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Source: Radiation Research, 163(3) : 258-265

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RR3311

RADIATION RESEARCH **163**, 258–265 (2005)
0033-7587/05 $15.00

A Hypothesis: Radiation-Related Leukemia is Mainly Attributable to the Small Number of People who Carry Pre-existing Clonally Expanded Preleukemic Cells

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Nakamura, N. A Hypothesis: Radiation-Related Leukemia is Mainly Attributable to the Small Number of People who Carry Pre-existing Clonally Expanded Preleukemic Cells. *Radiat. Res.* 163, 258–265 (2005).

Human leukemia frequently involves recurrent translocations. Since radiation is a well-known inducer of both leukemia and chromosomal translocations, it has long been suspected that radiation might cause leukemia by inducing specific translocations. However, recent studies clearly indicate that spontaneous translocations specific to acute lymphocytic leukemia (ALL) actually occur much more frequently than do leukemia cases with the same translocations. Moreover, the ALL-associated translocation-bearing cells are often found to have clonally expanded in individuals who do not develop ALL. Since radiation-induced DNA damage is generated essentially randomly in the genome, it does not seem likely that radiation could ever be responsible for the induction of identical translocations of relevance to ALL in multiple cells of an individual and hence be the primary cause of radiation-related leukemia. An alternative hypothesis described here is that the radiation-related ALL risk for a population is almost entirely attributable to a small number of predisposed individuals in whom relatively large numbers of translocation-carrying pre-ALL cells have accumulated. This preleukemic clone hypothesis explains various known characteristics of radiation-related ALL and implies that people who do not have substantial numbers of preleukemic cells (i.e. the great majority) are likely at low risk of developing leukemia. The hypothesis can also be applied to chronic myelogenous leukemia and to young-at-exposure cases of acute myelogenous leukemia.

INTRODUCTION

Radiation is one of the best-known etiological agents of leukemia (*1*). The most impressive example is fetal exposure in which diagnostic low-dose X-ray exposure [of the order of 10 mSv, ref. (*2*)] increased the background rate of childhood leukemia by 50% (*3*). The underlying mechanisms have not been clarified, however.

Three lines of evidence have been interpreted as indicating that radiation might increase the risk of leukemia in exposed people by inducing leukemia-specific translocations: (1) The dose response for the excess risk of leukemia as a whole is curvilinear (*1*), (2) human leukemia is frequently found in association with recurrent translocations (*4*), and (3) radiation is a known inducer of dicentrics or translocations, for which the dose response is curvilinear (*5*, *6*). There are also studies that show that radiation exposure is indeed capable of inducing *BCR/ABL* gene fusions that are specific to chronic myelogenous leukemia (CML) (*7*) and *RET/PTC1* gene fusions that are specific to thyroid cancer (*8*) in cultured cells *in vitro*, although the radiation doses used were quite large (i.e. 50 to 100 Gy).

This naive interpretation recently became open to challenge for two main reasons. The first is that most translocations associated with acute lymphocytic leukemia (ALL) do not convert the target cells that carry them into leukemia cells, while the second, and more important, is that translocation-bearing cells often seem to undergo clonal expansion in healthy individuals, most of whom will never acquire the disease [see ref. (*9*) for review]. Since it is well known that radiation exposure induces essentially random damage to DNA in the genome (*10*), there would appear to be no way in which a single exposure to radiation could produce multiple cells carrying precisely the same leukemia-specific translocation in any exposed individual.

The above considerations lead us to think that individuals carrying multiple cells with identical leukemia-specific translocations must be present in the population prior to radiation exposure, and that it is only these individuals (one can think of them as preleukemic cell carriers) who are at real risk of developing leukemia after radiation exposure. This would imply that radiation exposure is of critical importance in the causation of ALL *because of its ability to induce an additional mutation in a pre-existing preleukemic cell.* This preleukemic clone hypothesis explains a number of the known features of radiation-related leukemias that occur in radiation-exposed people.

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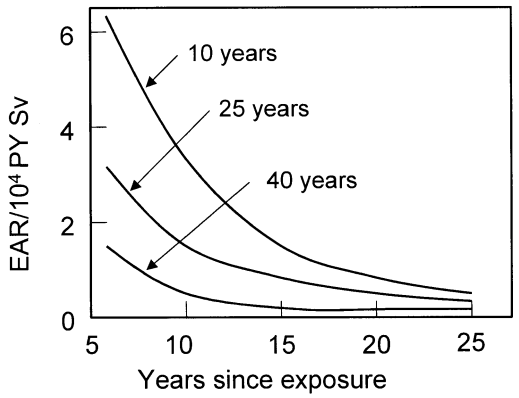

FIG. 1. Fitted temporal patterns of ALL risk in male survivors in relation to their age at the time of exposure [reproduced from ref. (*1*) with permission].

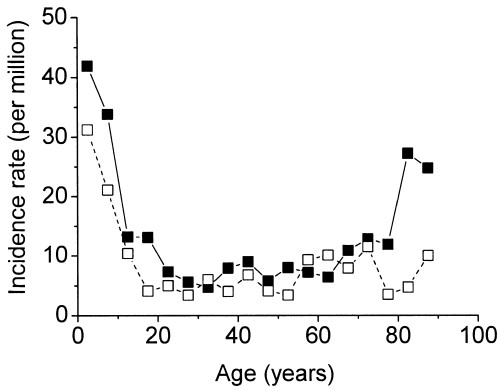

FIG. 2. Background incidence rate of ALL in Osaka, Japan (1993–1997) (*13*). Closed squares indicate males and open squares females.

RADIATION-RELATED ALL

Characteristics of ALL Risk in A-Bomb Survivors

Although risk estimation was not possible in the first 5 years after the bombings (i.e. 1945–1949), some information on leukemia was being collected, and there are indications that an excess risk of leukemia (all types together) had already begun to appear during the period from 1948 to 1950 (*11*). Only a limited number of leukemia cases appear to have been examined cytogenetically [see for example ref. (*12*)].

The estimated excess absolute risk (EAR) of ALL in A-bomb survivors has several characteristics (Fig. 1); namely, (1) the risk reached a peak within 5–10 years of radiation exposure and then declined continuously regardless of age at the time of exposure, (2) the magnitude of the risk appeared to be inversely related to age at the time of exposure, and (3) the risk in females was about half that in males (*1*).

Characteristics of Background ALL

ALL mainly affects children and (to a lesser extent) the elderly, so that the age-specific rate shows a broad U-shaped pattern [Fig. 2, ref. (*13*)]. The underlying mechanisms involved in the development of ALL appear to differ in children and adults. For example, while t(4;11) *AF4/MLL* is clearly associated with ALL in children who are less than a year old [50% to 80%, ref. (*14*)], t(9;22) *BCR/ABL* is rare in pediatric ALL but common in adult ALL patients who are $>$60 years old, where it is present in $>$20% of cases (*15*). Cases showing hyperdiploidy ($>$50 chromosomes) are common among young ALL patients but quite rare in older ones (*15*). The major ALL-specific translocations are t(12;21) (16–29% of total ALL), t(1;19) (6%), t(9;22) (3%–4%), and t(4;11) (2–3%), with hyperdiploidy (20–25%) and hypodiploidy (5%) also being observed (*4*). The karyotypes of some 30% of ALL cases appear to be normal (*15*).

Mori *et al.* recently screened cord blood samples for the presence of *TEL/AML1* fusion gene transcripts derived from t(12;21) (the most prevalent translocation in pediatric ALL) by RT-PCR and found six positive cases among nearly 600 samples (*16*). The frequency in cord blood samples is therefore ~1%; this is some two orders of magnitude higher than the frequency of pediatric ALL patients with this translocation, where estimates of the background cumulative risk for 0–15 years of age range from 1 in 10,000 (*16*) to 1 in 12,000 (*9*). Clearly possession of t(12;21) is not in itself enough to cause ALL. More importantly, Mori *et al.* also examined a few of the samples which contained *TEL/AML1* fusion gene transcripts by fluorescence *in situ* hybridization (FISH) and found that the translocation-bearing cells were present in about 1 per 1,000 B or pre-B cells (i.e. the target cells at risk of ALL) (*16*); this would appear to provide clear evidence of clonal expansion. Such findings would appear to indicate that the translocation-bearing cells are preleukemic but mostly nondetrimental; it may be that they remain quiescent, or even that the population of affected stem cells shrinks (see below) after birth unless and until additional mutational events initiate further expansion toward malignancy.

The fact that one cell in a thousand bears the ALL-specific translocation suggests that a large clonal expansion has occurred. However, if the cells do have a growth advantage, it is difficult to explain why the background rate of ALL declines with increasing age (Fig. 2). An alternative interpretation could be that the relevant translocations do not necessarily provide a strong growth advantage (positive selection) to the cells but rather that they occur at an early stage of fetal life when the number of hematopoietic stem cells (or their precursor cells) is still small. This is consistent with the fact that no overt signs of leukemia are observed in mice transgenic for the *TEL/AML1* fusion gene with an early B-cell-specific enhancer/promoter (*17*). This interpretation is attractive, because their being neutral would better explain the decreasing background rate of ALL, given that, for example, the absolute number of translocation-bearing stem cells could be expected to decrease when they commit to differentiation in the normal course

of development of the immune system, i.e. with increasing age.

Proposed Hypothesis for Radiation-Related ALL

As mentioned above, 1% of newborns may contain approximately one cell with a leukemia-relevant translocation per 1,000 target cells at risk (*16, 18*). It is therefore possible that any given population will include a small subgroup of individuals who have spontaneously acquired a clonal population of preleukemic cells that have nothing to do with their radiation exposure histories; the numbers of such individuals would be in proportion to the frequency of ALL cases in the total population, but there would be ~100 times as many of them; these individuals would be preleukemic cell carriers who are at real risk of developing ALL. The role of radiation would then be to induce *additional* mutations in target preleukemic cells, such that each additional mutation would push the affected individual one step further along the pathway to malignancy. One important implication of this hypothesis is that the great majority of people would not be in possession of clonally expanded populations of preleukemic cells and hence should be at little or no risk.

If we assume that this preleukemic clone hypothesis is correct, and (as has been reported) that the background rate of ALL declines relatively rapidly with age from birth until puberty (*13*), it would not seem unreasonable to assume that there will be a comparably rapid decline in the age-specific EAR for radiation with increasing age at the time of exposure. This is precisely what has been found in previous studies of the risk of ALL among A-bomb survivors (see Fig. 1). Similar reasoning could help to explain the sex difference in EAR [which is nearly twice as high in male survivors as female survivors; see ref. (*1*)], given that the background rates of ALL in males who are less than 20 years old are about 60% higher than we see in their female counterparts (Fig. 2). The relatively short latent period before leukemia appears is perhaps best explained on the basis that only a few events are likely to be involved in the conversion of pre-ALL cells to malignancy. Another issue concerns the rapidly declining trend in risk after the peak that occurs at 5–10 years postexposure (Fig. 1). This could be because those individuals who had acquired an additional, critical leukemia-relevant mutation in their pre-ALL clone developed leukemia within a limited lag time after exposure, and therefore, the longer the time elapsed, the less likely it is that they have acquired such mutation and consequently would develop leukemia.

One additional factor that could be important involves forced stem cell multiplication, which takes place mainly during a relatively short period of recovery from radiation-induced cell death; that is, the acquisition of leukemia-relevant genetic changes itself may not be enough to efficiently convert preleukemic cells into leukemic cells. Supporting evidence for my thinking stems from the development, albeit rare, of donor-derived secondary ALL in primary leukemia patients who receive bone marrow transplants from sex-mismatched siblings in the course of their treatment. Interestingly, these secondary ALL cases mainly occur within 3 years of receipt of the relevant transplant (*19*); this is a much shorter period than is usual for the emergence of ALL in A-bomb survivors, but its relative brevity can almost certainly be understood by taking into account the nearly complete depletion of hematopoietic cell populations in transplant recipients, whereas the extent of depletion in the A-bomb survivors must have been much less. A key point that should be borne in mind is that none of the donors developed leukemia. Thus any preleukemic cells that were present in the donors must have remained inert, and presumably, therefore, it was only when these cells were exposed to the unusual environment with plenty of growth factors in the recipient that they were converted into leukemic cells. Because the recovery processes from radiation injury facilitates clonal expansion of surviving cells, some of which may be preleukemic, such a scenario might well contribute, at least in part, to tumor promotion. This would be especially so if, as is suspected, radiation exposure causes genomic instability (*12, 20*). Our recent studies on clonal chromosome aberrations in T lymphocytes of A-bomb survivors indicate that expansions of cytogenetically marked clones took place mainly in the first few years after exposure and then simply stopped occurring (*21, 22*).

The present hypothesis also predicts that even though the *estimated* EAR of developing ALL in the 40-year-old group appears to be lower than it is in the 25-year-old group (Fig. 1), the *actual* risks may not differ very much given the similarities in their respective background rates (Fig. 2).

While the hypothesis assumes that radiation-related risk of leukemia is not caused by direct induction of leukemia-specific translocations but by additional mutations to preleukemic cells, a fraction of patients with secondary leukemia (both ALL and acute myelogenous leukemia; AML) after treatment of their primary malignancies with chemotherapy, especially with topoisomerase II (Topo II) inhibitors, are known to have specific translocations involving the *MLL* gene at 11q23 (*4*). Therefore, one might interpret the observations as indicating that acute exposure to some exogenous agents could directly induce specific translocations in human hematopoietic stem cells. This is probably not the case in a strict sense, however, because it is generally believed that Topo II inhibitors induce DNA breaks by interfering with the ligation through stabilizing the topoisomerase-DNA complexes, which is part of cellular responses [see ref. (*23*) for review].

Possible ALL Risk to Predisposed Individuals

Comparisons of the population risk of developing ALL with individual risks are summarized in Table 1. Here, in estimating the number of cumulative cases of ALL in the background, only those cases were considered that occurred

TABLE 1
Comparisons of the Number of Cumulative ALL Cases by Age 40 in Population as a Whole and Predisposed Individuals

	Frequency in a population	No. of cumulative background cases per 10^4 persons by age 40[a]	No. of cumulative excess cases during 25 years after radiation exposure per 10^4 males exposed to 1 Sv[b]	Relative risk
Population as a whole		5 (0.05%)	60 (0.6%) if exposed at age <20 30 (0.3%) if exposed at age 20–39	1
Individuals				
Noncarriers	>96%	~0	~0	
Carriers	<4%	125 (1.25%)	1,500 (15%) if exposed at age <20 750 (8%) if exposed at age 20–39	25

[a] The cumulative number of ALL in a population by age 40 was calculated from Fig. 2 as roughly 5 per 10^4 (0.05%). The corresponding value in predisposed individuals was estimated as 25 times the value for the population, or 1.25%.

[b] The radiation-related total of excess cases of ALL in a population was estimated from Fig. 1 assuming that the mean EAR in the first 5 years after the exposure was half of the peak value at the fifth year, i.e. about 60 excess ALL cases per 10^4 persons in a population (males) if exposed to 1 Gy at ages <20 years. If exposed at 20–39 years of age, the value was roughly half of the value for those exposed at <20 years of age. The corresponding values for the predisposed individuals were estimated by multiplying the value for the population as a whole by 25.

prior to the age of 40 because increasing background rates after the age of 40 (Fig. 2) are likely to be unrelated to mechanisms that originated in the fetus. From Fig. 2, a general population should contain about 5 persons per 10^4 who will develop ALL by age 40 (1 in 2,000 or 0.05%). This value is likely to vary somewhat from population to population but is of roughly the same order of magnitude as the widely accepted estimate of 1 in 2,000 for any leukemia by age 15 (i.e. in the age group where ALL predominates) (*9*). In the case of individuals who have acquired clonally expanded populations of preleukemic cells during fetal life, the cumulative probability of developing ALL by age 40 would appear to have increased by ~25-fold (see below) to become about 125 per 10^4 preleukemic cell carriers (1 in 80, 1.25%).

The estimate of a 25-fold increase is based on the following reasoning: (1) Pediatric ALL of both t(12;21) translocation-positive and -negative patients shows the same peak onset age of 1–5 years (*24*); (2) t(12;21) translocations are found in about one-fourth of total pediatric ALL cases (*24*); and (3) about 1% of newborns carry apparently expanded clonal preleukemic cells harboring the t(12;21) translocation (*16*, *18*). Consequently, about 4% of newborns should carry clonally expanded preleukemic cells of one type or another if it is true that all pediatric cases of ALL are preceded by expansion of a preleukemic cell population (see below). This means that both the background rate and the EAR for the development of ALL after radiation exposure are almost exclusively attributable to the 4% of predisposed individuals in a population; the RR of these predisposed individuals should therefore be roughly 25 times (1/0.04) higher than conventional estimates of the risk to the population as a whole.

In making the above calculations, I assumed that not only translocation-type ALL cases but also ALL cases involving hyperdiploid or normal karyotypes are likely to be preceded by clonal expansions of preleukemic cells. This is because the results of a recent study of T-cell ALL (*25*) indicated that abnormal expression of transcription factor genes (i.e. oncogenes that are frequently involved in the T-cell ALL-specific translocations) is not uncommon in ALL cells whose karyotypes are normal. By analogy, it is possible that certain other genes involved in pre-B- or B-cell ALL-specific translocations will also show up in deregulated mode in ALL cells that have a normal karyotype, in which case there may have been clonal expansion of the relevant preleukemic cells prior to birth. In the case of hyperdiploid ALL, the same Ig gene rearrangements seen in leukemia cells were also detected in tests of newborn blood spots (*26*). This finding indicates only that the leukemic cells and the small number of fetal cells were in the same B-cell lineage and does not prove that the fetal cells were preleukemic. There is one other important observation that supports the view that the hyperdiploid state is an early event. Namely, it relates to an ALL case involving trisomy 14 in which three different Ig gene rearrangements that were observed in the tumor and were specific to each chromosome 14 were all clearly evident in tests on newborn blood spots (*27*); this clearly shows that an event involving the gain of one chromosome 14 must have preceded the occurrence of all three of the Ig gene rearrangements that were observed in the tumor cells.

Let us return now to the risk of developing radiation-associated ALL in preleukemic cell carriers (Table 1). If we assume that carriers were exposed to 1 Gy of radiation at <20 years of age, the probability of their developing ALL can be expected to increase from the background level (~125 per 10^4) to ~1,500 per 10^4 carriers (i.e. 1 in 7, 15%) (see the footnote to Table 1 for an explanation of how this number was derived); by contrast, the probability of non-

predisposed people (i.e. the great majority who are not carriers of pre-leukemic cells) developing ALL will be, for all intents and purposes, zero. In carriers who were exposed when they were between 20 and 40 years old, the total excess number should be roughly half of the excess number observed in carriers exposed when <20 years old (see Fig. 1).

It is interesting to note that predisposed individuals are likely to have a cumulative risk of developing ALL within 25 years of radiation exposure that is about 25-fold higher than the risk experienced by the population as a whole, and that this difference may be independent of age of exposure. This is because both the background rate of ALL (Fig. 2)—which suggests a function of the proportion of predisposed individuals in a population—and the mean radiation-related EAR of ALL in a population (Fig. 1) tend to decrease with increasing age, at least up to age 20. In other words, the higher radiosensitivity of younger people, which has previously been difficult to explain, may no longer be quite so mysterious given that younger population groups are almost certain to contain a higher proportion of predisposed individuals than older population groups.

ALL-SPECIFIC TRANSLOCATIONS OCCUR MAINLY IN EARLY FETAL LIFE

It seems certain, at least in translocation-type pediatric ALL, that the majority of cases start from the emergence during fetal life of ALL-specific translocations [see ref. (*9*) for review]. Here I explain, based on the results of early epidemiological studies, that most of the initial translocation events occurred during the first trimester.

In the best known of these studies, Stewart *et al*. conducted a large-scale retrospective study of pediatric cancers (Oxford Survey of Childhood Cancers, OSCC) and found that fetal exposure to low-level diagnostic X rays [where the estimated dose was of the order of 10 mGy; see ref. (*2*) for review] increased the risk of leukemia and solid cancers by about 50% above the background rate before the exposed individuals attained the age of 15 years (*3*). About 50% of the pediatric leukemias were ALL and ~20% were AML (*3*). The present hypothesis assumes that the excess risk of ALL is closely associated with the presence of preleukemic cells prior to radiation exposure. That is, if it were not present in fetuses, the excess risk should be very much lower. In this respect, trimester 2 and 3 fetuses appear to have equal sensitivity, with a relative risk (RR) for trimester 2 and trimester 3 fetuses of 1.29 and 1.30, respectively (*28*). The RR for first-trimester fetuses [RR=3.19; see ref. (*28*)] appears to be higher than the values for later trimesters, although this higher value may well be complicated by the larger mean number of X-ray films taken (*28*) and the higher mean doses per film (*2*). Nonetheless, there is little doubt that first-trimester fetuses are at least as sensitive as later-stage fetuses. It can be concluded, therefore, that the predisposing events for pediatric ALL and AML occur mainly during the first trimester. This may explain the apparent discrepancy between (1) the finding by Mori *et al.* that the pre-ALL condition is present in one out of 1,000 pre-B or B cells at birth, a finding that indicates a significant clonal expansion (*16*), and (2) the finding by Andreasson *et al.* that the possession of an ALL-specific fusion gene does not provide the affected cells with a growth advantage in transgenic mice or with growth factor independence in cultured cells (*17*).

As discussed below, in addition to explaining the risk of ALL, this hypothesis also explains in straightforward ways the radiation-associated risk of both CML and, to a lesser extent, AML.

APPLICATION OF THE HYPOTHESIS TO THE RISK OF CML

CML is a disease that mainly affects elderly people [the incidence increases with the square of age, ref. (*29*)], and t(9;22) or *BCR/ABL* gene fusions are detected in virtually all cases of CML (*4*). Further, *BRC/ABL* fusion transcripts are frequently present in the white blood cells of ordinary individuals (*30, 31*), and the translocation itself does not seem to cause the cells to become malignant [cited in ref. (*29*)]. Four major observations concerning CML risks in A-bomb survivors (*1*) are discussed in turn.

The temporal risk pattern for CML is similar to that for ALL. Given that *BCR/ABL* translocations are common and that the number of additional mutations which are required for the conversion of translocation-bearing preleukemic cells into tumor cells may well be quite small (*29*), the present hypothesis explains the rapid initial increase followed by the decay in the EAR of CML using exactly the same reasoning as was used for ALL.

An EAR was detected in Hiroshima survivors but not in Nagasaki survivors. There are, however, two major factors that may explain this difference; namely, (1) the Nagasaki cohort was about half the size of the Hiroshima cohort, and (2) the background rate of CML in the former population was less than about one-third the rate in Hiroshima. These factors combine to make the absolute number of predisposed individuals smaller in Nagasaki, so that the EAR probably could not have reached statistical significance. This reasoning agrees with that of the study authors (*1*). It should be noted that the RRs observed in the two cities do not appear to be significantly different (*1*).

The EAR observed in Hiroshima survivors was lower in females than in males. This observation is in accord with expectation, given that the background rate in females was about 50% lower than in males (*1*).

The EAR appears not to depend on age. Although the present hypothesis predicts that the EAR should be larger for exposures at older ages because the background rate of CML increases with the square of age (*29*), it may be that a discrepancy results from the problems that are typically

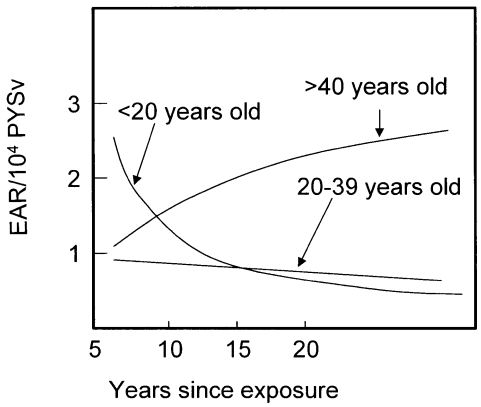

FIG. 3. Fitted temporal patterns of AML risk in A-bomb survivors [reproduced from ref. (*1*) with permission].

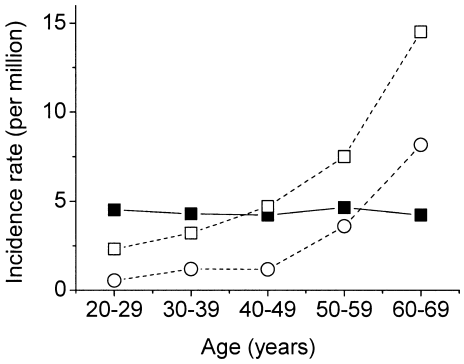

FIG. 4. Age-specific incidence rate for *de novo* AML. Open squares represent normal karyotypes, open circles chromosome gain or loss-type, and closed squares translocation-type AML [reproduced from ref. (*37*) with permission from Nature Publishing Group].

encountered in dealing with small numbers of CML cases in this cohort: 62 cases in all, 26 estimated as excess (*1*).

APPLICATIONS OF THE HYPOTHESIS TO THE RISKS OF AML

Recurrent translocations are also known in AML. They include t(8;21)*AML1/ETO* [predominantly found in young patients, ref. (*4*)]; t(15;17)*PML/RARα* [observed mainly in acute promyelocytic leukemia (APL) cases (*4*), and with a background rate that is independent of age (*32*)]; t(11q23) [fusions of the *MLL* gene to one or other of >30 different genes (*4*) that are present in about 50% of infant cases of AML (*33*), with the proportion declining rapidly from ~65% at <1 year old to ~5% after the age of 1 [cited in refs. (*34*) and (*35*)]; and inv(16) or t(16;16) [present in 10–12% of AML cases irrespective of age (*4*)].

Moreover, as observed for ALL-specific translocations, an AML-specific translocation seems insufficient to cause malignancy. Thus, for example, when Mori *et al.* screened cord blood samples for the presence of AML-specific transcripts from the t(8;21) *AML1/ETO* fusion gene, they found one positive case in about 500 samples (~0.2%) (*16*)—a frequency that is again two orders of magnitude higher than the frequency of pediatric AML patients with the same translocation, given that the cumulative risk for age 0–15 years is one in 80,000 (*9, 16*). Interestingly, Mori *et al.* (*16*) also found that about one per 1,000 panmyeloid cells in the one RT-PCR positive sample for AML examined was carrying the t(8;21) translocation.

On the other hand, the changes in EAR for AML with age at exposure to radiation are quite complex (Fig. 3). If people were exposed to radiation at <20 years of age, the EAR appeared to peak within 5–10 years of exposure, only to decline thereafter as in ALL and CML. By contrast, for exposures at 20–39 years of age, the risk stayed fairly constant after exposure. In people exposed at age 40 or older, EAR increased steadily with years since exposure, with a pattern like that in solid cancers (*36*).

The key information that is needed to explain the risk pattern includes the peculiar age dependence in background rate of AML subtypes according to chromosomal characteristics. Thus, although AML as a whole mainly affects elderly people, the background rate of translocation-type AML remains almost constant with age (*37, 38*), and the net effect is that translocation-type AML is predominant among young patients (Fig. 4).

The temporal pattern of EAR for young survivors exposed when <20 years old is therefore best explained by the presence of clonally expanded translocation-type pre-AML cells in individuals prior to their being exposed to radiation. By contrast, non-translocation-type AML probably predominates in middle-aged and older patients (Fig. 4). This may mean that the temporal pattern of EAR for survivors exposed at >40 years resembles that of solid cancers in developing through a multistep process in which radiation exposure is responsible for one step (*39*), in which case the EAR of AML would be expected to increase with years since exposure (*36*). People exposed at 20–40 years of age might well then be a mixture of translocation-type and non-translocation cases and hence could be expected to give rise to an EAR that remains more or less constant with time.

While there is evidence for an increased EAR for ALL, AML and CML in the A-bomb survivor population, it is only in AML that we see a curvilinear dose response that is statistically significant (*1*). Since we assume that radiation exposure adds one event to the series of events that lead to the malignant phenotype, one might well expect that the dose–response curve would be indicative of single-hit kinetics. The fact that it turns out to be curvilinear does not rule out the involvement of a single hit, however, given that mutation induction at a single gene does not always give rise to a linear dose response. Support for this thinking comes from a review of the data on the induction of mutations at one particular *HPRT* locus; such data when plotted may be indicative of either a linear dose response [in normal skin fibroblasts; ref. (*40*)] or a curvilinear dose response [in T lymphocytes; refs. (*41, 42*)]. Another possible

source of curvature in the dose response of the EAR involves forced stem cell division at high doses after radiation-induced tissue injury. Such conditions are likely to facilitate positive selection of large clones (*21*) which may include pre-AML cells and in this way can be expected to assist in the development of AML.

POSSIBLE APPROACHES TO TEST THE HYPOTHESIS

If clonal expansion of cells with ALL-specific translocation was the only predisposing condition and the other two types, hyperdiploid and apparently normal karyotype, were not predisposing, then the present hypothesis predicts that the translocation-type ALL would predominate among the excess cases of early-onset ALL, especially among survivors exposed when <20 years old. On the other hand, if all three types were preceded by the expansion of preleukemic cells, it is unlikely that the spectra of ALL subtypes would differ between early- and late-onset cases. This is particularly true for CML, where essentially all cases are positive for the *BCR/ABL* gene fusion (*4*). In this regard, it might be only for AML that we can test the hypothesis; namely, early-onset cases among the survivors exposed at age 20 or younger would be expected as preferentially translocation-type AML. By contrast, since non-translocation-type AML seems to be dependent upon the accumulation of multistep changes, they would not be expected to give rise to excess cases with a short latent period after exposure at ages <20 years.

In practice, frozen specimens of these early cases do not exist, so leukemia cells in paraffin-embedded autopsy tissue samples are probably the only choice available for analysis. Since recent studies indicate that relevant translocations or fusion gene transcripts can be detected on paraffin-embedded tissues with RT-PCR (*43*) or FISH (*44*), respectively, the hypothesis might be tested in these samples.

A complementary approach would be to create mouse models in which human ALL- or AML-specific fused genes are expressed. Transgenic mice expressing a *TEL/AML1* fusion cDNA exist, and the animals did not develop ALL spontaneously (*17*). Also, transgenic mice expressing an *AML1/ETO* fusion cDNA did not develop spontaneous AML but did so at high frequency after treatment with ethylnitrosourea (*45*). It seems likely that the animals in question are also predisposed to radiation-induced leukemia, but to my knowledge such studies have not been reported.

CONCLUSION

The hypothesis that a small fraction of the human population consists of people who are predisposed to radiation-related leukemia neatly explains virtually all of the characteristics of the EAR of leukemia seen in the A-bomb survivor cohort. If this hypothesis is correct, it may become important to think about, in addition to the current risk estimation to a population, individual risk estimation in which the key factor will be whether any given individual is a carrier of clonally expanded preleukemic cells. Detecting individuals at risk will not be easy, because adult carriers of leukemia-specific translocations of kinds of relevant to ALL (*18*) or CML (*30, 31*) are relatively common in blood cell examinations. The present hypothesis also suggests that while the risk to the small proportion of people who carry preleukemic cells is high, the risk to most non-predisposed people of developing radiation-induced ALL or CML (or AML if young) would be very low.

ACKNOWLEDGMENTS

Special thanks are given to Dr. D. G. MacPhee for his advice on preparation of the manuscript and to Dr. D. L. Preston for his encouragement to the study and his kind help in interpreting the leukemia data for A-bomb survivors. The author also thanks Drs. C. A. Waldren, D. A. Pierce, K. Koyama, Y. Kusunoki and S. Izumi for their suggestions and Drs. Y. Kodama and A. Noda for discussions. The Radiation Effects Research Foundation (RERF), Hiroshima and Nagasaki, Japan, is a private, nonprofit foundation funded by the Japanese Ministry of Health, Labour and Welfare (MHLW) and the U.S. Department of Energy (DOE), the latter through the National Academy of Sciences. This publication was supported by RERF Research Protocol RP8-93.

Received: August 30, 2004; accepted: October 2, 2004

REFERENCES

1. D. L. Preston, S. Kusumi, M. Tomonaga, S. Izimi, E. Ron, A. Kuramoto, N. Kamada, H. Dohy and K. Mabuchi, Cancer incidence in atomic bomb survivors. Part III: Leukemia, lymphoma and multiple myeloma, 1950–1987. *Radiat. Res.* **137** (Suppl.), S68–S97 (1994).
2. R. Doll and R. Wakeford, Risk of childhood cancer from fetal irradiation. *Br. J. Radiol.* **70**, 130–139 (1997).
3. J. F. Bithell and A. M. Stewart, Pre-natal irradiation and childhood malignancy: A review of British data from the Oxford Survey. *Br. J. Cancer* **31**, 271–287 (1975).
4. E. S. Jaffe, N. L. Harris, H. Stein and J. W. Vardiman, *Pathology and Genetics of Tumors of Haematopoitic and Lymphoid Tissues.* IARC Press, Lyon, 2001.
5. Y. Kodama, D. Pawel, N. Nakamura, D. Preston, T. Honda, M. Itoh, M. Nakano, K. Ohtaki, S. Funamoto and A. A. Awa, Stable chromosome aberrations in atomic bomb survivors: Results from 25 years of investigation. *Radiat. Res.* **156**, 337–346 (2001).
6. D. C. Lloyd, R. J. Purrott, G. W. Dolphin, D. Bolton, A. A. Edwards and M. J. Corp, The relationship between chromosome aberrations and low LET radiation dose to human lymphocytes. *Int. J. Radiat. Biol.* **28**, 75–90 (1975).
7. T. Ito, T. Seyama, T. Mizuno, T. Hayashi, K. S. Iwamoto, K. Dohi, N. Nakamura and M. Akiyama, Induction of BCR-ABL fusion genes by *in vitro* X-irradiation. *Jpn. J. Cancer Res.* **84**, 105–109 (1993).
8. T. Mizuno, K. S. Iwamoto, S. Kyoizumi, H. Nagamura, T. Shinohara, K. Koyama, T. Seyama and K. Hamatani, Preferential induction of RET/PTC1 rearrangement by X-ray irradiation. *Oncogene* **19**, 438–443 (2000).
9. M. F. Greaves and J. Wiemels, Origins of chromosome translocations in childhood leukaemia. *Nat. Rev. Cancer* **3**, 639–649 (2003).
10. M. N. Cornforth, K. M. Greulich-Bode, B. D. Loucas, J. Arsuaga, M. Vazquez, R. K. Sachs, M. Bruckner, M. Molls, P. Hahnfeldt and D. J. Brenner, Chromosomes are predominantly located randomly with respect to each other in interphase human cells. *J. Cell Biol.* **159**, 237–244 (2002).
11. J. H. Folley, W. Borges and T. Yamawaki, Incidence of leukemia in

survivors of the atomic bomb in Hiroshima and Nagasaki, Japan. *Am. J. Med.* **13**, 311–321 (1952).

12. M. Nakanishi, K. Tanaka, T. Shintani, T. Takahashi and N. Kamada, Chromosome instability in acute myelocytic leukemia and myelodysplasic syndrome patients among atomic bomb survivors. *J. Radiat. Res.* **40**, 159–167 (1999).
13. D. M. Parkin, S. L. Whelan, J. Ferlay, L. Teppo and D. B. Thomas, Eds., *Cancer Incidence in Five Continents*, Vol. VIII. Scientific Publication No. 155, IARC, Lyon, 2002.
14. C. A. Felix and B. J. Lange, Leukemia in infants. *Oncologist* **4**, 225–240 (1999).
15. L. M. Secker-Walker, H. G. Prentice, J. Durrant, S. Richrad, E. Hall and G. Harrison, Cytogenetics adds independent prognostic information in adults with acute lymphoblastic leukemia on MRC trial UKALL XA. *Br. J. Haematol.* **96**, 601–610 (1997).
16. H. Mori, S. M. Colman, Z. Xiao, A. M. Ford, L. E. Healy, C. Donaldson, J. M. Hows, C. Navarrete and M. Greaves, Chromosome translocations and covert leukemic clones are generated during normal fetal development. *Proc. Natl. Acad. Sci. USA* **99**, 8242–8247 (2002).
17. P. Andreasson, J. Schwaller, E. Anastasiadou, J. Aster and D. G. Gilliland, The expression of ETV6/CBFA2 (TEL/AML1) is not sufficient for the transformation of hematopoietic cell lines *in vitro* or the induction of hematologic disease *in vivo*. *Cancer Genet. Cytogenet.* **130**, 93–104 (2001).
18. M. Eguchi-Ishimae, M. Eguchi, E. Ishii, S. Miyazaki, K. Ueda, N. Kamada and S. Mizutani, Breakage and fusion of the TEL(ETV6) gene in immature B lymphocytes induced by apoptogenic signals. *Blood* **97**, 737–743 (2001).
19. L. D. Cooley, D. A. Sears, M. M. Udden, W. R. Harrison and K. R. Baker, Donor cell leukemia: Report of a case occurring 11 years after allogeneic bone marrow transplantation and review of the literature. *Am. J. Hematol.* **63**, 46–53 (2000).
20. M. A. Kadhim, S. J. Marsden and E. G. Wright, Radiation-induced chromosomal instability in human fibroblasts: Temporal effects and influence of radiation quality. *Int. J. Radiat. Biol.* **73**, 143–148 (1998).
21. M. Nakano, Y. Kodama, K. Ohtaki, M. Itoh, A. A. Awa, J. Cologne, Y. Kusunoki and N. Nakamura, Estimation in the number of hematopoietic stem cells giving rise to clonal chromosome aberrations in blood T lymphocytes. *Radiat. Res.* **161**, 273–281 (2004).
22. N. Nakamura, M. Nakano, Y. Kodama, K. Ohtaki, J. Cologne and A. A. Awa, Prediction of clonal chromosome aberration frequency in human blood lymphocytes. *Radiat. Res.* **161**, 282–289 (2004).
23. C. A. Felix, Leukemias related to treatment with DNA topoisomerase II inhibitors. *Med. Pediatr. Oncol.* **36**, 525–535 (2001).
24. A. Borkhardt, G. Cazzaniga, S. Viehmann, M. G. Valsecchi, W. D. Ludwig, L. Burci, S. Mangioni, M. Schrappe, H. Riehm and A. Biondi, Incidence and clinical relevance of TEL/AML1 fusion genes in children with acute lymphoblastic leukemia enrolled in the German and Italian multicenter therapy trials. *Blood* **90**, 571–577 (1997).
25. A. A. Ferrando, D. S. Neuberg, J. Staunton, M. L. Loh, C. Huard, S. C. Raimondi, F. G. Behm, C. H. Pui, J. R. Downing and A. T. Look, Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. *Cancer Cell* **1**, 75–87 (2002).
26. E. R. Panzer-Grumayer, K. Fasching, S. Panzer, K. Hettinger, K. Schmitt, S. Stockler-Ipsiroglu and O. A. Haas, Nondisjunction of chromosomes leading to hyperdiploid childhood B-cell precursor acute lymphoblastic leukemia is an early event during leukemogenesis. *Blood* **100**, 347–349 (2002).
27. J. W. Taub, M. A. Konrad, Y. Ge, J. M. Naber, J. S. Scott, L. H. Matherly and Y. Ravindranath, High frequency of leukemic clones in newborn screening blood samples of children with B-precursor acute lymphoblastic leukemia. *Blood* **99**, 2992–2996 (2002).
28. W. A. Gilman, G. W. Knearle, E. G. Knox and A. M. Stewart, Pregnancy x-rays and childhood cancers: Effects of exposure age and radiation dose. *J. Radiat. Prot.* **8**, 3–8 (1988).
29. M. Vickers, Estimation of the number of mutations necessary to cause chronic myeloid leukaemia from epidemiological data. *Br. J. Haematol.* **94**, 1–4 (1996).
30. C. Biernaux, M. Loos, A. Sels, G. Huez and P. Stryckmans, Detection of major bcr-abl gene expression at a very low level in blood cells of some healthy individuals. *Blood* **86**, 3118–3122 (1995).
31. S. Bose, M. Deininger, J. Gora-Tybor, J. M. Goldman and J. V. Melo, The presence of typical and atypical BCR-ABL fusion genes in leukocytes of normal individuals: Biologic significance and implications for the assessment of minimal residual disease. *Blood* **92**, 3362–3367 (1998).
32. M. Vickers, G. Jackson and P. Taylor, The incidence of acute promyelocytic leukemia appears constant over most of a human lifespan, implying only one rate limiting mutation. *Leukemia* **14**, 722–726 (2000).
33. J. A. Martinez-Climent, M. J. Thirman, R. Epstein, III, M. M. Le Beau and J. D. Rowley, Detection of 11q23/MLL rearrangements in infant leukemia with fluorescence *in situ* hybridization and molecular analysis. *Leukemia* **9**, 1299–1304 (1995).
34. J. A. Ross, Dietary flavonoids and MLL gene: A pathway to infant leukemia? *Proc. Natl. Acad. Sci. USA* **97**, 4411–4413 (2000).
35. R. Strick, P. L. Strissel, S. Borgers, S. L. Smith and J. D. Rowley, Dietary bioflavonoids induce cleavage in the MLL gene and may contribute to infant leukemia. *Proc. Natl. Acad. Sci. USA* **97**, 4790–4795 (2000).
36. D. L. Preston, Y. Shimizu, D. A. Pierce, A. Suyama and K. Mabuchi, Studies of mortality of atomic bomb survivors. Report 13: Solid cancer and noncancer disease mortality: 1950–1997. *Radiat. Res.* **160**, 381–407 (2003).
37. A. V. Moorman, E. Roman, R. A. Cartwright and G. J. Morgan, Age-specific incidence rates for cytogenetically-defined subtypes of acute myeloid leukemia. *Br. J. Cancer* **86**, 1061–1063 (2002).
38. C. Schoch, W. Kern, P. Krawitz, M. Dugas, S. Schnittger, T. Haferlach and W. Hiddemann, Dependence of age-specific incidence of acute myeloid leukemia on karyotype. *Blood* **98**, 3500 (2001).
39. D. A. Pierce and M. L. Mendelsohn, A model for radiation-related cancer suggested by atomic bomb survivor data. *Radiat. Res.* **152**, 642–654 (1999).
40. J. Thacker and R. Cox, Mutation induction and inactivation in mammalian cells exposed to ionizing radiation. *Nature* **258**, 429–431 (1975).
41. Vijayalaxmi and H. J. Evans, Measurement of spontaneous and X-irradiation-induced 6-thioguanine-resistant human blood lymphocytes using a T-cell cloning technique. *Mutat. Res.* **125**, 87–94 (1984).
42. B. J. Sanderson, J. L. Dempsey and A. A. Morley, Mutations in human lymphocytes: Effect of X- and UV-irradiation. *Mutat. Res.* **140**, 223–227 (1984).
43. K. S. Iwamoto, T. Mizuno, T. Ito, M. Akiyama, N. Takeichi, K. Mabuchi and T. Seyama, Feasibility of using decades-old archival tissues in molecular oncology/epidemiology. *Am. J. Pathol.* **149**, 399–406 (1996).
44. Y. Matsumoto, K. Nomura, S. Matsumoto, K. Ueda, M. Nakao, K. Nishida, H. Sakabe, S. Yokota, S. Horiike and M. Taniwaki, Detection of t(14;18) in follicular lymphoma by dual-color fluorescence *in situ* hybridization on paraffin-embedded tissue sections. *Cancer Genet. Cytogenet.* **150**, 22–26 (2004).
45. Y. Yuan, L. Zhou, T. Miyamoto, H. Iwasaki, N. Harakawa, C. J. Hetherington, S. A. Burel, E. Lagasse, I. L. Weissman and D. E. Zhang, AML1-ETO expression is directly involved in the development of acute myeloid leukemia in the presence of additional mutations. *Proc. Natl. Acad. Sci. USA* **98**, 10398–10403 (2001).