions of the lymphocyte subsets, the disease histories, both before and after the AHS examination, of the 108 participants were investigated. Ultimately, analysis was based on 104 persons, excluding four with diseases considered to affect lymphocyte subsets: One case each diagnosed as having chronic thyroiditis and multiple myeloma at time of examination; one case who died of stomach cancer about four months after examination; and one case who

## 材料及び方法

### 対象集団

調査対象者は成人健康調査集団(AHS)から選定した．AHSでは，最初約20,000人で構成された固定集団に対して，1958年以来2年ごとに検診を行っている．

診察時年齢及び被曝放射線量(T65DR総線量)[4]をそれぞれ3群及び4群に分類した(年齢：36～55，56～75，76歳以上；線量：0，1～49，50～99，100＋rad)．原爆投下時広島にいなかった者，又はT65DR線量推定値が算定できない者は，本調査から除外した．最近，T65DRの精度が問題になっているが，[5,6]いかなる新しい線量測定法のもとでも，非被爆，低，中等度，高線量の一般的分類は存続されるものと思われる．

1981年8月24日から10月8日までの期間にAHS検診を広島放影研で受けている群から約120人の無作為な層化集団(年齢-線量区分あたり10人)を選定する試みを行った．調査集団として合計129人を選定したが，時間的制限，及びAHS集団の年齢-線量分布の偏りのために56歳以上の者及び50～99rad線量区分の者は限られていた．6人は受診を取り消し，4人は本調査への協力を拒否した．したがって，実際には119人から検査用の血液標本を入手した．幾つかの検査(6例)は失敗し，5例は必要なデータが入手できなかったので，完全な検査結果が得られたのは108例であった．これら除外した21例は，年齢-線量区分の全体に，無作為に散在していた．したがって，これらの例を除外することで我々の推論がゆがめられる機会は小さいものと思われる．

更に，幾つかの疾患はリンパ球サブセットの割合に影響を及ぼすことが知られているので，108人の受診者のAHS検診前後における病歴を調べた．最終的には，リンパ球サブセットに影響を及ぼすと考えられる疾患を有する次の4例を除く104例に基づいて解析を行った：すなわち，1例は診察時に慢性甲状腺炎，1例は多発性骨髄腫を診断された者であり，受診後約4か月を経て胃癌で死亡した1例と，受診

died of pancreas cancer six months after examination and who was presumed to have developed cancer before the examination. Two cases, who had cervical cancer and breast cancer resections in 1971 and 1973, respectively, were included in this study, since no clinical abnormalities were observed at the time of their AHS examinations (conducted 10 years and 8 years after surgery, respectively) and no relapse has been observed 4 years thereafter. These two cases were considered to represent complete cures and their past cancers were assumed to impart little or no effect on the test results.

後6か月を経て膵臓癌で死亡した1例は受診前に発癌していたと思われる者である．1971年及び1973年にそれぞれ子宮頚部癌及び乳癌切除術を受けている2例は，AHS 検診時(外科手術後それぞれ10年及び8年を経て実施)に臨床的異常が認められず，その後4年間に再発も認められていないので，本調査の対象に含めた．これら2症例は完治しており，既往の癌は検査結果に影響をほとんど，あるいは全く与えないと思われた．

Table 1 shows the distribution of the 104 participants by age, dose, and sex. Since the number of cases is small in the 50-99 rad dose group, the cases of this group were combined with the 1-49 rad group for statistical analysis. Moreover, since sex was not controlled at the time of selection, the proportion of males is extremely small which made it difficult to investigate possible sex effects conclusively.

表1は，104人の対象者の年齢，線量，及び性別分布を示すものである．50～99rad 群では症例数が少ないので，この群の症例を 1～49rad 群と組み合わせて統計的解析を行った．更に，選定時に性別の調整を行わなかったので，男性の割合は極めて小さく，そのため，性別の影響を詳細に調査することは困難であった．

TABLE 1 DISTRIBUTION OF STUDY PARTICIPANTS BY AGE AT EXAMINATION, RADIATION DOSE, AND SEX
表1 調査対象者の分布；診察時年齢，放射線量，及び性別

| T65DR | Age at Examination | | | |
|---|---|---|---|---|
| | 36-55 | 56-75 | 76+ | Total |
| 0 | 9 (3) | 11 (2) | 9 (2) | 29 (7) |
| 1-49 | 9 (3) | 8 (2) | 11 (3) | 28 (8) |
| 50-99 | 11 (3) | 5 (1) | 2 (1) | 18 (5) |
| 100+ | 10 (3) | 11 (2) | 8 (2) | 29 (7) |
| Total | 39 (12) | 35 (7) | 30 (8) | 104 (27) |

Number of males is parenthesized. 括弧内は男性数を示す．

**Isolation of Peripheral Blood Lymphocytes**

Five milliliters of venous blood were drawn from each subject, defibrinated using glass beads, and mixed with an equal volume of phosphate-buffered saline (PBS) in 15 ml centrifuge tubes (Corning Co.). Four milliliters of Ficoll-Hypaque (gravity: 1.077) were underlaid and the tubes were centrifuged at 400 x g for 30 minutes at room temperature. The mononuclear cells at the interface were harvested and diluted with PBS; the mixture was centrifuged once at 510 x g for 10 minutes and twice at 240 x g for 10 minutes, and the cells were finally resuspended in PBS at a final concentration of $1 \times 10^7$/ml.

**末梢血リンパ球の分離**

各対象者から 5ml の静脈血を採取し，ガラスビーズを用いて線維素除去を行い，15ml の遠心管(Corning 社製)中で同量の phosphate-buffered saline (PBS) と混合した．4ml の Ficoll-Hypaque (重力：1.077) をその下に入れ，遠心管を室温で30分間，400×g で遠心分離にかけた．境界面における単核細胞を採取し，PBS で希釈した．これを 510×g 10分間で1回，また 240×g 10分間で2回，遠心分離にかけ，最後にその細胞を最終濃度 $1 \times 10^7$/ml になるよう PBS 中に再浮遊した．

**Analysis of Peripheral Blood Lymphocyte Subsets Using Indirect Immunofluorescence Antibody Assays**

The monoclonal antibodies used were anti-Leu-1, anti-Leu-2a, anti-Leu-3a, and anti-HLA-DR produced by Becton Dickinson Co. Anti-Leu-1 is a monoclonal antibody specific to T cells. It responds to more than 95% of peripheral blood T cells, but does not respond to B cells, null cells, monocytes, and granulocytes.[7] Anti-Leu-2a responds to cytotoxic/suppressor T cells[8] and anti-Leu-3a to helper/inducer T cells without exception.[9] Since anti-Leu-2a sometimes responds feebly to natural killer (NK) cells as well as cytotoxic/suppressor T cells, and since Leu-1 does not react with all T cells, e.g., those identified by sheep red blood cells (SRBC) receptor, the sum of the proportions of Leu-2a positive cells and of Leu-3a positive cells exceeds the proportion of Leu-1 positive cells. Anti-HLA-DR reacts with B cells, monocyte/macrophages, and activated T cells.[10]

First, to 0.1 ml of four different lymphocyte suspensions ($1 \times 10^6$ cells) described above, 50$\mu$l of each monoclonal antibody (anti-Leu-1, anti-Leu-2a, anti-Leu-3a, and anti-HLA-DR) diluted to 1/10 was added, allowed to react at 4°C for 20 minutes, centrifuged, and washed twice in PBS. Then, 10$\mu$l of antimouse IgG fluorescein isothiocyanate (FITC, 1:10, Tago Co.) was added to each tube and allowed to react at 4°C for 15 minutes, after which the mixture was centrifuged and washed twice before being fixed with 1 ml of 1% paraformaldehyde. More than 200 mononuclear cells were counted under the fluorescence microscope, and the proportion of membrane fluorescence positive cells was determined.

**間接免疫蛍光抗体法による末梢血リンパ球サブセットの解析**

使用したモノクローナル抗体は，Becton Dickinson 社製の抗 Leu-1，抗 Leu-2a，抗 Leu-3a，抗 HLA-DR であった．抗 Leu-1 はT細胞に特異的なモノクローナル抗体であり，末梢血T細胞の95%以上に反応するが，B細胞，null 細胞，単球，顆粒球には反応しない．[7] 抗 Leu-2a は cytotoxic/suppressor T細胞に，[8] 抗 Leu-3a は helper/inducer T細胞に，例外なく反応する．[9] 抗 Leu-2a は cytotoxic/suppressor T細胞に対してと同様に natural killer (NK) 細胞にも弱く反応することがあり，Leu-1 もヒツジ赤血球(SRBC) 受容体によって同定されるT細胞など，すべてのT細胞に反応するわけではないため，Leu-2a 陽性細胞及び Leu-3a 陽性細胞の割合の総計は Leu-1 陽性細胞の割合より多い．抗 HLA-DR は B細胞，単球/マクロファージ及び活性化されたT細胞に反応する．[10]

まず，上記リンパ球浮遊液を 0.1ml ($1 \times 10^6$ 細胞) ずつ4本に分注し，10倍稀釈した各モノクローナル抗体 (抗 Leu-1，抗 Leu-2a，抗 Leu-3a，抗 HLA-DR) 50$\mu$l を加え，4°C で20分間反応させ，遠心分離し，PBS で2回洗浄した．10$\mu$l の抗マウス IgG フルオレセイン・イソチオシアネート (FITC，1：10，Tago 社) を各チューブに加え，4°C で15分間反応させた後，その混合液を遠心分離し，2回洗浄後1%のパラホルムアルデヒド 1 ml で固定した．蛍光顕微鏡で200以上の単核細胞を算定し，膜蛍光陽性細胞の割合を調べた．

## METHOD OF STATISTICAL ANALYSIS

Data employed in the analysis were sex, age, A-bomb exposure dose, and the four determinations obtained through indirect immunofluorescence antibody assays, as described above. Those determinations were the proportions of lymphocytes identified by monoclonal antibodies, anti-Leu-1, anti-Leu-2a, anti-Leu-3a, and anti-HLA-DR.

In this report, effects of the independent variables, sex, age, and exposure dose on lymphocytes percentages, the dependent variables, were studied using analysis of variance and regression

## 統計的解析方法

解析に用いたデータは，性，年齢，原爆被爆線量，並びに上記の間接免疫蛍光抗体法で得た4種類の測定値である．これらの測定値は抗 Leu-1，抗 Leu-2a，抗 Leu-3a，抗 HLA-DR などのモノクローナル抗体によって確認されたリンパ球の割合である．

本論では，分散分析と回帰分析を用いて独立変数である性，年齢及び被曝線量が従属変数であるリンパ球百分率に及ぼす影響を調べた．従属変数の適当な

analysis. Appropriate normalizing transformations for the dependent variables were selected by examination of normal probability plots. For Leu-2a and HLA-DR positive cells, logarithmically transformed values were used as dependent variables in regression analyses; since the percentage of lymphocytes reactive with the other two antibodies seemed to show relatively normal distributions, and were used as they were. Furthermore, regression analyses were augmented with investigation to detect highly influenced points, using tools of residual analyses such as Cook's distance and studentized residuals.[11] The commonly applied practice at RERF of substituting 600 rad for dose exceeding this value[12] was also employed for the regression analyses.

正規化変換は，正規確率表を検討して選定した．Leu-2a と HLA-DR 陽性細胞については，対数変換値を回帰分析の独立変数として用いた．他の二つの抗体に反応したリンパ球の割合は比較的正規分布に近い分布を示しているので，そのままの値を用いた．更に，Cook の距離とスチューデント化された残差[11]のような残差分析の方法を用いて影響の大きかった点を探知することにより，回帰分析の妥当性が確かめられた．放影研では通常，線量が 600rad を超える場合 600rad を用いているが，この回帰分析においても，その方法[12]を採用した．

## RESULTS

Shown in Table 2 are the mean, standard deviation, and maximum and minimum values for each lymphocyte subset. Leu-1 positive cells accounted for approximately 50.4% of mononuclear cells. The proportions of Leu-2a and Leu-3a positive cells, which together comprise the peripheral T cell pool, were 15.7% and 42.9% of mononuclear cells, respectively. HLA-DR positive cells accounted for 17.7% of the mononuclear cells.

## 結果

表2は，各リンパ球サブセットの平均値，標準偏差，並びに最大値，及び最小値を示す．Leu-1 陽性細胞は単核細胞の約50.4%を占めた．Leu-2a 及び Leu-3a 陽性細胞は共にT細胞サブセットを構成するものであるが，これらの割合は，それぞれ単核細胞の15.7%及び42.9%であった．HLA-DR 陽性細胞は単核細胞の17.7%であった．

TABLE 2 AVERAGES AND RANGES OF MEASUREMENTS
表2 測定値の平均及び範囲

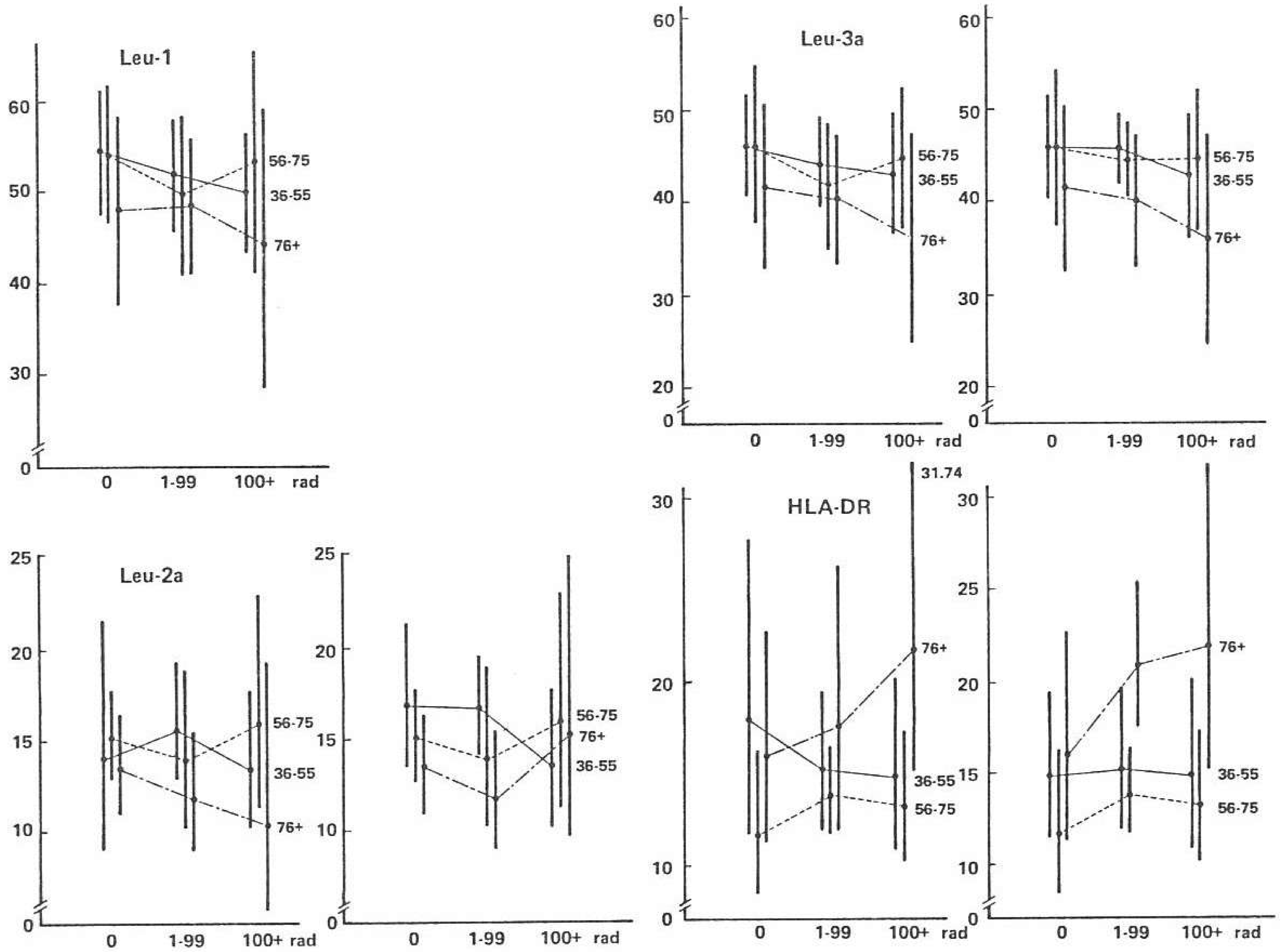
| | Number | Mean | Standard Deviation | Min | Max |
|---|---|---|---|---|---|
| Leu-1 (%) | 104 | 50.421 | 14.850 | 13.6 | 86.0 |
| Leu-2a (%) | 104 | 15.732 | 7.794 | 3.0 | 41.2 |
| log-transformed | 104 | 2.628 | 0.531 | 1.099 | 3.718 |
| Leu-3a (%) | 104 | 42.868 | 12.384 | 2.0 | 67.8 |
| HLA-DR (%) | 104 | 17.716 | 10.518 | 2.0 | 79.0 |
| log-transformed | 104 | 2.731 | 0.548 | 0.693 | 4.369 |
| Age | 104 | 61.875 | 14.207 | 36.0 | 86.0 |
| Dose | 104 | 87.846 | 121.061 | 0 | 600* |

*T65DR dose estimates greater than 600 rad (1100 rad) are truncated to 600 rad.
600rad 以上(1100rad)のT65DR 線量推定値は 600rad とした．

Figure 1 shows means of proportions and their 95% confidence intervals for each subset defined by the age and dose categories. From this figure, one might notice any trend or any effect in means, but it should be emphasized the corresponding confidence intervals are so wide that the trend or effect are not conclusive unless we examine it carefully.

図1は，各サブセットに関する割合の平均値とその95%信頼区間を年齢及び線量区分別に示したものである．この図から，平均値に何らかの傾向や影響が認められるかもしれないが，対応する信頼区間があまりにも広いために，その傾向や影響については，慎重に検討しなければ結論を得られないことを強調したい．

FIGURE 1 PROPORTIONS OF MONOCLONAL ANTIBODY POSITIVE CELLS BY AGE AND RADIATION DOSE
図1 年齢，被曝線量別モノクローナル抗体陽性細胞の割合




LEFT FIGURE:（左図）
Based on the entire 104 cases 全104例に基づく

RIGHT FIGURE:（右図）
Excluding extreme points 端点を除く
Leu-2a: lowest 4 cases
Leu-3a: lowest 2 cases
HLA-DR: maximum and minimum cases
Details of those points are given in the text and later figures. これらの点の詳細は本文及び後出の図に示す.

Table 3 shows the summary results of analyses of variance relating sex, age, and radiation dose to the proportions of Leu-1, Leu-2a, Leu-3a, and HLA-DR positive cells, based on all 104 cases. This table indicated that 1) a change with age is observed in the proportion of HLA-DR positive cells, 2) a difference by sex is also observed in the proportion of HLA-DR positive cells, 3) radiation effects are not evident, and 4) there is no significant interaction between age and radiation exposure. Since the number of males studied was not sufficient, as mentioned in Materials and Methods, we could not study the interaction of sex with other variables.

表3は，全104症例に基づいて，性，年齢及び被曝線量とLeu-1, Leu-2a, Leu-3a 及びHLA-DRの各陽性細胞の割合との関係を調べた分散分析の要約結果を示す．この表は，次のことを示している．1) HLA-DR陽性細胞の割合には年齢に伴う変化が認められた．2) HLA-DR陽性細胞の割合には性差も認められた．3) 放射線による影響は明らかでなかった．4) 年齢と放射線被曝との有意な相互効果は認められなかった．材料と方法の項で述べたように，対象となった男性の数が十分でなかったので，性別とほかの変数との相互効果は調査できなかった．

TABLE 3 SUMMARY ANALYSIS OF VARIANCE TABLES (Based on entire 104 cases)
表3 分散表の解析の要約(全104例に基づく)

| Effects | Sums of Squares[1)] | df | Mean Square | F | P |
|---|---|---|---|---|---|
| Leu-1 | | | | | |
| Sex | 12.34 | 1 | 12.34 | 0.0531 | 0.8 |
| Age | 510.1 | 2 | 255.0 | 1.133 | 0.3 |
| Dose | 144.0 | 2 | 71.99 | 0.3199 | 0.7 |
| Error 1[2)] | 22054 | 98 | 225.0 | | |
| Age-Dose Interaction | 215.4 | 4 | 53.85 | 0.2318 | 0.9 |
| Error 2[3)] | 21838 | 94 | 232.3 | | |
| Leu-2a (log-transformed) | | | | | |
| Sex | 0.2167 | 1 | 0.2167 | 0.7482 | 0.4 |
| Age | 1.112 | 2 | 0.556 | 1.963 | 0.15 |
| Dose | 0.0546 | 2 | 0.0273 | 0.0963 | 0.9 |
| Error 1 | 27.76 | 98 | 0.2832 | | |
| Age-Dose Interaction | 0.5275 | 4 | 0.1319 | 0.4553 | 0.8 |
| Error 2 | 27.23 | 94 | 0.2897 | | |
| Leu-3a | | | | | |
| Sex | 21.01 | 1 | 21.01 | 0.1317 | 0.7 |
| Age | 463.7 | 2 | 231.9 | 1.501 | 0.2 |
| Dose | 178.6 | 2 | 89.3 | 0.5781 | 0.6 |
| Error 1 | 15138 | 98 | 154.5 | | |
| Age-Dose Interaction | 146.3 | 4 | 36.58 | 0.2294 | 0.9 |
| Error 2 | 14992 | 94 | 159.5 | | |
| HLA-DR (log-transformed) | | | | | |
| Sex | 4.304 | 1 | 4.304 | 16.92 | <0.0001 |
| Age | 1.430 | 2 | 0.7150 | 2.848 | 0.06 |
| Dose | 0.0948 | 2 | 0.0474 | 0.1888 | 0.8 |
| Error 1 | 24.62 | 98 | 0.2512 | | |
| Age-Dose Interaction | 0.7006 | 4 | 0.1752 | 0.6885 | 0.6 |
| Error 2 | 23.92 | 94 | 0.2544 | | |

1) Sums of squares for each effect are calculated as the difference of the residual sum of squares between the model excluding the effect and the model including it.
各影響の二乗和は，影響を除いたモデルと含めたモデルとの間における残差二乗和の差として計算した.

2) The variance estimates (mean square) corresponding to this error were used to evaluate effects of age and dose factors. Therefore, F-tests for these two factors are based on (2,98) degrees of freedom.
この誤差に相当する分散推定値(平均二乗)を，年齢及び線量の影響の評価に用いた．したがって，これら二つの因子に関するF検定は，自由度(2,98)に基づく.

3) The variance estimates of this error were used to test sex and age-dose interaction effect. Degrees of freedom for these tests are thus (1,94) and (4,94), respectively.
この誤差の分散推定値は，性及び年齢-線量相互作用効果を調べるのに用いた．したがって，これらの検定の自由度は，それぞれ(1,94)及び(4,94)である.

Further analyses of each lymphocyte subpopulation are described in the following section. Since no significant interactions were observed, we focused our attention on the main effects of the individual factors.

各リンパ球subpopulationのより詳細な解析については，次項で述べる．有意な相互効果は認められなかったので，各因子の主な影響に主眼を置いた.

**Leu-1 Positive Cells**

As shown in Table 3, changes in the proportion of Leu-1 positive cells by age, radiation dose, and sex were not observed. As shown in Figure 1, the 76+ age-group and the exposed groups presented lower values, but the differences were not significant. The female group also showed lower values, but the difference was not significant either in the analysis of variance (Table 3) or in the regression analysis (Appendix Table 1).

**Leu-2a Positive Cells**

As mentioned earlier, since normal probability plotting suggested that logarithmically transformed values of the proportion of Leu-2a positive cells are roughly normally distributed, the proportions of Leu-2a positive cells were analyzed after log transformation.

From the results of regression analysis and analysis of variance, there is no evidence of simple sex, age, or dose effects on the proportion of Leu-2a positive cells. None of these factors individually was significantly related to this measurement (Table 3), nor were they jointly significant ($p=0.5$) when considered in an additive model (Appendix Table 2, 1st and 2nd columns). However, in a quadratic regression model of the age effect, the coefficient of the square of age was marginally significantly less than zero ($p=0.03$, Appendix Table 2, 4th column). The values of the estimated regression coefficients for this model imply that the proportion of Leu-2a positive cells tends to be lower in the high age range. However, the four lowest values are concentrated at the extreme of the age range (Figure 2), and hence they strongly influence the quadratic model. Thus analyses excluding these four points were also made (Appendix Table 3). As in the aforementioned results, no effects due to exposure dose or sex were observed, and in addition no significant effects of age were evident.

**Leu-3a Positive Cells**

As the distribution of the proportion of Leu-3a positive cells is almost normal, the measured values were employed in the analysis without transformation.

Results of regression analysis show that age, sex, and exposure dose did not significantly affect the proportion of Leu-3a positive cells, as expected from Figure 1. For example, sex, age, and dose are jointly nonsignificant ($p=0.5$) when

Leu-1 陽性細胞

表3に示すように，Leu-1 陽性細胞の割合には年齢，被曝線量，性別による変動は認められなかった．図1にみられるように，76歳以上の年齢群と被爆群は低い値を示したが，その差は有意なものではなかった．女性群も低値を示したが，その差は分散分析(表3)においても回帰分析(付表1)においても有意なものではなかった．

Leu-2a 陽性細胞

前に述べたように，Leu-2a 陽性細胞の割合は対数変換することによりほぼ正規分布に従うことが正規確率表により示唆されたため，Leu-2a 陽性細胞の割合は対数変換後に解析した．

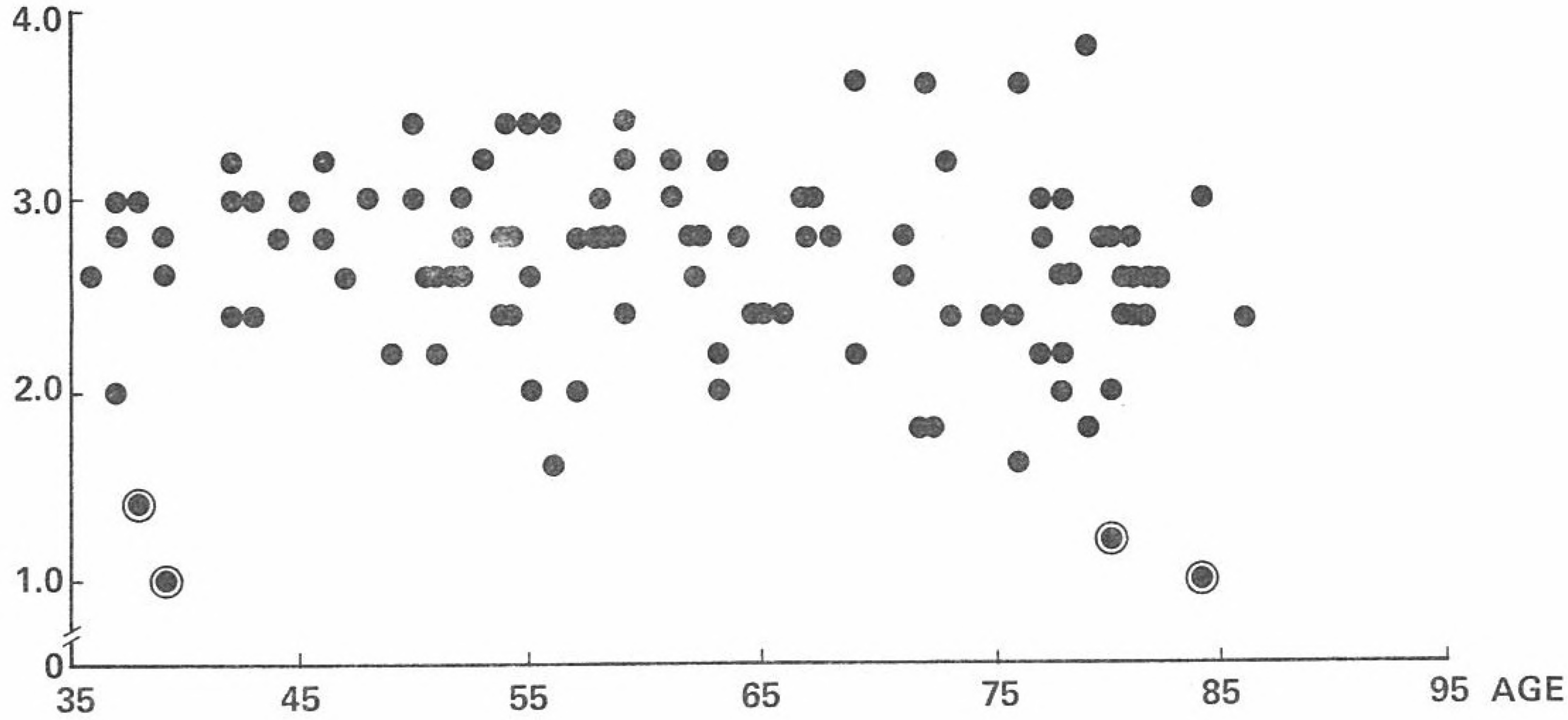
回帰分析及び分散分析の結果では，Leu-2a 陽性細胞の割合に性，年齢又は，線量が単独で与える影響は認められない．これらの変量には，個別にこの測定値(表3)と有意な関連を示すものはなく，加算的モデルで考えると，それらが総合的に有意な関連をもつことも認められなかった($p=0.5$; 付表2，第1，2欄)．しかし，年齢効果に関する二次回帰モデルにおいては，年齢の二次の項の係数は0よりも辛うじて有意に負の値となった($p=0.03$; 付表2，第4欄)．このモデルで推定された回帰係数の値は，Leu-2a 陽性細胞の割合が高年齢層では低くなる傾向があることを示唆している．しかし，四つの最低値は年齢幅の両極に集中しているので(図2)，それらは二次モデルに強い影響を与えている．したがってこれらの4点を除いた解析をも行った(付表3)．前述の結果同様，被曝線量や性別による影響は認められず，また，年齢による有意な影響も明らかでなかった．

Leu-3a 陽性細胞

Leu-3a 陽性細胞の割合の分布はかなり正規分布に近い分布であるため，測定値は変換を行わずに解析に用いた．

回帰分析の結果は，図1から期待されたように，年齢，性，被曝線量が Leu-3a 陽性細胞の割合に有意な影響を与えていないことを示している．例えば，加算的モデルで考えた場合は，性，年齢，及び線量の

FIGURE 2 SCATTER PLOT OF THE PROPORTION OF Leu-2a POSITIVE CELLS (log-transformed) VS AGE
図2 Leu-2a 陽性細胞(log-transformed)の割合と年齢を示す散在図



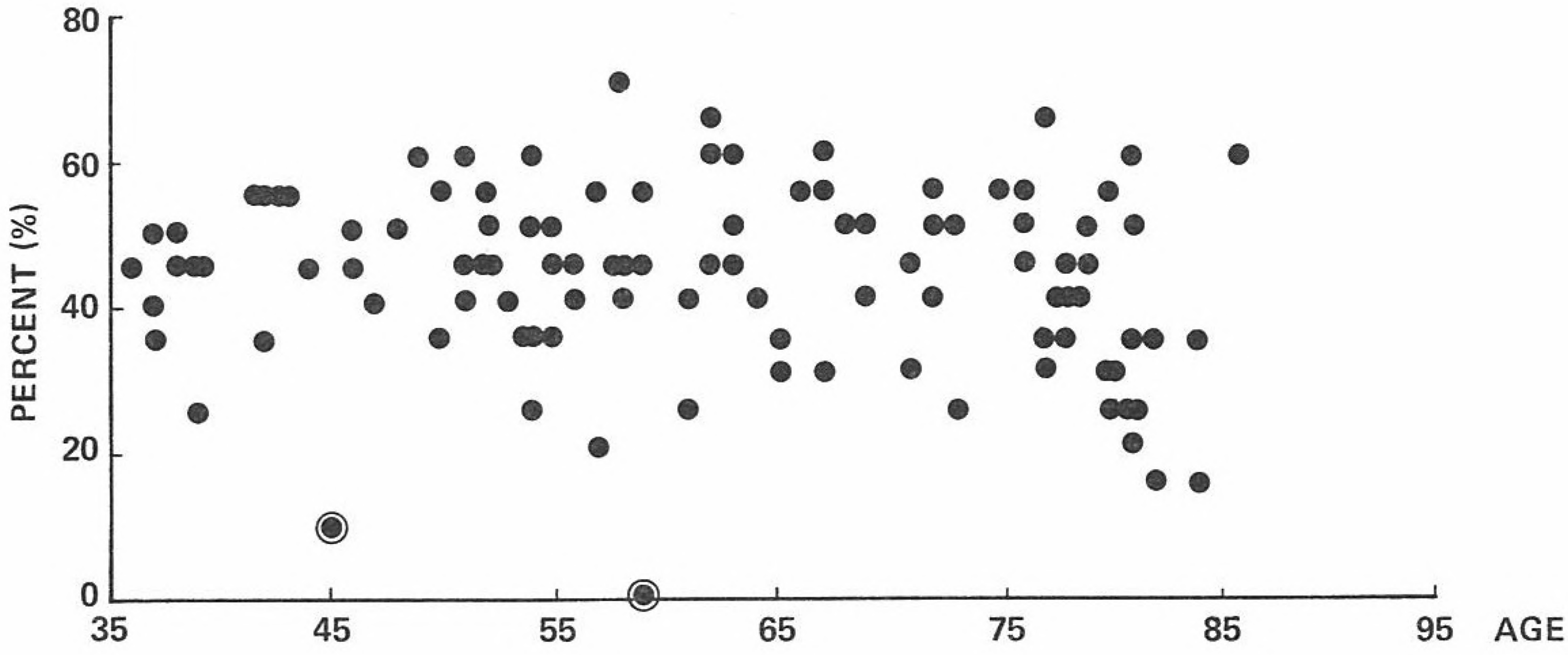

Circled four points are 丸で囲む4点は

| MF | Sex | Dose | Age | Leu-1 | Leu-2a | Leu-3a | HLA-DR |
|---|---|---|---|---|---|---|---|
| [redacted] | F | 493 | 84 | 14.9 | 3.0 | 12.6 | 25.2 |
| | F | 0 | 39 | 55.0 | 3.0 | 45.6 | 12.9 |
| | F | 133 | 80 | 47.8 | 3.2 | 53.6 | 26.7 |
| | M | 66 | 38 | 62.9 | 4.2 | 52.1 | 27.2 |

considered in an additive model (Appendix Table 4, 1st and 2nd columns). The large negative coefficient associated with the 76+ age-group (e.g., Appendix Table 4, 4th column) indicates an especially low average proportion of Leu-3a positive cells in the elderly, although the effect is not statistically significant (p=0.08). As shown in Figure 3, there is a possibility that the two lowest proportions are masking the effect of age. Therefore, analyses excluding these two points were made (Appendix Table 5). Those results indicate that the effects of exposure dose and sex remain nonsignificant (p=0.9, Appendix Table 5, 2nd and 3rd columns). On the other hand, although heterogeneity among the three age categories was not significant (p=0.08), the mean proportion in the 76+ age group was about 6% points lower than that of the other two groups, a marginally significant decrease whether or not adjustment was made for the effects of the other covariates (Appendix Table 5, 2nd, 3rd, and 4th columns, p=0.04, 0.04, and 0.02,

組み合わせは有意ではない(p=0.5; 付表4, 第1, 2欄). 76歳以上の年齢群における大きい負の係数(付表4, 第4欄)は, 高年齢層における Leu-3a 陽性細胞の割合の平均値が特に低いことを示しているが, その影響は統計的に有意なものではない(p=0.08). 図3に示したように, 二つの低値が年齢の影響を隠している可能性がある. したがって, これら二つの値を除いた解析を行った(付表5). これらの結果は, 被曝線量と性の影響が依然として有意でないことを示している(p=0.9; 付表5, 第2, 3欄). 一方, 三つの年齢区分での相違性は有意でなかったが(p=0.08), 76歳以上の年齢群における平均値は, 他の二つの群より約6%低く, 他の共変量の影響の修正に関係なく, 辛うじて有意な減少を示している(付表5, 第2,3,4 欄; それぞれ p=0.04, 0.04及び

FIGURE 3 SCATTER PLOT OF THE PROPORTION OF Leu-3a POSITIVE CELLS VS AGE
図3 Leu-3a 陽性細胞の割合と年齢を示す散在図




Circled two points are 丸で囲む2点は

| MF | Sex | Dose | Age | Leu-1 | Leu-2a | Leu-3a | HLA-DR |
|---|---|---|---|---|---|---|---|
| [redacted] | F | 6 | 59 | 20.0 | 25.8 | 2.0 | 8.9 |
| | F | 52 | 45 | 36.3 | 19.0 | 11.4 | 10.3 |

respectively). We also attempted fitting of regression models in which the proportion of Leu-3a positive cells decreases continuously as a linear or quadratic function of age (Appendix Table 5, 5th and 7th columns). In both models age term was statistically significant ($p=0.04$ and $p=0.02$, respectively). However, when both linear and quadratic terms for age are included, neither term is individually significant (Appendix Table 5, 6th column). It may be considered that over-parameterization has occurred in this attempt to examine the decreasing tendency by age. However, in view of the negligible difference between the youngest and middle age-groups (Appendix Table 5, 3rd and 4th columns) it is suspected that the model based on two age-groups (≦75 and 76+) is more appropriate than the models in which the proportion of Leu-3a positive cells decreases linearly (1.6% points per 10 years) with age. This statement is supported by the smaller residual sum of squares value (12410) for the former model, compared to that (12542) of the model assuming linear decreasing trend for age (Appendix Table 5, 4th and 5th columns).

0.02). Leu-3a 陽性細胞の割合が，年齢の直線又は二次関数的に継続的に減少する回帰モデルの適合をも試みた(付表5，第5，7欄)．いずれのモデルにおいても，年齢に対応する項は統計的に有意であった(それぞれ $p=0.04$, $p=0.02$)．しかし，年齢の直線あるいは二次の項の双方が含まれる場合は，そのいずれにも単独には有意性はみられない(付表5，第6欄)．年齢別の減少傾向を調べる上で，over-parameterization が起こったとも考えられる．しかし，最も年齢の若い群と中間の年齢群間(付表5，第3，4欄)の差は無視できるものであることから見て，二つの年齢群(75歳以下及び76歳以上)に基づくモデルは，Leu-3a 陽性細胞の割合が年齢に伴って直線的に減少する(10年で1.6%)モデルより適当ではないかと思われる．この所見は，前述のモデルの残差二乗和(12410)が，年齢に関する直線的減少傾向を想定したモデルの残差二乗和(12542)に比べて低いことによって裏付けられる(付表5，第4，5欄)．

### HLA-DR Positive Cells

The logarithmically transformed proportion of HLA-DR positive cells was used as the dependent variable in the regression analyses described below.

A large effect of sex was apparent, with the proportion being higher in males (geometric means: 22.2% for males, 13.5% for females) this effect was highly significant ($p<0.0001$) whether or not adjustments were made for the other covariates (Appendix Table 6, 2nd, 3rd, and 4th columns). After adjustment for sex and age, differences between the three dose groups were not statistically significant ($p=0.8$, Appendix Table 6, 2nd and 3rd columns). Attention should be paid to the proportion of HLA-DR positive cells in relation to age, again. That is, as shown in Figure 4, since one value at each end point of age range seems to deviate from the general pattern, the effect of age was possibly distorted. After adjusting for the sex difference, heterogeneity among the age-groups was not statistically

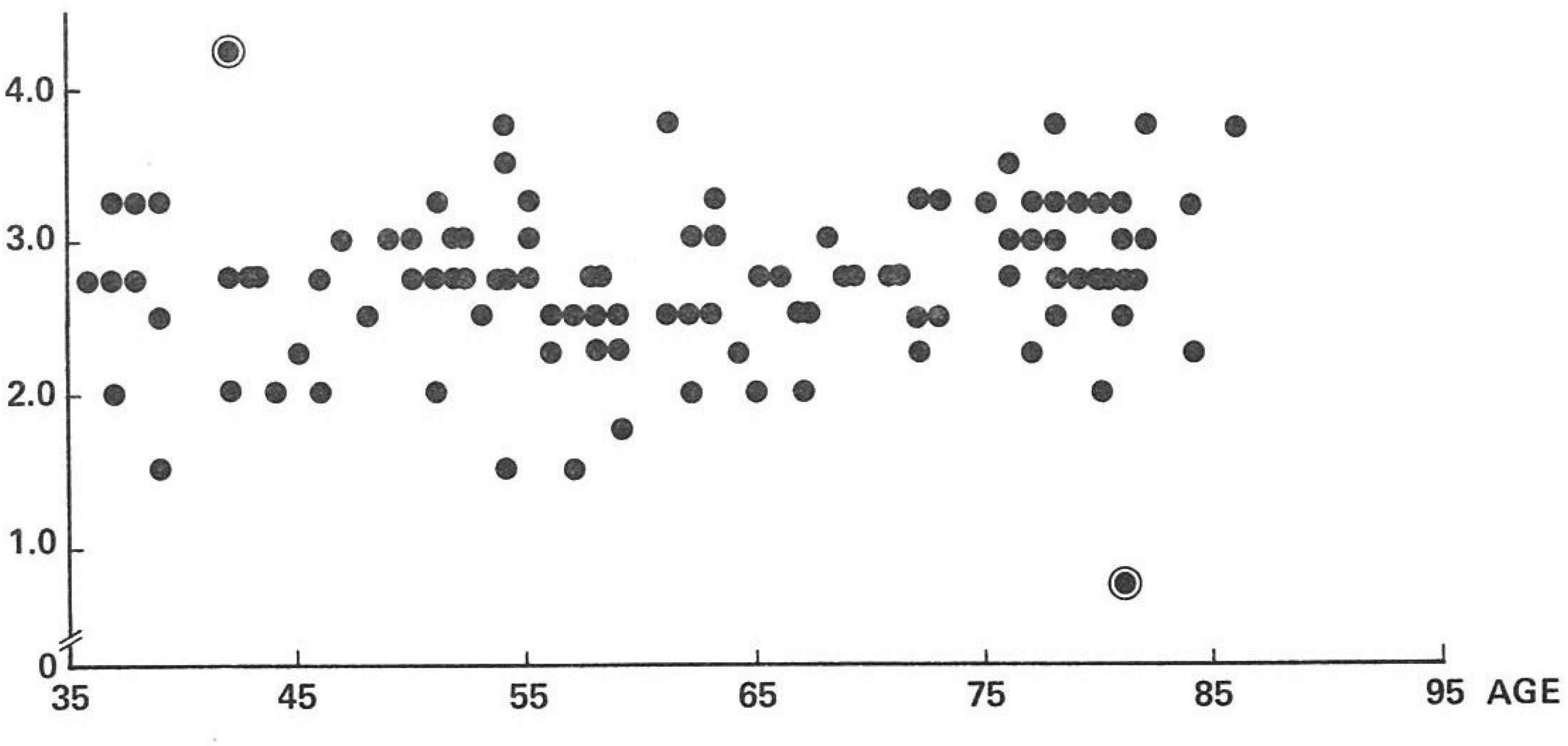
### HLA-DR 陽性細胞

下記の回帰分析では対数変換を行った HLA-DR 陽性細胞の割合を従属変数として用いた.

性による大きな影響があり，その割合が男性に高い(幾何平均値：男性22.2%，女性13.5%). その他の共変数の修正にかかわりなく，この影響は極めて有意であった(p< 0.0001；付表6，第2-4 欄). 性と年齢について補整を行った後の三つの線量群間の差は統計的に有意なものではなかった(p=0.8,付表6，第2，3欄). HLA-DR 陽性細胞の割合と年齢との関係に再び注目する必要がある. すなわち，図4で示すように，各年齢区分の端点の値は，一般的パターンから外れており，年齢の影響をゆがめている可能性がある. 性差を補正した後の年齢群間の相違性は，統計的には有意でなかった(p=0.06，付表

FIGURE 4 SCATTER PLOT OF THE PROPORTION OF HLA-DR POSITIVE CELLS (log-transformed) VS AGE

図4 HLA-DR 陽性細胞(log-transformed)の割合と年齢を示す散在図




Circled two points are 丸で囲む2点は

| MF | Sex | Dose | Age | Leu-1 | Leu-2a | Leu-3a | HLA-DR |
|---|---|---|---|---|---|---|---|
| [redacted] | F | 1 | 81 | 49.0 | 13.0 | 37.0 | 2.0 |
| | M | 0 | 42 | 53.0 | 26.0 | 54.0 | 79.0 |

significant ($p=0.06$, Appedix Table 6, 3rd and 4th columns). On the other hand, according to analyses excluding these two values, there was significant heterogeneity ($p=0.003$), with the mean for the 76+ age-group significantly higher than that of the other two groups combined by a factor of $e^{.3234}=1.4$ ($p=0.01$, Appendix Table 7, 4th column). The difference between the 56-75 and ≦55 age-groups was not statistically significant ($p=0.3$, Appendix Table 7, 2nd and 3rd columns). The regression models including linear and/or quadratic terms in age were also studied (Appendix Table 7, 6th, 7th, and 8th columns), and all showed an increasing trend with age. When both linear and quadratic were included, neither coefficient was individually significant, although both together were significant ($p=0.008$). As was the case for Leu-3a this may be due to over-parameterization. The model which allows higher values in the oldest age-group seems most appropriate for HLA-DR positive cells, as seen in Figure 1, and by comparing the residual sums of squares for the various models (Appendix Tables 6 and 7). In each case the residual sum of squares for a model based on age-groups is less than that of the corresponding model based on linear and/or quadratic trends in age. Regarding the effects of sex and exposure dose, exclusion of the two individuals indicated in Figure 4 does not affect the conclusions described above.

## DISCUSSION

Whether immune competence is disturbed in A-bomb survivors as a late effect of exposure to ionizing radiation has not yet been fully elucidated, although currently available evidence is all negative. The authors analyzed lymphocyte subsets as markers of immune competence of A-bomb survivors.

Rosette formation techniques, methods using heterogeneous antiserum, and other methods, have been employed in the past for the identification of lymphocyte subsets. However, with the development of the hybridoma production technique by Köhler and Milstein,[13] a large number of monoclonal antibodies have now been produced, and using these antibodies, studies on lymphocyte subsets can now be conducted in greater detail.

Clinically, the changes that occur in lymphocyte subsets due to various diseases are being clarified.

6，第3，4欄）．一方，これら二つの値を除いた解析では，有意な相違性が認められ（$p=0.003$），76歳以上の年齢群の平均値は他の2群を一緒にした平均値より $e^{.3234}=1.4$，有意に高かった（$p=0.01$，付表7，第4欄）．56-75歳と55歳以下の年齢群間の差は統計的に有意ではなかった（$p=0.3$，付表7，第2,3欄）．年齢に直線及び二次項を含めた回帰モデルをも調べたが（付表7，第6-8欄），それらはすべて年齢とともに上昇する傾向を示した．直線及び二次項を含める場合は，両者を一緒にしたときは有意であったが（$p=0.008$），単独では，いずれの係数も有意でなかった．Leu-3a の場合と同様，これも over-parameterization によるのかもしれない．図1でも分かるが，各モデルの残差二乗和（付表6，7）と比較すると高齢者群に高値が認められるモデルが，HLA-DR 陽性細胞に最適であると思われる．どの場合においても，年齢群に基づくモデルの残差二乗和は，対応する直線及び二次の年齢傾向をもつモデルの残差二乗和より小さくなってくる．性及び被曝線量の影響に関して，図4に示した2人の対象者を除外しても上記の結論には影響しない．

## 考　察

原爆被爆者に電離放射線被曝の後影響としての免疫能障害があるかどうかは，まだ完全には解明されていないが，現在得られている所見はすべて陰性である．著者らは，原爆被爆者の免疫能のマーカーとしてのリンパ球サブセットを解析した．

過去，リンパ球サブセットの同定にはロゼット形成法，異種抗血清を用いる方法，その他が用いられてきた．しかし，Köhler 及び Milstein[13] によるハイブリドーマ作製法の開発に伴って，現在多数のモノクローナル抗体が作製されており，今日ではこれらの抗体を用いてリンパ球サブセットに関する検索をより詳細に行うことが可能である．

各疾患によってリンパ球サブセットに起こる変化は，臨床的にはしだいに明らかにされている．例えば，

For example, a decrease in the number of cytotoxic/suppressor T cells is observed in autoimmune diseases, such as systemic lupus erythematosus (SLE),[14] rheumatoid arthritis (RA),[15] and Sjogren syndrome,[16] and an increase of cytotoxic/suppressor T cell and a decrease of helper/inducer T cell numbers are reported in infectious viral diseases such as infectious mononucleosis,[17] cytomegalovirus infection,[18] and hepatitis B (HB) viral hepatitis.[19] In this regard it is of interest that the proportions of total T cells were low in the two cases who were not under treatment for cancer at the time of examination and died of cancer within a year after examination, though these cases were excluded from the analysis of this study (Table 4).

全身性エリテマトーデス(SLE),[14] 慢性関節リウマチ(RA),[15] 及びシェーグレン症候群[16] などの自己免疫疾患では，cytotoxic/suppressor T 細胞数の減少が認められ，伝染性単核症,[17] サイトメガロウイルス感染,[18] 及びB型肝炎(HB)ウイルス肝炎[19] などの伝染性ウイルス性疾患では cytotoxic/suppressor T 細胞の増加及び helper/inducer T 細胞数の減少が報告されている．本調査の解析対象から除外された，検診時に癌治療を受けていなかったが，検診後1年以内に癌で死亡した者2例で，総T細胞の割合が低かったのは興味深いことである(表4)．

TABLE 4 CASES EXCLUDED FROM ANALYSIS

表4 解析から除外した症例

| Age | Sex | Leu-1 | Leu-2a | Leu-3a | HLA-DR | |
|---|---|---|---|---|---|---|
| 81 | Female | 31.2 | 4.3 | 2.9 | 13.8 | Died on 5 January 1983 due to stomach cancer with metastasis to the liver |
| 75 | Female | 25.9 | 10.2 | 30.8 | 28.0 | Died on 26 March 1982 due to caudal pancreatic cancer |
| 82 | Male | 51.4 | 11.0 | 28.0 | 7.3 | Multiple myeloma (IgA-λ) |
| 82 | Female | 52.1 | 4.5 | 39.0 | 32.5 | Chronic thyroiditis |

The results of the present study revealed no effect of radiation exposure on the proportions of peripheral blood lymphocyte subsets positive for Leu-1 (T cells), Leu-2a (cytotoxic/suppressor T cells), Leu-3a (helper/inducer T cells), or HLA-DR (B cells and monocytes) in A-bomb survivors. Concerning the lymphocyte subsets of A-bomb survivors, Akiyama et al[20] reported that the proportions of T and B cells derived by the rosette technique were not affected by exposure. With regards to the function of T cell subsets, activity of suppressor T cells is reported to be more sensitive to radiation than activity of helper T cells.[21] On the other hand, concerning the proportion of T cell subsets, Wasserman et al[22] reported that the proportion of T cells possessing IgG·Fc receptors derived by the rosette technique decreased after X-irradiation of 1,600 rad in vitro, and that no change was observed in the proportion of either Leu-2a or Leu-3a positive cells.

本研究の結果では，原爆被爆者の Leu-1 (T細胞), Leu-2a (cytotoxic/suppressor T 細胞), Leu-3a (helper/inducer T細胞)又は HLA-DR (B細胞及び単球)に陽性を示す末梢血リンパ球サブセットの割合には，放射線の影響は認められなかった．原爆被爆者のリンパ球サブセットに関して秋山ら[20] は，ロゼット法により得られたT細胞及びB細胞の割合には，被曝の影響はみられなかったと報告している．T細胞サブセットの機能については，suppresser T 細胞活性は helper T 細胞活性よりも放射線感受性が高いことを報告している．[21] 他方，T細胞サブセットの割合について Wasserman ら[22] は，試験管内で 1,600rad の放射線を照射後ロゼット法により得られた IgG·Fc 受容体保有T細胞の割合は減少したが，Leu-2a や Leu-3a 陽性細胞の割合には変化はみられなかったと報告した．

Concerning the change of lymphocyte subsets due to aging, many reports state that T cells

加齢によるリンパ球サブセットの変化については，

generally decrease with age,[23-26] but some reports state that they remain unchanged.[27,28] Nagel et al,[29] using monoclonal antibodies, reported that although the proportion of OKT4 (helper/inducer T cells) showed no change, OKT3 (T cells) and OKT8 (cytotoxic/suppressor T cells) decreased with age. Concerning the proportion of B cells, some investigators reported no difference with aging,[26,30] while others reported an increase.[24] Our results showed that there was no difference in the proportions of Leu-1 positive cells and Leu-2a positive cells with age, although the proportion of Leu-3a positive cells tended to decrease and that of HLA-DR positive cells tended to increase. The effect of age is more concentrated primarily in the oldest age-group (76+) for both measurements.

Matsumoto et al[31] have reported on the sex difference in the subsets of lymphocytes, stating that higher values of OKT4 positive cells are observed in females and of OKT8 cells in males and no sex difference are observed in OKT11 (total T cell) and OKIa (B cell). Our results showed that the proportion of HLA-DR positive cells (B cells and monocytes) is higher in males.

In this study, lymphocyte subsets were determined using a fluorescence microscope. To minimize errors in the determination, a single trained viewer counted all lymphocytes. Identification was made blindly on dose, age, sex, etc. Thus, the results obtained by the same tranined technician were almost identical to those obtained using an Fluorescence-activated Cell Sorter.

Next, the method of statistical analysis will be discussed. The method employed in this study was based on the assumption that the percentage of fluorescent lymphocytes obtained for each individual (or its transformed value) is distributed normally around the mean of a certain group. If the denominators of the proportion, i.e., the numbers of cells counted, are known, one possible method for analyzing such data is logistic regression analysis, based on the assumption that the numerators of the proportions, i.e., the numbers of positive cells, are binomially distributed. This method is, however, ordinarily based on the additional assumption that each individual in a group has the same true, underlying proportion of positive cells. When logistic regression analysis was applied to the present data under

T細胞はおおむね年齢とともに減少するとの報告が多いが,[23-26] 不変であるという報告も認められる.[27,28] Nagel ら[29] はモノクローナル抗体を用いて研究を行い，OKT4 (helper/inducer T細胞)の割合には変化はみられなかったが，OKT3 (T細胞)及びOKT8 (cytotoxic/suppressor T細胞)は年齢とともに減少したと報告した．B細胞の割合については，加齢に伴う差は認められないという報告,[26,30] 増加するという報告[24] がみられる．今回の結果では，Leu-1陽性細胞及びLeu-2a陽性細胞の割合には年齢による差はなかったが，Leu-3a陽性細胞の割合は年齢とともに減少し，HLA-DR陽性細胞の割合は増加する傾向があった．これら二つに対する加齢の影響は主として高齢群(76歳以上)に著明であった．

松元ら[31] は，リンパ球のサブセットの男女差に関して報告し，OKT4 陽性細胞の値は女性に高く，OKT8 の値は男性に高いが，OKT11(総T細胞)及びOKIa (B細胞)では男女差はみられないと述べている．我々の研究結果は，HLA-DR陽性細胞(B細胞及び単球)の割合は男性に高いことを示した．

本研究では，蛍光顕微鏡を用いてリンパ球サブセットの測定を行った．測定誤差を最小限にするため，1人の熟練した観察者がすべてのリンパ球数を算定した．線量，年齢，性などに関して盲目的に同定を行った．したがって，その熟練技術員による結果は，FACS (Fluorescence-activated Cell Sorter)を用いて得られたものとほとんど同じであった．

次に統計的解析法について述べる．本研究で用いた方法は，各対象者について入手した蛍光リンパ球の百分率(又はその変換値)が特定の群の平均値周辺に正規分布しているとの仮定に基づくものであった．割合の分母，すなわち算定した細胞数が分かっている場合は，そのようなデータの解析方法は，割合の分子，すなわち陽性細胞数が二項分布を呈するとの仮定に基づくロジスティック回帰分析が考えられる．ただし，この方法は通常，ある特定の群の各対象者はすべて反応細胞の真の割合として同じ陽性細胞率をもつことを仮定している．割合は200個の

the assumption that the proportions are based on observation of 200 cells, even the models giving consideration to effects of sex, age, exposure dose, and interaction of age and exposure (as in Table 3) gave very poor fits. This is demonstrated by the extremely large values of Pearson's $\chi^2$ statistic (1,756, 893.5, 1,228, and 989.4, for Leu-1, Leu-2a, Leu-3a, and HLA-DR, respectively, all with 94 degrees of freedom). Therefore, it is apparent that there is an extra-binomial variation in this data. While various methods have been proposed recently as direct approaches to eliminating this variation, the regression analyses for this report were based on the assumptions first mentioned in this study. These assumptions consider that the percentages or their appropriately transformed values are approximately normally distributed. This method was employed since direct correction for extra-binomial variation was not possible, because the exact denominator values used to calculate proportions were not recorded. However, based on the hypothetical assumption mentioned above that exactly 200 mononuclear cells were observed for each individual, fitting models with the correction for extra-binomial variation proposed by Williams[32] in 1982 gave results very similar to those of this study. Thus, it is expected that results of this study are similar to those that would have been obtained with appropriate correction for extra-binomial variation, and that conclusions of this report are valid.

Except for a finding of reduced phytohemagglutinin reactivity of peripheral blood lymphocytes with age in the exposed group,[20] studies of cellular immunity of A-bomb survivors conducted so far have shown no effect of exposure on the proportions of lymphocyte subsets, the differentiation ability of B lymphocytes, or the function of concanavalin A-induced suppressor T lymphocytes.[33] Based on the current data, there was no significant effect due to radiation exposure. A difference, if any exists, in immune competence between A-bomb survivors and the nonexposed may be too small to be detected by the immunological tests at the present time, some 40 years after the A-bombings.

However, the high incidence of breast, lung, and thyroid cancers in A-bomb survivors is reported even at present. It is clear that immuno-

細胞の観察に基づくものと仮定して，今回のデータにロジスティック回帰分析を適用した場合は，性，年齢，被曝線量，並びに年齢と被曝の相互作用(表3)の各影響を考慮したモデルによってさえも，適合はほとんど得られなかった．これは，Pearson $\chi^2$ 統計の値が極めて高いことによって立証される(Leu-1, Leu-2a, Leu-3a, 及び HLA-DR に対してはそれぞれ 1,756, 893.5, 1,228, 及び 989.4 であり，自由度はすべて94である)．したがって，このデータでは二項外の変動があるように思われる．最近この変動を除去する直接のアプローチとして色々な方法が提案されているが，本報の回帰分析は本研究で最初に述べた仮定に基づくものである．これらの仮定では，百分率又はそれを適当に変換した値がほぼ正規分布に従うことを考慮している．割合の算定に用いられた正確な分母値が記録されておらず，二項外の変動を直接に補正できなかったので，この方法を用いた．ただし，各対象者にちょうど200個の単核細胞が認められたという上記の仮の設定に基づき，1982年にWilliams[32]が提唱した二項外変動の補正によってモデルに適合させたところ，本研究のものに非常に近い結果が得られた．したがって本研究の結果は，二項外変動を適当に補正して得られているものと同様であり，本報の結論は妥当なものであると思われる．

これまでに行われた原爆被爆者の細胞免疫に関する研究では，被爆群では年齢とともに末梢血リンパ球の PHA 反応が減少するという所見[20]を除けば，リンパ球サブセットの割合，Bリンパ球の分化能，又は Con A 誘発性 suppressor Tリンパ球機能に及ぼす被曝の影響は認められていない．[33] 現在のデータによれば，放射線被曝による有意な影響はなかった．原爆被爆者と非被爆者の免疫能にもし差があるとしても，それは原爆投下後約40年を経た現在，免疫検査によって探知されるにはあまりにも小さいものであるかもしれない．

しかし，現在でも原爆被爆者においては乳癌，肺癌，甲状腺癌の発生率が高いことが報告されている．免疫

competence is important in carcinogenesis. The immunologic surveillance theory[34] of defense against cancer is now recognized as not applicable to all cancer types, but persons whose immune systems are suppressed by drugs have increased risk of some neoplasms.[35] Then, we cannot rule out the possibility that A-bomb exposure may still have some effects on immunocompetence. Therefore, if the crucial relationship of carcinogenesis and A-bomb survivors should be studied in the future, it is felt that more sensitive and specific immunologic tests should be conducted.

能が発癌において重要な役割を果たすことは明らかである．癌防御に関する免疫学的監視論[34]は，すべての癌型には適用できないことが現在では認められているが，免疫系が薬剤によって抑制されている者には幾つかの新生物のリスクは増大している.[35] したがって，原爆被爆が依然として免疫能に幾らかの影響を及ぼしている可能性は除くことができない．それゆえ，将来，発癌と原爆被爆者との極めて重大な関係について研究を行う場合は，より感度が高く，より特定の免疫学的検定を行う必要があると考えられる．

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## APPENDIX TABLES 1-7

## 付表 1〜7

In these appendix tables, the results of regression analyses of the proportions of antibody positive cells are shown. We have defined several indicator variables for use in these analyses as follows:

これらの付表では，抗体陽性細胞の割合に関する回帰分析の結果を示した．これらの解析に用いる幾つかの指標を次のように定義した：

| | | | | |
|---|---|---|---|---|
| Sex 性 | | = | 1 | female 女性 |
| | | | 0 | male 男性 |
| Age 年齢 | 56-75 | = | 1 | 55 < age 年齢 < 76 |
| | | | 0 | otherwise その他 |
| Age 年齢 | 76+ | = | 1 | 75 < age 年齢 |
| | | | 0 | otherwise その他 |
| Dose 線量 | 1-99 | = | 1 | 0 < dose 線量 < 100 (rad) |
| | | | 0 | otherwise その他 |
| Dose 線量 | 100+ | = | 1 | 99 < dose 線量 (rad) |
| | | | 0 | otherwise その他 |

In addition the covariate names Age and Dose were used to indicate continuous variables. Values shown in the Appendix Tables are least squares estimates of the coefficients of corresponding variables; the standard errors of these estimates are given in parentheses. The Sums of Squares shown at the bottom of each table are the residual sums of squares for the models expressed in the corresponding columns; df indicates the degrees of freedom for that Sum of Squares.

In the analyses involving indicator variables, the Grand Mean is the mean for the reference group, i.e., the group for which all the indicator variables included in the model are zero. For example, when only Sex is involved, the reference group consists of all males in the analysis; similarly the group of males aged less than 56 and not exposed to radiation is the reference group when a model involves Sex, Age 56-75, Age 76+, Dose 1-99, and Dose 100+.

When the continuous variables are used in the analysis, the Grand Mean is merely the value when those variables are set to zero. Thus in some instances, such as when Age is used in the analysis, the attempt to interpret the Grand Mean itself should be avoided since we have examined people aged only in the range of 36 to 86 in this study.

このほか，年齢及び線量という共変量を用いて連続変量を示した．付表に示した値は，変数に対応する係数の最小二乗推定値である．これらの推定値の標準誤差は括弧内に示した．各表の下部に示した二乗和は，対応する欄に示したモデルにおける残差二乗和である．df は二乗和の自由度を示す．

指標変数に関する解析では，総平均値は対照群，すなわち，モデルに含まれるすべての指標がゼロである集団の平均値である．例えば，性のみが含まれる場合は，対照群は解析では男性全員からなる；同様に，モデルが性，年齢56〜75，年齢76＋，線量1〜99，線量100＋を含む場合は，対照群は56歳未満の男性で放射線に被曝しなかった者となる．

解析に連続変量を用いる場合，総平均はそれらの変量がゼロと推定されたときの値にすぎない．したがって，解析に年齢を用いる場合においては，総平均値自体を解釈することは避けるべきである．なぜなら，本研究では36〜86歳の群を調べたにすぎないからである．

The fitted mean for a particular group of interest or at particular covariate values of interest is calculated as the sum of the corresponding parameter estimates or products of the covariate values and corresponding coefficients. For example, if we are interested in the mean percentage of Leu-1 positive cells for 50-year-old females exposed to 100 rad of A-bomb radiation, estimated from the model expressed in the last column of Appendix Table 1, the estimated mean value is

興味ある特定の群，又は興味ある特定の共変量値の適合される平均値は，対応するパラメーター推定値の和，又は共変量値とそれに対応する係数の積として算定される．例えば，100radの原爆放射線に被曝した50歳の女性におけるLeu-1陽性細胞の百分率平均値を付表1の最後の欄に示すモデルから推定すると，推定平均値は次のようになる：

$$51.82 = 59.34 + (-0.5042) + (-0.1323) \times 50 + (-0.00405) \times 100.$$

Based on the grouped data analysis (column 2 in Appendix Table 1), the estimated mean percentage for such persons is

群別データ解析（付表1の第2欄）に基づき，このような例の百分率の平均の推定値は次のようになる．

$$50.41 = 54.24 + (-0.8189) + (-3.015).$$

APPENDIX TABLE 1 REGRESSION COEFFICIENTS FROM ANALYSES OF THE PROPORTION OF Leu-1 POSITIVE CELLS

付表1 Leu-1陽性細胞の割合の解析による回帰係数

| | | | |
|---|---|---|---|
| Grand Mean | 50.42<br>(1.456) | 54.24<br>(4.229) | 59.34<br>(7.044) |
| Sex | | −0.8189<br>(3.378) | −0.5042<br>(3.354) |
| Age 56-75 | | 0.08377<br>(3.507) | |
| Age 76+ | | −4.899<br>(3.685) | |
| Dose 1-99 | | −2.294<br>(3.598) | |
| Dose 100+ | | −3.015<br>(3.975) | |
| Age | | | −0.1323<br>(0.1038) |
| Dose | | | −0.004056<br>(0.01219) |
| Sum of Squares | 22713 | 22054 | 22321 |
| df | 103 | 98 | 100 |

APPENDIX TABLE 2 REGRESSION COEFFICIENTS FROM ANALYSES OF THE PROPORTION OF Leu-2a POSITIVE CELLS (log-transformed)

付表2 Leu-2a 陽性細胞(log-transformed)の割合の解析による回帰係数

| | | | | | |
|---|---|---|---|---|---|
| Grand Mean | 2.628<br>(0.05212) | 2.780<br>(0.1500) | 2.925<br>(0.2332) | 0.7270<br>(1.017) | 3.024<br>(0.2505) |
| Sex | | −0.1015<br>(0.1198) | | | −0.09184<br>(0.1193) |
| Age 56-75 | | 0.02913<br>(0.1244) | | | |
| Age 76+ | | −0.2151<br>(0.1307) | | | |
| Dose 1-99 | | −0.02094<br>(0.1277) | | | |
| Dose 100+ | | −0.06048<br>(0.1410) | | | |
| Age | | | −0.004795<br>(0.003674) | 0.07108<br>(0.03440) | −0.004728<br>(0.003693) |
| $(Age)^2$ | | | | −0.0006198<br>(0.0002795) | |
| Dose | | | | | −0.0003933<br>(0.0004336) |
| Sum of Squares | 29.10 | 27.76 | 28.62 | 27.29 | 28.24 |
| df | 103 | 98 | 102 | 101 | 100 |

APPENDIX TABLE 3 REGRESSION COEFFICIENTS FROM ANALYSES OF THE PROPORTION OF Leu-2a POSITIVE CELLS (log-transformed), EXCLUDING FOUR INDIVIDUALS WITH LOW VALUES

付表3 Leu-2a 陽性細胞(log-transformed)の割合(低値の4例を除く)の解析による回帰係数

| | | | | | |
|---|---|---|---|---|---|
| Grand Mean | 2.686<br>(0.04552) | 2.857<br>(0.1328) | 3.024<br>(0.2084) | 2.463<br>(0.9394) | 3.093<br>(0.2304) |
| Sex | | −0.08547<br>(0.1050) | | | −0.08007<br>(0.1041) |
| Age 56-75 | | −0.05708<br>(0.1084) | | | |
| Age 76+ | | −0.1989<br>(0.1162) | | | |
| Dose 1-99 | | −0.05931<br>(0.1116) | | | |
| Dose 100+ | | −0.02357<br>(0.1249) | | | |
| Age | | | −0.005464<br>(0.003284) | 0.01380<br>(0.03161) | −0.005485<br>(0.003328) |
| $(Age)^2$ | | | | −0.0001570<br>(0.0002562) | |
| Dose | | | | | −0.00009352<br>(0.0003975) |
| Sum of Squares | 20.52 | 19.70 | 19.95 | 19.88 | 19.83 |
| df | 99 | 94 | 98 | 97 | 96 |

APPENDIX TABLE 4 REGRESSION COEFFICIENTS FROM ANALYSES OF THE PROPORTION OF Leu-3a POSITIVE CELLS

付表 4 Leu-3a 陽性細胞の割合の解析による回帰係数

| | | | | | | |
|---|---|---|---|---|---|---|
| Grand Mean | 42.87<br>(1.214) | 45.83<br>(3.504) | 44.33<br>(1.973) | 44.17<br>(1.416) | 50.81<br>(5.418) | 50.59<br>(5.846) |
| Sex | | 1.022<br>(2.798) | | | | 1.240<br>(2.783) |
| Age 56-75 | | −0.6097<br>(2.906) | −0.3229<br>(2.848) | | | |
| Age 76+ | | −4.967<br>(3.053) | −4.826<br>(3.022) | −4.671<br>(2.682) | | |
| Dose 1-99 | | −2.679<br>(2.981) | | | | |
| Dose 100+ | | −3.274<br>(3.294) | | | | |
| Age | | | | | −0.1283<br>(0.08537) | −0.1320<br>(0.08617) |
| Dose | | | | | | −0.005425<br>(0.01012) |
| Sum of Squares | 15795 | 15138 | 15337 | 15339 | 15453 | 15373 |
| df | 103 | 98 | 101 | 100 | 102 | 100 |

APPENDIX TABLE 5 REGRESSION COEFFICIENTS FROM ANALYSES OF THE PROPORTION OF Leu-3a POSITIVE CELLS, EXCLUDING TWO INDIVIDUALS WITH LOW VALUES

付表 5 Leu-3a 陽性細胞の割合（低値の 2 例を除く）の解析による回帰係数

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Grand Mean | 43.58<br>(1.127) | 45.13<br>(3.182) | 45.19<br>(1.816) | 45.20<br>(1.304) | 53.67<br>(4.981) | 12.15<br>(21.75) | 49.42<br>(2.792) | 53.20<br>(5.324) |
| Sex | | 2.046<br>(2.544) | | | | | | 2.289<br>(2.526) |
| Age 56-75 | | −0.1791<br>(2.669) | 0.01075<br>(2.623) | | | | | |
| Age 76+ | | −5.781<br>(2.780) | −5.692<br>(2.761) | −5.697<br>(2.445) | | | | |
| Dose 1-99 | | −0.940<br>(2.724) | | | | | | |
| Dose 100+ | | −3.285<br>(2.983) | | | | | | |
| Age | | | | | −0.1625<br>(0.07823) | 1.271<br>(0.7355) | | −0.1699<br>(0.07853) |
| $(Age)^2$ | | | | | | −0.01171<br>(0.005974) | −0.001442<br>(0.0006327) | |
| Dose | | | | | | | | −0.008520<br>(0.009170) |
| Sum of Squares | 13084 | 12165 | 12410 | 12410 | 12542 | 12074 | 12438 | 12318 |
| df | 101 | 96 | 99 | 100 | 100 | 99 | 100 | 98 |

APPENDIX TABLE 6 REGRESSION COEFFICIENTS FROM ANALYSES OF THE PROPORTION OF HLA-DR POSITIVE CELLS (log-transformed)

付表 6 HLA-DR 陽性細胞(log-transformed)の割合の解析による回帰係数

| | | | | | |
|---|---|---|---|---|---|
| Grand Mean | 2.731<br>(0.05372) | 3.046<br>(0.1413) | 3.080<br>(0.1111) | 3.092<br>(0.09742) | 2.723<br>(0.2372) |
| Sex | | −0.4697<br>(0.1128) | −0.4688<br>(0.1119) | −0.4886<br>(0.1132) | −0.4920<br>(0.1129) |
| Age 56-75 | | −0.1368<br>(0.1172) | −0.1363<br>(0.1156) | | |
| Age 76+ | | 0.1627<br>(0.1231) | 0.1625<br>(0.1219) | | |
| Dose 1-99 | | 0.02661<br>(0.1202) | | | |
| Dose 100+ | | 0.07946<br>(0.1328) | | | |
| Age | | | | | 0.005455<br>(0.003497) |
| Dose | | | | | 0.0003885<br>(0.0004105) |
| Sum of Squares | 30.91 | 24.62 | 24.71 | 26.14 | 25.31 |
| df | 103 | 98 | 100 | 102 | 100 |

APPENDIX TABLE 7 REGRESSION COEFFICIENTS FROM ANALYSES OF THE PROPORTION OF HLA-DR POSITIVE CELLS (log-transformed), EXCLUDING TWO INDIVIDUALS WITH EXTREME VALUES

付表 7 HLA-DR 陽性細胞(log-transformed)の割合(極値の2例を除く)の解析による回帰係数

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Grand Mean | 2.735<br>(0.04834) | 2.880<br>(0.1278) | 2.990<br>(0.09944) | 2.994<br>(0.09008) | 3.043<br>(0.08933) | 2.518<br>(0.2095) | 3.844<br>(0.8675) | 2.751<br>(0.1324) | 2.471<br>(0.2138) |
| Sex | | −0.3848<br>(0.09917) | −0.3899<br>(0.09898) | −0.3999<br>(0.09864) | −0.4143<br>(0.1035) | −0.4223<br>(0.1003) | −0.4008<br>(0.1005) | −0.4185<br>(0.09982) | −0.4145<br>(0.1005) |
| Age 56-75 | | −0.09763<br>(0.1020) | −0.1099<br>(0.1012) | | | | | | |
| Age 76+ | | 0.2805<br>(0.1084) | 0.2704<br>(0.1079) | 0.3234<br>(0.09633) | | | | | |
| Dose 1-99 | | 0.1340<br>(0.1060) | | | | | | | |
| Dose 100+ | | 0.1336<br>(0.1161) | | | | | | | |
| Age | | | | | | 0.008587<br>(0.003119) | −0.03768<br>(0.02955) | | 0.008683<br>(0.003118) |
| $(Age)^2$ | | | | | | | 0.0003778<br>(0.0002400) | 0.00007350<br>(0.00002522) | |
| Dose | | | | | | | | | 0.0003881<br>(0.0003618) |
| Sum of Squares | 24.07 | 18.051 | 18.40 | 18.62 | 20.75 | 19.27 | 18.79 | 19.11 | 19.05 |
| df | 101 | 96 | 98 | 99 | 100 | 99 | 98 | 99 | 98 |