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Etiology of Leukaemias

with Special Reference to Genetic Problems

L. W. LAW [1]

A critical review is made of the present knowledge of the etiology of neoplasms of the haematopoietic system in experimental animals and man. Genetic factors play a dominant role in the origin of leukaemias in mice. A Mendelian interpretation of the data is excluded and several genes appear to be involved in susceptibility. The data available on leukaemias in man are equivocal so far as the role of genetic factors is involved. The author discusses the value of family and twin studies—which suggest the operation of rare, highly penetrant, recessive genes—and of cytogenetic studies in contributing to a fuller understanding of the nature and etiology of leukaemia.

The contributions made by genetics to cancer research at the experimental level have been striking. Many years, however, have been spent in developing pure strains of animals and in elaborating satisfactory techniques of study in order to make such contributions meaningful. It is quite unlikely that the methods used, except in rare cases in statistical and genetic studies of cancer in man, have as yet reached the degree of sophistication necessary to yield satisfactory data. On the other hand, recent cytogenetic studies in man, revealing a relationship of altered karyotypes to neoplasia, have again refocused attention upon the role of genetic factors in cancer and particularly among the leukaemias.

STUDIES IN EXPERIMENTAL ANIMALS

Incontrovertible evidence exists to show that genetic factors play a dominant role in the origins of certain neoplasms. Those neoplasms occurring in inbred strains of mice, upon which adequate genetic studies have been done, include mammary tumours, pulmonary neoplasms and leukaemia (Heston, 1948; Law, 1954). The facts obtained to date definitely exclude any direct Mendelian interpretation of the data. Many genes appear to be involved, determining susceptibility to each of the above neoplasms, and the genes appear to be cumulative in effect, resembling closely the inheritance of such characters as polydactylism (Wright, 1934) and specific skeletal defects (Gruneberg, 1952). Linkage studies have led to the identification of specific genes concerned with susceptibility to neoplasms (Heston & Deringer, 1947; Law, 1952).

One characteristic of the type of inheritance depending upon the expression of multiple genes with a threshold for manifestation of the character is that the character is sensitive to influences of the environment. This, indeed, has led to the discovery of several important non-genetic factors of interest such as the Bittner milk agent (Bittner, 1936), the maternal resistance factor in leukaemia (MacDowell & Taylor, 1948) and the influences of maternal age and parity upon the expression of neoplasms (Strong, 1951). The sensitivity of various neoplasms to the influences of environment has erroneously led to the impression that non-genetic variables are all-important. It should be recognized, however, that only in a critical analysis is it possible properly to evaluate most environmental effects.

Several neoplastic diseases in man clearly behave as if determined by single dominant genes; these will be discussed later. In contrast, no tumour has yet been found in experimental animals the occurrence of which is determined by a single gene, nor which shows the dramatic effects of certain rare genes in man, such as those concerned, for example, with retinoblastoma, polyposis intestini, and xeroderma pigmentosum.

[1] Head, Leukemia-Studies Section, National Cancer Institute, Bethesda, Md., USA.

Distinct morphological and biological entities of the lymphomas and leukaemias are encountered in the experimental animal and many of their histological and clinical features mimic these diseases of man. Engelbreth-Holm (1942) and Dunn (1954) have discussed details concerning the pathological aspects of this complex group of neoplasms. The most common morphological form appearing in inbred strains of mice is lymphocytic leukaemia, and even among this morphologically distinct group evidence is accumulating to indicate the existence of different biological entities (" spontaneous " *versus* virus-induced; those of thymic origin *versus* those of non-thymic origin, etc.). Certain facts have been established concerning the influence of genetic factors in the etiology of these diseases in inbred strains of mice. High-leukaemic strains of mice, such as C58, AKR, C3Hf/$_{Fg}$ and F, and low-leukaemic strains, such as RF, C3H and STOLI, have been used in investigations of attempts to evaluate the role of genetic factors. Results of experiments employing reciprocal matings, backcrosses, foster-nursings, and reciprocal transfers of fertilized ova have revealed the following: (1) susceptibility to leukaemia has a genetic basis; (2) the pattern of inheritance is not Mendelian, but several genes appear to be involved; (3) linkage tests have revealed that two known genes of the mouse are plus modifiers of susceptibility—a gene *f*, which ordinarily produces a transitory siderocytic anaemia, and a gene *d*, which influences body growth; (4) the male parent contributes the potentiality to induce leukaemia equally as well as the female (as determined by reciprocal matings) except in situations where the low-leukaemic parent contributes a maternal resistance factor (MRF); (5) neither high-leukaemic nor low-leukaemic sublines may be established by foster-nursings or by transfer of fertilized ova from high-leukaemic donors to low-leukaemic recipients, or by the reciprocal transfer. (See Law, 1952, 1959.)

Thymic tissue plays a decisive role in the genesis of lymphocytic leukaemia and thymic lymphosarcomas in certain inbred strains of mice (Law, 1959; Kaplan, 1959); in one high-leukaemic strain, C3H/$_{Fg}$, it plays little if any role, however (Law, 1957). The indirect effect of thymic tissue in spontaneous and radiation-induced lymphocytic leukaemias is nevertheless expressed genetically as an intrinsic property of the thymus in leukaemogenesis. Thymic tissue from low-leukaemic strains grafted into compatible, but potentially leukaemic, hosts does not call forth lymphomas, whereas thymic tissue from high-leukaemic strains (AKR and C57BL, for example) does (Law, 1959). It is highly unlikely that a counterpart exists in man of this model system in mice; nevertheless the " sphere of influence " of the thymus has been a fruitful tool for studies of pathogenesis and mechanisms of leukaemogenesis.

Carcinogenic hydrocarbons, irradiation and estrogens are definitely leukaemogenic agents, and it is becoming clear that urethane (Fiore-Donati et al., 1961), mineral oils (Potter & Boyce, 1962) and plastic films (Merwin & Algire, 1959) play a prominent role in the etiology of certain neoplasms of the haematopoietic system. Recently much attention has been directed towards leukaemogenic viruses (Gross, 1957; Graffi, 1957; Moloney, 1960) and attempts made to devise unifying concepts (Gross, 1958; Zilber, 1961). Since leukaemogenic agents have been isolated from leukaemic tissues arising in irradiated C57BL mice (Lieberman & Kaplan, 1959) and C3H/$_{Gs}$ mice (Gross, 1959), it is argued that perhaps all " spontaneous " leukaemias and those arising following introduction of carcinogens, estrogens or other leukaemogens, result from " activation " of leukaemogenic viruses, or induction (in the bacteriophage sense of induction); the apparent widespread distribution of infectious leukaemogenic viruses, their apparent predilection for transplantable neoplasms of the mouse and the lack of rapid *in vitro* and *in vivo* tests fo these viruses make it difficult to test such concepts.

Some recent work in our laboratory (Law & Moloney, 1961) concerning the congenital transmission of one leukaemogenic virus—that of Moloney—throws light on the adequacy or inadequacy of some recently developed concepts. The findings here should be considered in terms of the epidemiological problems in human leukaemia. Generalized lymphocytic (stem cell) neoplasms result in many strains of mice, either with high- or low-leukaemic potentialities following parenteral introduction of virus; some strains, however, nearly completely resist virus infection. Transfer of virus occurs in vertical fashion from mother to offspring in several C3H strains of mice and is most efficiently transferred through the mother's milk. Transfer from mother to offspring during the prenatal period and through the paternal line has not been observed. High-leukaemic and low-leukaemic lines may be established simply by foster-nursings. Some strains—for example, RFM and C57BL—are susceptible to virus but are not able to replicate and transmit virus to their offspring. In these studies

there was found no indication of mouse-to-mouse infectivity. Findings with the Gross virus isolated from AKR leukaemic mice are similar (Gross, 1961; Law—unpublished results), except for a relatively inefficient maternal transfer of the Gross virus. These results concerning the pattern for congenital transmission of the Moloney and Gross viruses stand in sharp contrast to the patterns for transfer of potentialities to develop leukaemia in the high-leukaemic strains AKR, C58, F and C3Hf/$_{Fg}$. The concepts that leukaemia is transmitted through the germinal cells or that it is an "egg-borne" disease (Gross, 1956) are therefore inadequate in face of the present evidence. Also, although superficially in some aspects the induction of leukaemia by virus may resemble lysogeny in bacteria, the above results discourage the use of the lysogeny model system as an analogy in designing investigations of the mechanism of leukaemogenesis.

GENETIC STUDIES IN MAN

While it is known, from controlled genetic studies in the experimental animal, that certain specific genes modify the frequency and expression of certain neoplasms, none have been described which show the striking effects of certain genes in the human population. Some of these examples are the genes determining retinoblastoma, xeroderma pigmentosum and polyposis intestini. It is important to note, however, that among these diseases, and particularly retinoblastoma, some cases probably do not represent genetic mutants (Griffith & Sorsby, 1944).

Certain forms of cancer have been reported to occur with a high frequency in families. The well-known "G" family of Warthin (Hauser & Weller, 1936) and the familial leukaemias reported by Anderson (1951), Steinberg (1960) and Stewart (1961) are examples. In any form of cancer distributed at random among a population, it would be expected that some families would have several cases. For the leukaemias, it has been estimated that, of the 11 396 persons dying in the USA from leukaemias and lymphomas in 1956, somewhat more than 100 would be expected to have relatives with leukaemia. These approximations are based upon the probability of developing leukaemia by age 35 and having 10 relatives. The approximations therefore do not apply to the rare familial cases mentioned above, which indeed require more of our attention.

Since it is obvious that malignant diseases do not yield to a direct Mendelian interpretation—that is, good approximations to Mendelian ratios are not obtained except for the rare cases mentioned above—two general methods have been employed generally in genetic studies: (1) a statistical comparison of families (proband method) and (2) a study of twins. Both types of study are beset with difficulties. Ideal controls are usually unobtainable and the validity of controls must always be questioned. A heterogeneity of skills is characteristic with a large number of investigators involved in such studies. Errors in diagnosis are not uncommon. Reliability of the data obtained from death certificates and questionnaires is not known. These variables are probably reflected in the different conclusions which have been drawn from the various published studies concerning the role of genetic factors for any given organ or site of neoplasm.

Videbaek (1947) first examined the familial occurrence in man of leukaemia (of all types and morphological forms) from a statistical and genetic approach and concluded, among other things, that there was an increased frequency among his proband material (relatives) over that of his controls. Videbaek reaffirmed his views in his 1958 publication. The Danish data were re-analysed by Busk (1948), who did not agree with Videbaek's general conclusions.

Subsequently to the work of Videbaek the results of several studies have been published on frequencies of leukaemia (all types and forms usually grouped together) in families of leukaemic patients compared with several different kinds of control groups (Amiotti, 1953; Morganti & Cresseri, 1954; Gausch, 1954; Revol et al., 1954). Again, evidence was obtained for and against genetic factors in the causation of this group of diseases.

Of more importance and interest is a recent study by Steinberg (1960), who limited his study to an estimation of the role of heredity in the causation of acute leukaemia in children (who became ill before the age of 16 years) and the further examination of the frequency of cancer in general, of pernicious anaemia and of blood dyscrasias in general among close relatives of his patients with acute leukaemia. Although this study revealed that the families of children with acute leukaemia did not suffer an increased frequency of leukaemia, of cancer in general, or of blood dyscrasias, an extraordinary concentration was found of leukaemia (in three or more children) in three families. Similar findings were reported by Videbaek (1947). Indeed it is important to point out that *many of these*

families resulted from consanguineous matings. These striking concentrations in families and the isolated cases of identical morphological forms of leukaemia in monozygotic twins (Anderson, 1951; Gausch, 1954; B. MacMahon [1]) lead one to suspect the operation of rare, highly penetrant, recessive genes. It is therefore suggested that more attention should be directed towards the study of this possibility of the existence of rare genetic mutants in the population and the likelihood of detection of apparently "normal carriers". Also, new procedures now available for analysing data concerning effects of consanguineous marriages (Norton, 1961) allow of more precise analyses of genetic influences and should be pursued.

Stewart (1961) has recently called attention to the probable existence among childhood leukaemias of two distinct varieties of the disease, the numerically important one being responsible for the early peak of leukaemia mortality at 2-4 years of age and apparently derived from undifferentiated blast cells. This common type is stated to be "familial". This concept of Stewart, along with the recent implications derived from cytogenetic studies of leukaemias in man, suggest that future genetic studies should be more concerned with attempts to define specific entities perhaps related to age, cell morphology, chromosome karyotype, etc., within this group of diseases and that attention should be confined to these. It is conceivable that one entity (subdivision) may be inherited in Mendelian fashion and others not, or that different entities may be inherited by different mechanisms.

A study of twins is an excellent method of assessing the importance of genetic factors in disease, especially where it is expected that simple Mendelian ratios will not be obtained. If a malignant disease or leukaemia is concentrated in a family, there should be an excess of concordant pairs in both monozygotic and dizygotic twins. If such concentration is the result of genetic factors, the concordance should be greater for monozygotic twins. There are several examples in the literature of monozygotic twins developing the same morphological forms of leukaemia almost simultaneously. These appear not to be chance events and should not be disregarded.

Two sets of investigations in which twins were studied systematically and with unbiased samples are those in Germany (von Verschner & Kober, 1940) and in Denmark (Busk et al., 1948). These samples, however, are not large. The results published to date have been preliminary in nature since in many cases one twin is still alive; many twins have been under observation for too short a period of time; and significant questions have been raised concerning reasons for excluding certain twin pairs (Gorer, 1943). Unfortunately there is little information from twin studies to indicate precisely the influence of genetic factors in leukaemia and the lymphomas.

With the considerable interest now being shown in the geographical distribution of cancer (and leukaemia) and in studies of epidemiology of malignant diseases it is important to know the role of genetic variables. Twin studies will provide such information. They need to be done systematically, with patience, and on an international scale.

CYTOGENETIC STUDIES

Progress in cytogenetic studies in man has been rapid following the introduction of newer techniques and the stimulus of the discovery in 1956 (Tjio & Levan) that man had 46 chromosomes and not 48. A series of discoveries relating chromosomal changes to various metabolic and developmental diseases have been published. The changes in sex-chromosome patterns leading to human intersexualities are well documented (Ferguson-Smith, 1961).

The potential value of cytogenetic studies in improving our understanding of the nature of leukaemia is suggested in recent reports relating to chronic myeloid leukaemias and leukaemic mongols. Nowell & Hungerford (1960) first described a specific pseudodiploid pattern of chromosomes among most of the chronic myeloid leukaemics studied by them. A minute chromosome (Ph'), belonging to either pair 21 or 22 (Group VII) was identified. This abnormal, small, acrocentric chromosome apparently results from either deletion or translocation, but it is difficult at the moment to distinguish between these two possibilities. More than 40 patients have now been characterized from several sources (Tough et al., 1961; Sandberg et al., 1961; Kinlough & Rabson, 1961) as showing the marker chromosome, but with normal diploid karyotypes in their non-malignant tissues. Of interest also are those patients with chronic myeloid leukaemia who do not show this anomaly. Assuming that those leukaemics with the Ph' chromosome represent "genetic diseases" resulting from genic imbalance (as a

[1] To be published.

result of deletion or translocation), those without the anomaly must represent different clinical or morphological subdivisions or a different cause.

Acute leukaemia appears to be more common among mongols than among normal children (Stewart, 1961). Most mongoloid children are autosomal trisomics, that is, they have 47 chromosomes, an extra chromosome existing along with either pair 21 or 22 (Group VII). It has been suggested that the same chromosome is involved in both chronic myeloid leukaemia and the increased liability toward leukaemia among mongoloids (Tough et al., 1961). This concept appears highly likely. Leukaemia among mongols is apparently not associated with any chromosome abnormalities apart from that of the typical mongol karyotype. Nevertheless, the extra chromosome could account for both of these effects in children and need not be related to the causation of leukaemia in normal (non-mongoloid) children.

Two other trisomic syndromes have now been established and these are similar to mongolism in that mental defect and multiple congenital anomalies are present in both groups. One of these involves chromosome 13, 14 or 15 (Group IV), and the other trisomic either chromosome 17 or 18 (Group V) (see Patau et al., 1960, and Smith et al., 1960). These are rare occurrences; nevertheless, their relationship to acute leukaemia should be watched carefully.

There have been no reports of other consistent chromosome aberrations in other leukoses or lymphomas or in other forms of cancer in man or in the experimental animal, except in a group of murine lymphocytic leukaemias which were found to deviate extensively from the normal murine karyotype (Wakonig & Stich, 1960), and in several acute leukaemias in man in which a visible chromosomal abnormality appeared to be a unique finding (Ford, 1960). It should be recognized, however, that the difficulties and obstacles inherent in cytological techniques, in tissues to be examined and in the interpretations of normal karyotypes in certain species do not allow for rapid progress in this field.

RÉSUMÉ

Les facteurs génétiques jouent un rôle prépondérant dans l'origine de certains néoplasmes, en particulier les leucémies, de certaines lignées consanguines de souris. La réceptivité aux tumeurs ne peut s'expliquer par des facteurs mendéliens, et divers gènes semblent y participer, ainsi que l'indiquent des tests de linkage. Chez l'homme, les choses se passent comme si certains néoplasmes étaient déterminés par des gènes uniques, dominants. Tel n'est pourtant pas le cas des leucémies, dont la génétique est encore mal élucidée. La comparaison statistique de familles (selon la méthode proband), et l'étude des jumeaux univitellins comportent de grandes difficultés (celle d'obtenir des témoins comparables, le grand nombre de recherches et de compétences nécessaires, les éventuelles erreurs de diagnostic et celles des causes de décès des certificats, par exemple). Ce que l'on sait toutefois des familles résultant de mariages consanguins, et l'apparition des mêmes formes de leucémies chez les jumeaux monozygotes laissent supposer qu'il peut s'agir de quelques gènes récessifs, rares et très pénétrants.

Les hydrocarbures cancérogènes, les rayonnements ionisants et les oestrogènes sont des agents leucémigènes chez les animaux d'expérience, et il s'avère que l'uréthane, les huiles minérales et les pellicules de plastique jouent un rôle de premier plan dans l'induction de certains néoplasmes du système hématopoïétique de la souris. Récemment, on a isolé des virus leucémigènes dans divers laboratoires. On ne sait pas dans quelle mesure ces virus et les autres agents leucémigènes, physiques et chimiques, interviennent dans les leucémies spontanées. Ces études seront d'autant plus difficiles que les virus sont extrêmement répandus, dans le monde et dans les laboratoires; ils ont une prédilection pour les néoplasmes greffables de la souris, et ne peuvent encore être décelés rapidement par des tests in vitro ou in vivo.

La transmission congénitale du virus leucémigène de Moloney a été étudiée. Ce virus se propage par le lait maternel, mais non par le placenta ou les tissus extra-embryonnaires, ni par filiation paternelle. Ainsi se trouve infirmée l'hypothèse selon laquelle les virus seraient transmis par les cellules germinales, à l'image des systèmes lysogènes.

La valeur que peuvent acquérir les méthodes cytogénétiques dans l'élucidation des processus leucémigènes est mise en lumière par les récentes observations concernant le rôle de chromosome 21 (ou 22) dans les leucémies myéloïdes chroniques et chez les mongols leucémiques. Aucune autre relation précise entre chromosomes et cancer — de quelle forme que ce soit — n'a été mise en évidence chez l'homme.

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