On the Origin of Human Acute Myeloblastic Leukemia: Virus-"Hot Spot" Hypothesis

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Cellular Aspects

These remarks are directed only to one specific type of leukemia, acute myeloblastic (granulocytic) leukemia (AML). In this disease, as discussed elsewhere in this workshop, there is a progressive expansion of myeloblasts that appear morphologically somewhat different from normal myeloblasts. The important concept that these cells are *usually* not rapidly dividing but, in fact, may even have generation times much longer than normal bone marrow myeloblasts has led us to speculate (1–3) that the pathophysiology of this disease most likely involves a block in the normal process of maturation and differentiation of these cells to granuloctyes. Normally, myeloblasts make up less than 5 % of the total nucleated cell population of the bone marrow. The size of the pool of these cells appears to be constant. These cells, of course, are capable of DNA synthesis and of mitosis. During differentiation, a process that occurs in the bone marrow for the life time of the organism, it appears that on usual division of these cells, one daughter cell feeds back into this pool while the other begins to mature to a more differentiated end stage cell, the granulocyte. During the maturation process, several cell stages can be morphologically identified. These are listed below: myeloblast ——→ promyelocyte ——→ myelocyte ——→ metamyelocyte ——→ band cell ——→ mature granulocyte

The major morphological changes are: (1) the nucleus becomes progressively more condensed and stains more darkly (so-called pyknosis) while nucleoli disappear; (2) the cytoplasm becomes less basophilic (presumably less RNA) and begins to show the appearance of granules some of which are lysosomes. Functionally, the nuclear morphological changes indicate that the nuclear material is becoming "metabolically inert," i. e., DNA synthesis terminates and the cells are no longer able to divide. The functional cytoplasmic changes are manifold but, in general, I like to think of it as representing a change in the pattern of proteins that are made. The immature cells, for instance, contain high amounts of enzymes involved in the anabolic processes for DNA and RNA synthesis, while the proteins in the more mature cells are primarily concerned not with the "selfish" process of self-reproduction, but in serving the whole organism. There are many other analogous cell systems, e. g., in the formation of the anucleated mature red blood cell from the proliferative erythroblast the entire process appears directed to the purpose of forming a carrier of hemoglobin for the benefit of the organism while quite obviously losing its proliferative potential. The granulocyte

contains, for instance, high concentrations of hydrolytic enzymes (within the lysosomes) which, of course, are essential to the normal function of the granulocyte, phagocytosis and digestion of foreign material, especially micro-organisms. The capacity to synthesize DNA and divide probably terminates at the metamyelocyte stage, and the cell which is released under normal circumstances into the peripheral blood from the bone marrow is the granulocyte. This cell is thought to live approximately eight days. Now, in AML we see an expanding population of myeloblasts, abnormal in appearance, which have variable generation times but most of which apparently divide no more rapidly and often slower than normal myeloblasts. It is obvious why we proposed that to account for these observations, the simplest explanation is that these myeloblasts are blocked in their maturation process so that a pool of immature cells capable of proliferation gradually expands and results in the clinical disease we call acute myeloblastic leukemia. I naturally do not infer that these cells are not abnormal or very "sick" cells as Dr. Torelli likes to call them, I only emphasize that to account for their expansion the simplest explanation is the one described above.

The "Inciting" Agent

In thinking about the origin of the above described cellular changes, we should take into account pertinent information in man and in animal model systems. Some of these are listed here. 1) Some chemicals, e. g., benzene and radiation, can increase the incidence of leukemia in man and animals. 2) Genetic considerations are clearly of great significance both in animal and human leukemias. The most striking example is with identical twins. If one twin develops leukemia the other has a very high risk of subsequently developing leukemia. Genetic factors are also critical to animal leukemias, e. g., very high frequency of leukemia in AKR inbred mice and the almost non-existence of the disease in NIH Swiss mice, yet no greater amount of virogenes was detected in high frequency leukemia strains (AKR) than in intermediate (C-57) or low frequency strains such as the NIH Swiss mice (6). 3) In animals of several species, acute leukemias can be induced by certain type-C RNA tumor viruses. (Although it must be admitted that these are generally lymphocytic leukemias, and we really have no appropriate models for myeloblastic leukemias.) It appears that at least some normal tissues of at least some animals may contain *endogenous* type-C viruses which so far do *not appear to be tumorigenic*. Thus, it appears that type-C viruses may or may not contain oncogenic information. Presumably, this depends on the nature of the genomic RNA, and there is evidence that this RNA can be changed depending on its recent "history." For example, infecting a different cell with virus may lead to aquisition of new sequences from this cell (4). *(It may be best to look upon these viruses as all potentially capable of picking up information that leads to oncogenic potential, if they are not already frankly oncogenic.)*

It appears that type-C viruses are most frequently found in transformed cells but components of virus (in the absence of complete virions) have been noted in many normal tissues, especially embryonic, and as stated above, whole virions can be induced from some previously non-virus producing normal cells. It should also be noted that type-C mammalian *leukemia* viruses, generally do not transform cells *in vitro*; type-C

sarcoma viruses transform *in vitro*, yet leukemia viruses are, of course, leukemogenic *in vivo*. Moreover, Duesberg has emphasized that sarcoma viruses contain extra information apparently not found in leukemia viruses (5), although this evidence is limited to avian systems. 4) Type-C RNA tumor virus components have now, I believe, been unequivocally demonstrated in human acute leukemic cells (see below).

Evidence for Type-C Virus Related Information in Human Acute Leukemic Cells

In 1970, shortly after the discovery of reverse transcriptase in type-C viruses, we reported on the detection of an RNA-dependent DNA polymerase activity in some fresh human acute leukemic cells, an activity we could not detect in normal lymphocytes even after stimulation with PHA (7). Since that report, this enzyme has been purified and extensively characterized, particularly in AML (8–14). We can say that it has the known biochemical properties of virus reverse transcriptase. These biochemical properties include the following: a) it catalyzes an endogenous (i. e., using a *native* RNA template-primer) RNA primed-RNA directed DNA synthesis in a cytoplasmic particle; b) the enzyme purified from this particle will transcribe heteropolymeric portions of viral 70S RNA; c) the enzyme responds to the relatively specific synthetic template-primer dG_{12}.rC and favors dT_{12}.rA over dT_{12}.dA. These are all characteristics like viral reverse transcriptase and different from the major DNA polymerases of normal cells or *other* DNA polymerases from leukemic cells; d) the molecular weight of a recently purified enzyme from a patient with acute myelomonocytic leukemia (AMML) was shown to be approximately 70,000 daltons (see R. Gallagher, et al., elsewhere in this book), this is the known estimate of virus reverse transcriptase; e) the RNA template appears to be 70S (15, 16) or 35S (14, 15) in size, the size of virus RNA and its subunits, respectively. Recently, immunological observations have shown that this enzyme is specifically related to the reverse transcriptase from two known primate type-C RNA tumor viruses, the woolly monkey (simian sarcoma virus) and gibbon ape leukemia virus (17, and R. Gallagher, et al., elsewhere in this book).

Nucleotide sequence relatedness has been demonstrated between the nucleic acids of these particles from human acute leukemic cells and the genomic RNA of murine (10, 15, 16) and especially primate (simian sarcoma) virus (10). In these recent studies, we showed that a small amount of homology exists between leukemia virus RNA and the DNA product of the human leukemic reverse transcriptase. However, to our surprise, more than 50 % homology was found with the RNA of some (primate and murine) *sarcoma* type-C viruses (10).

Finally, we have noted that these viral related components are present in an intracytoplasmic particle which bands with the density typical of type-C virus (1.14 to 1.17 g/ml) (8, 10, 11, 14, 16). We interpret these results as indicative that at least some human acute leukemic cells contain defective (i. e., doesn't infect, replicate, etc., no budding form membrane and release is seen) type-C virus which is sarcoma related (Mammalian sarcoma viruses are not released in the absence of helper type-C "leukemia" virus.) A recent observation from Spiegelman's lab indicates that leukemic cells DNA contains sequences different (or extra) than found in normal cell DNA. Some of the DNA sequences made from leukemic cytoplasmic RNA by the

human leukemic cells reverse transcriptase apparently are not found in normal cell DNA but are detected in the DNA of leukemic cells (20). These results were interpreted as evidence against the oncogene theory of Huebner and Todaro (21). The evidence I have discussed here for type-C virus related components in human leukemic cells has been recently reviewed in more detail elsewhere (18, 19).

Virus "Hot Spot" Hypothesis

I believe the following model may be useful in thinking about leukemogenisis in man. In any case, it is in keeping with what we know and accounts for some apparent discrepencies on the obervations of factors which induce leukemia.

(1) There are "virogenes" in normal cells. These "viral" genes play an important role in growth and perhaps early differentiation of primitive embryonic cells. We know that type-C viruses may bud from cell membranes. Presumably, a genetic component of these viruses may affect host cell membranes. Normally, these gene products are repressed with the progression of undifferentiated primitve embryonic type cells to more mature cells. These genes are all included in type-C leukemia viruses and most are included also in type-C sarcoma viruses. In addition, other genomes repressed in the more primitive cells become induced during maturation. The active "virogenes", characteristic of undifferentiated cells, are functional in maintaining the growth of these cells, and are critical to transformation.

(2) Operationally, myeloblasts and similar cells of the normal bone marrow may be considered as "embryonic" cells (similar metabolic state) while the granulocyte (myeloblast derived) may be regarded as a "mature adult" cell.

(3) Physically adjacent or functionally related to the virogenes, resides a set of nucleotide sequences ("hot spot") which are unusually sensitive to events which alter nucleotide sequence (by mutation or by recombination). Among other things, these regions are sensitive to influence of some chemicals, radiation and hereditary factors. These changes in sequences may also influence chromosomal structure. These genes (virogenes and "hot spot") are not limited to one chromosome. The chromosomal changes frequently observed in leukemias (and many other neoplasias) are probably secondary to these events and need not in themselves be causally related to leukemia. Regulator genes may control the expression of both virogene and "hot spot". The regulator genes need not be in close proximity to the virogene or "hot spot." In this respect, Rowe and his associates have evidence of genes controlling viral gene expression which apparently are present in different chromosomes. The *normal* function of the "hot spot" may involve genetic diversity, useful to the embryonic state, and in antibody producing cells perhaps also to generation of antibody diversity. Some component directly bears on membrane function and or structure.

(4) The "hot spot" then is present and is susceptible to modification by recombination and/or by mutagenesis in *all* individuals. In some individuals, there is "hypermutability" of the "hot spot" (again by either mutation or recombination). These people then may already contain aberrent sequences and by heredity or by congenital factors may be passed on from one generation to another. Direct modification of the "hot spot" (by radiation, chemical carcinogenes, or by integration of new information as from virus infection) or indirect, e. g., by mutation in the

regulator gene which controls the "hot spot" and virogene may account for expression of these sequences at a time when they should be "repressed," leading to a return to, or maintenance of, the "embryonic" state. Some individuals are predisposed due to a hypermutable "hot spot," depending on genetic factors (e. g., hereditary ataxia telagniectasia, Bloom's syndrome, Down's syndrome [mongoloids]-all hereditary or congenital disorders with a high incidence of leukemia may already contain aberrant sequences within the "hot spot" or are highly susceptible to develop changes). The congenital problems such as mongoloids occur because of *in utero* changes. Thus, "normal" cells *could* contain oncogenic information (as the oncogene proposal demands) but not necessarily, and by this model *usually* they would not, but they would *always* contain the set of "viral" genes. Thus, the presence and expression of the virogene is not sufficient for leukemia, and nucleotide sequence change is a pre-requisite. In hereditary disorders, the event which modified the "hot spot" (directly or indirectly) occured generations ago.

(5) The type-C leukemia RNA tumor viruses may contain only these virogenes, but by virtue of the propensity of the provirus DNA (synthesized from the genomic RNA via reverse transcriptase after infection) to integrate in the region of the "hot spot" (which is subject to change not only by mutagenesis but also by recombination) it may induce transformation by modifying the "hot spot." The type-C sarcoma viruses contain most of the nucleotide sequences as the leukemia viruses, but they also contain the modified information from the "hot spot."

When virogene activity is expressed completely, virus particles may form. Normally, even in embryonic type cells complete expression does not occur. Partial expression can lead to defective (non-infectious) non-tumorigenic particles, e. g., viral-like particles such as the so-called A particles, or infectious particles such as some of the endogenous type-C viruses referred to earlier, or virus specific products not assembled into particulate forms. The *normal* information from the "hot spot" relates to normal membrane function and expressed with the viral genes can affect virus replication (budding from cell membrane, etc.).

(6) When the "viral gene" expression occurs these RNA transcripts have a propensity to physically and functionally associate with RNA transcripts from the "hot spot". When the "hot spot" is aberrant and expressed with virogene, sarcoma viruses may be produced as well as endogenous type-C viruses which may or may not contain oncogenic information. Mechanisms which induce expression of the "hot spot" may co-induce components of the virogene. With infection from without by type-C viruses, the new virogenes will have a propensity to integrate around this "hot spot" region. The "hot spot" will also have high content of reiterated nucleotide sequences. With expression of an *aberrant* "hot spot" only partial expression of the virogene usually occurs. This component is essential to formation of mature budding virions, hence sarcoma genome products are defective, unable to be released as mature virions to infect additional cells, leaving the organism "protected" to a transformation of only one cell. When complete virogene expression occurs, sarcoma virions are formed and released, potentially transforming several clones or even spreading the disease from organism to organism (exogenous infection). The type-C endogenous virus may also be formed after transformation *without sarcoma virus.* Presumably in this instance, there are other mechanisms which inhibit the

the formation of the mature sarcoma virus. These different possibilities then are:

1) Transformation (expression of aberrant "hot spot")→→→→no virus but virus components.
2) Transformation (expression of aberrant "hot spot")→→→→endogenous virus.
3) Transformation (expression of aberrant "hot spot")→→→→infectious sarcoma virus.

(7) For neoplastic transformation of any one cell then, whole virus is not needed nor is virus infection from without. Transformation will be accompanied, however, by the expression of at least some virogenes when they normally should be repressed-since induction of the expression of the "hot spot" is accompanied usually, if not always, by induction of some of the virogenes. (Virogenes and "hot spot" may be under the co-ordinate control of one regulatory gene.) Thus, leukemia occurs with at least some virogene expression (the result of the transformation rather then the cause), which may occur with release of virions which are either infectious, defective, or tumorigenic. If the latter, these particles may transform other cells and on occasion, infect another person so that in this instance the disease is directly caused by exogenous infection.

(8) Some type-C viruses may be without immediate oncogenic potential. In the past few years, some endogenous type-C viruses have been induced from some normal cells (22–24). These induced apparently endogenous virions in most instances do not appear to be oncogenic for their species of origin. However, they do contain reverse transcriptase and hence the ability to synthesize a DNA provirus some or all of which may integrate into the "hot spot" region. They, therefore may evolve into oncogenic particles. They may acquire nucleotide sequences (by repeated RNA→DNA DNA→RNA reactions) which allows them to eventually induce expression of an aberrant "hot spot" or make a normal "hot spot" aberrant. It is proposed that in most cases type-C leukemia viruses which are, in fact, leukemogenic *in vivo* act by affinity of this information to integrate around the "hot spot" sequences and induce expression of these sequences (see number 6). The critical factor to leukemogenesis then is the control of the expression of this "hot spot". The information when abnormal may be because of sequence change or addition of new sequences.

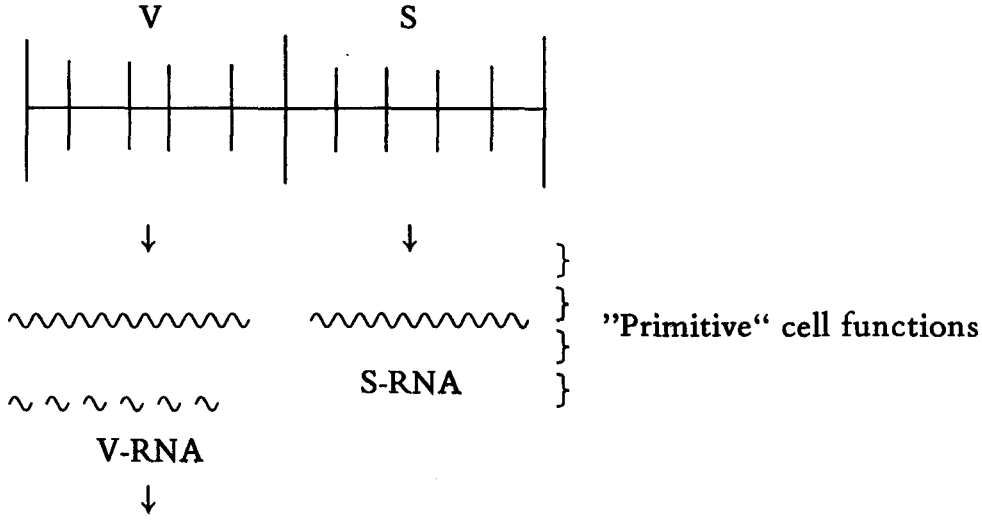
(10) One function of the "hot spot" (as well as one component of the virogenes) as described above has, at least in part, an affect on cell membrane function and/or structure. When altered, cell membranes are modified so that they do not respond properly to the usual regulator proteins. For instance, a greater concentration of the "maturation" proteins (colony stimulating activity) may be needed for normal maturation. Maturation arrest ensues which is potentially reversible *in vitro*, but should not have long term therapeutic benefits *in vivo* because of the need for its continued presence in relatively high concentrations. In this respect, another disease should be mentioned. Paroxysmal nocturnal hemoglobinuria, an apparent "in born error" is a membrane alteration and terminates in leukemia in a greater than predicted frequency.

A schematic illustration of the model is shown below where V = virogene and S = hot spot.

This model is testable in some of its components, and it predicts the following:

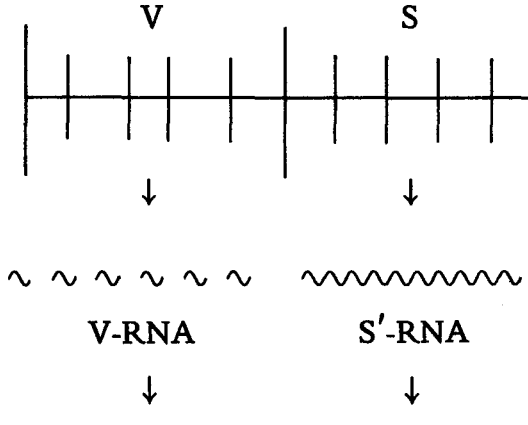
(1) Leukemic cells will contain some nucleic acid sequences different from normal cells of most, but not all, people, and these special nucleotide sequences in leukemic cells may be found in some "normal" cells of certain hereditary disorders, or congential disorders, e. g., possibly in mongoloids.

1) Normal Cell (immature)



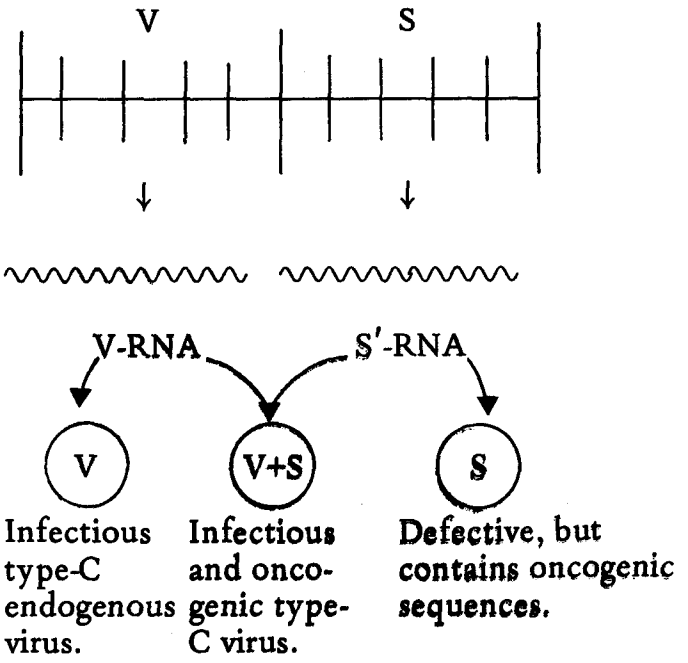
Some degradation of V-RNA (virus particles usually not found)

2) Normal Cell (mature)

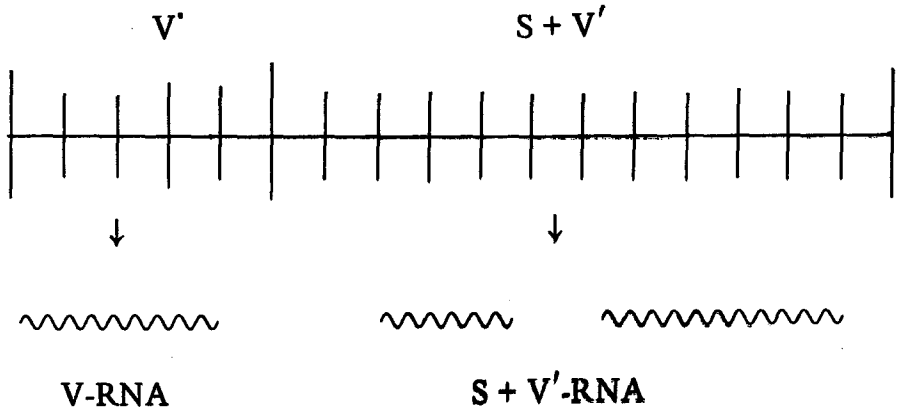


Most is degraded or it is not even expressed.

3) Transformed Cell (by mutation)



4) Transformed Cell (by recombination-addition of sequences, e. g., by infection with virus)



(Consequences are as for 3.)

(2) Information within sarcoma viruses is the oncogenic information not only for sarcomas but also leukemias.

(3) Normal cells always contain some genes in common with type-C viruses. These genes are normally expressed in embryonic type cells to some degree and always in transformed cells, but not necessarily completely. When completely expressed, viruses are formed. The viruses then are the *result* of the transformation in this case.

(4) Infection from without by oncogenic type-C viruses may be a direct cause of

leukemia in some instances. It is possible, that it is always caused by exogenous viral infection. The model does not bear on the question – wether this is common or not.

(5) Type-C leukemia viruses activate or create "sarcoma" sequences.

(6) More type-C virus information is expressed in transformed cells than in normal adult tissues, and perhaps some virogene expression occurs in some normal mature cells.

(7) The sequences in the "hot spot" or in a regulator gene controlling the "hot spot" and virogenes of *some* hereditary disorders with high incidences of leukemia will be *different* (nucleotide sequence mutational change or addition by recombination of new sequences) than normal cell DNA.

(8) Finally, I propose that unlike some traditional concepts of viral transformation *in vitro*, a more mature cell is not induced to de-differentiate, but instead the target cell for a leukemia inducing agent will be the pluripotential stem cell or the progenitor blast cell (myeloblast) which will be blocked in its maturation.

The virus "hot spot" proposal differs from the oncogene theory of Huebner and Todaro (21) in the following ways: a) It is aimed chiefly at a specific neoplasia – leukemia in man in contrast to all neoplasia; b) It attempts to explain the phenotype changes in the cells and not just the "genotypic" origin of neoplasia; c) Most importantly, in the consideration of the genotypic origin of neoplasia, it does not demand oncogenes in all somatic cells of all vertebrates, as the oncogene theory proposes. The latter states that regulator (switch on- switch off mechanisms) are sufficient to cause neoplastic transformation in any somatic cell. In contrast, this proposal states that this certainly may occur *but* nucleotide sequence change or addition in DNA is a pre-requisite; although this change could be "in born" it is not present in most cells of most people. The virus "hot spot" proposal differs from Temin's protovirus hypothesis (25) as follows: a) Again, its aim is somewhat different. The protovirus hypothesis is directed primarily at explaining the origin, evolution, and replication of RNA viruses that may become oncogenic; b) The protovirus hypothesis also does not attempt to deal with the phenotypic changes in leukemia; c) The requirement for DNA nucleotide sequence change in the virus "hot spot" proposal is similar to the protovirus theory. However, the protovirus theory puts the emphasis on somatic mutation and reverse transcriptase and its RNA template as pivotal to this change, i. e., the emphasis for mutation is in the RNA template for the reverse transcriptase, reverse transcriptase itself, or the DNA product made by reverse transcriptase. The virus "hot-spot" proposal states that the nucleotide sequence change critical to leukemogenesis may be by mutation or and perhaps more commonly, by addition of information through recombination. In addition, the change may be brought about by modification of nuclear DNA sequences not involving reverse transcriptase reactions.

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