

Review Article

Leukaemogenesis in Down's Syndrome

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SUMMARY

Due to the fixed karyotype and documented malignancy risk in patients with Down's syndrome, recently described aetiological factors can be assigned to their proper places in a conceptual framework for leukaemogenesis in these individuals. This is a more profitable approach than those in which various types of karyotypic patterns are matched to different malignancies. It seems that viruses may play a special role, but they need interaction with other factors, most of which are present in Down's syndrome. A unifying concept which may be helpful in establishing research priorities is presented.

S. Afr. med. J., 51, 158 (1977).

Much has been recorded about chromosomal abnormalities as a cause or consequence of malignant transformation, and about the correlation of tumours with specific karyotypic patterns.

Special circumstances associated with Down's syndrome allow a different approach to the problem. In Down's syndrome a fixed karyotype is found and the increased risk of different types of malignant change has been documented.^{45,14} The various possible aetiological factors can be assembled in a unified concept to form a working hypothesis of leukaemogenesis in these individuals. The purpose of this review is to discuss recent clinical and experimental findings and to present such a unifying concept for leukaemogenesis in Down's syndrome.

GENERAL FACTORS IN VIRAL ONCOGENESIS

Although evidence for a viral aetiology for human leukaemia has accumulated, it seems highly unlikely that leukaemia is contagious in the usual sense of the term. It appears that tumour initiation may result from a complex inter-

action of 3 conditions: (a) genetic predisposition to cancer; (b) infection by a tumour virus, especially in immunodeficient individuals; and (c) environmental factors.³⁶ (The first 2 conditions have definitely been proved in mice, where infection causes leukaemia or immunisation. Susceptible mice can then be inoculated against leukaemia.) All of these 3 conditions may be evident in Down's syndrome.

Genetic Predisposition

Krivit and Good³³ first reported the association between Down's syndrome and leukaemia in 1957. Holland *et al.*²⁸ followed 2 033 probands with Down's syndrome for periods ranging from 1 to 14 years. During this period 7 index patients died of leukaemia and 7 from other forms of cancer. The death rate for leukaemia was 18 times greater in those with Down's syndrome than in the general population, while the mortality from other types of cancer was 2.6 times above the national rate for the USA. The several studies which support the association between Down's syndrome and leukaemia have recently been reviewed by Rosner and Lee.⁴⁵ Acute myelofibrosis may also occur with unusual frequency in patients with Down's syndrome.¹⁴

Tumour Viruses

There is evidence that leukaemic children who have received whole-body irradiation and bone marrow from their siblings develop leukaemia of donor cell type.¹⁷

The oncornaviruses represent the leukaemogenic RNA viruses. Human cells can be infected with these viruses and can be transformed by viral gene products obtained from animal cells.³⁰

A type cRNA virus was recently isolated from cultured cells of a patient with myeloblastic leukaemia, but who did not have Down's syndrome.²⁰

The evidence of oncogenic viral infections in humans is important, in view of accumulating evidence that antiviral immunodeficiency occurs in individuals with Down's syndrome.^{8,31,35,48} It is probably significant that the trisomic chromosome in persons with Down's syndrome also carries the locus for interferon-induced antiviral activity.⁵⁰ The existence of 2 types of individuals with Down's syndrome with lesser and greater degrees of cellular immunodeficiency has been reported.^{10,48} The latter type may be more likely to become chronic hepatitis B surface antigen (HBsAg) carriers. HBsAg is also found in excess in patients with lymphocytic leukaemia.⁵ It has not yet been shown that HBsAg carrier status indicates an immune deficit which could be implicated in leukaemia, but the significance of HBsAg carrier status in Down's syndrome must be fully investigated.

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Environmental Factors

In viral leukaemogenesis in animals, vertical transmission of infection has led to the oncogene hypothesis, which states that transmission via the zygote results in a viral or proviral genome being present in a repressed state in every somatic cell.³⁰ Malignancy can result when de-repression is induced by environmental influences such as irradiation, chemical carcinogens and ageing. Vertical transmission has not been conclusively demonstrated in humans, but is suggested by reports such as a recent epidemiological investigation carried out in California,³ which revealed a possible leukaemia risk to 0-4-year-olds due to their mothers having suffered an influenza infection during the first trimester of pregnancy. In this case, the virus may also have exerted its oncogenic effect through increased chromosome breakage. This aspect will be dealt with further on.

ASSOCIATION BETWEEN DOWN'S SYNDROME AND LEUKAEMIA

Since the 1890s chromosomal and mitotic irregularities have been recognised as the one common feature in cancer. This fact stimulated the mutation theory of cancer at an early date.³⁴ In 1947, the higher incidence of leukaemia in relatives of leukaemia patients led to the consideration of genetic leukaemogenic factors.³⁵ In the early sixties, the chromosome which is present in triplicate in cells of patients with Down's syndrome was thought to be the same one which exhibits a long-arm deletion in chronic myeloid leukaemia (Philadelphia chromosome). Since the leucocyte alkaline phosphatase level is generally increased in patients with Down's syndrome and decreased in patients with chronic myeloid leukaemia, this was thought to prove the existence of a leucopoiesis locus on this chromosome.

In 1971, O'Riordan *et al.*⁴² showed the Philadelphia chromosome to be a deleted No. 22. The extra chromosome in Down's syndrome shows delayed uptake of radioisotopes, is more often satellited and shows more intense staining in its long arms when examined by means of fluorescence techniques, and thus represents a different G-group autosome — No. 21.³⁹ In spite of the fact that the association between chromosome 21 and leukaemia has not been explained in its initial hypothesis, recent findings emphasise the possibility that chromosome 21 is important in the onset of the leukaemic process,^{6,54} or is concerned with abnormal haematopoiesis.^{29,43,45} The association between Down's syndrome and leukaemia does not seem to be a direct cause-effect relationship, but involves an interaction between many factors.

VIRUSES, CHROMOSOME BREAKAGE AND VIRAL GENOME INCORPORATION

Evidence of a viral aetiology in human leukaemia was recently provided by Gallagher and Gallo,²⁰ who isolated a type of cRNA virus from cultured blood cells of a patient with acute myelocytic leukaemia. Since the investigators had only used biological materials of human origin, contamination with animal viruses was unlikely. Immunological evidence in this case also indicates that the virus was of human origin. The major proteins were identical to pro-

teins previously isolated from other patients with acute myelocytic leukaemia.

Proteins from the new virus were immunologically closely related to proteins in two viruses that cause leukaemia in subhuman primates, but were more distantly related to proteins from a mouse leukaemia virus. The authors postulate that interaction with other factors may be required to produce myelogenous leukaemia in man.

Hypotheses concerning the infectious transmission of leukaemia have been tested, but the findings were mostly negative. Statistical studies of leukaemic clustering have not provided solid evidence for an infectious mode of spread. The incidence of leukaemia among marital partners of leukaemic persons or in children born of women with leukaemia during pregnancy is not excessive.³⁸ These observations do not exclude the possibility of a viral aetiology for leukaemia, but may be due to the complex interplay of many factors.

Chromosome Breakage

The importance of genetic factors in some cases is illustrated by the pedigree reported by Zuelzer *et al.*⁵⁵ These investigators did chromosomal studies of relatives of an infant with myeloblastic leukaemia and with cytogenetic features of partial D trisomy. They showed excessive breakage, chromatid exchange, endoreduplication and fragment formation in 3 generations of the affected child's family. This suggested a genetic factor acting in the manner of a breakage gene and constituted a possible link between leukaemogenesis, congenital anomalies and chromosome aberrations. In this special situation the breakage gene '... could have facilitated the occurrence of exogenous mutagenic events, e.g. the entry of hypothetical leukaemia viruses into the genome of rapidly replicating cells such as that of bone marrow'. Very little is known about norms of chromosome breakage in the 'average' population. Breakage is the phenomenon which occurs before an abnormal chromosome forms, and at least one of these, a centric fusion of the D-D type, is believed to be one of the commonest forms of autosomal heterozygosity found in the population at large.³² These individuals are believed to be completely normal. Patients with genetic conditions in which there is a clearly recognisable increase in chromosome breakage, such as Bloom's syndrome, Fanconi's anaemia and ataxia telangiectasia, have a notable susceptibility to cancer, especially of the lymphoreticular system. Chronic myeloid leukaemia is known to be associated with a specific type of breakage chromosome, the Philadelphia chromosome.

Viruses have been demonstrated to cause at least 3 types of chromosome breakage *in vitro*.⁴⁰ Chromosome breakage and breaking agents are important, but complex phenomena and the relationship between breakage and abnormal growth are very difficult to define. Evidence for increased breakage in Down's syndrome has been provided by Higurashi *et al.*²⁵ who found that cultured cells from patients with Down's syndrome display more chromosome breakage after virus infection than those of normal subjects. We have found²² nuclear membrane abnormalities in 30 patients with Down's syndrome. Membrane abnormalities may be

an underlying factor in the increased chromosome breakage, since chromosome replication is a membrane-associated phenomenon.

Viral Genome Incorporation

Hypothetical incorporation of the virus genome into the chromosome, nucleus or cytoplasm of the host cell is the second major area of importance.³⁰ Early maternal virus infection may cause non-disjunction. This may or may not be the same virus that is subsequently incorporated into the genome of the host cell. Non-disjunction may occur through persistence of the nucleolus during meiosis, or by an effect of the ovum on the nucleolus, leading to a failure of separation of half bivalents during meiosis.³¹

Clinical and laboratory evidence for this concept includes the following: Collmann and Stoller³¹ have shown that a cyclical incidence of infective hepatitis was followed 9 months later by the same cyclical pattern in the incidence of the births of children with Down's syndrome. Recent investigations have shown that the nucleoli in man are organised on the short arms of the acrocentric chromosomes in groups D and G, and this may account for the apparent non-random involvement of these autosomes in trisomy, primarily by means of 'nucleolus dysfunction'. If the trisomy in Down's syndrome is a consequence of the mechanical prevention of disjunction due to nucleolar persistence, then this immediately suggests a possible reason why virus infection could be important in relation to the aetiology of this condition, particularly if the viruses are DNA viruses that replicate within the host cell nucleus. The nucleolus is assumed to play an important part in the development of the virus. Polani *et al.*⁴³ have suggested in 1960 that the persistence of nuclear remnants might be an important factor in the development of Down's syndrome. Factors that are responsible for the dissolution of the nucleolus at mitosis or meiosis are not well understood. It has been shown that nucleoli can be made to persist at mitosis by the use of cobalt salts and that their propensity for fusion in interphase can be enhanced by treatment with thyroxin.

Heneen and Nichols³⁴ have shown that virus infection can increase persisting nucleoli during mitosis in culture. This holds chromatin together and impedes separation during anaphase, which can lead to non-disjunction and trisomy. Bjønness *et al.*⁴ have described a neonate with congenital leukaemia and a G trisomy without phenotypic manifestations of Down's syndrome. These authors postulated that the extra chromosome was of postzygotic origin due to non-disjunction or selective endoreduplication and that a mutagenic factor such as a virus could have been important in the initiation of both conditions.

PRIMARY ANTIVIRAL IMMUNODEFICIENCY AND CANCER

A relationship between experimental immunodeficiency and DNA virus-induced tumours was first reported by Vandeputte *et al.*⁵² Allison *et al.*¹ induced tumours by adenovirus in susceptible mice after using antilymphocyte serum or neonatal thymectomy to produce immunodeficiency.

McEndy *et al.*³⁷ demonstrated that thymectomy often results in a decrease of mouse leukaemogenesis. Other works suggest that intact immunological defences are important to control RNA virus-induced leukaemias and lymphomas in the mouse.^{2,13,43}

Immunodeficiency has been studied extensively in graft-versus-host (GVH) reactions in animals. Recent experiments have demonstrated that the development of GVH-induced lymphomas is associated with murine leukaemia virus activation.²⁶ In mixed leucocyte culture systems, blast formation is often associated with leukaemia virus activation.²⁷

A finding which may be important in understanding the relationship between virus immunity and cancer risk, is the localisation by Nabholz³⁹ and by Tan *et al.*⁵⁰ of the interferon-induced antiviral locus on chromosome 21. Tan *et al.*⁵¹ illustrated a gene dosage effect for this locus by demonstrating an enhanced antiviral response in trisomy 21 fibroblasts *in vitro* as compared with trisomy 13, trisomy 18 and diploid control fibroblasts. It is possibly significant that the gene which regulates the expression of the antiviral state is situated on the same chromosome which is associated with a high leukaemic risk in the trisomic state. It is not clear how the observations of enhanced antiviral response in cultured cells with trisomy 21 can be related to findings which indicate abnormal virus immunity in Down's syndrome. Knowledge of the effect of regulator genes on separate chromosomes and of the factors which could influence these regulator genes would be important in this context.

Evidence of disturbed virus immunity in Down's syndrome includes increased susceptibility to *in vitro* transformation after exposure to oncogenic SV40 virus.³¹ It is interesting that monosomy of G-group chromosomes in primary human cells transformed by SV40 virus has been reported by Shein and Enders³⁷ in an early study. Possibly the gene responsible for antiviral activity could exhibit less functional activity in monosomy than when it displays a gene dosage effect in trisomy. We have shown that 46% of reported patients with the 21 deletion syndrome suffered from repeated infections.²¹ Yamada and Furusawa⁵⁴ have found an unusually high figure of involvement of the No. 21 chromosome in 8 patients with leukaemia; in 5 of these, monosomy for No. 21 was present.

Other important findings are the depressed reactivity to skin test antigens *in vivo*,⁴⁵ impaired PHA response,⁸ and viral-like inclusions found in the blasts of a patient with Down's syndrome and leukaemia.⁷ Such a virus may be of aetiological importance or it may simply represent a passenger unrelated to the leukaemic process. Levin *et al.*³⁵ substantiated previous reports of a deficient T-cell system in individuals with Down's syndrome. They found the T-cell population in children with Down's syndrome to be only 60% of that in normal children. The infections seen were not of the type usually found in children with grossly deficient T-cell systems and thymic aplasia, such as moniliasis or progressive vaccinia, but rather the common bacterial and viral respiratory infections. In individuals with Down's syndrome who develop leukaemia it may be that transformation frequency of target cells is of such magnitude that other immunological surveillance mecha-

nisms (T- and B-cell systems) may be inadequate to prevent malignancy. On the other hand, whether it is a transient or a permanent phenomenon, all children with Down's syndrome may not exhibit the same degree of cellular deficiency.

A high prevalence of Australian antigenaemia in individuals with Down's syndrome has been established. Sutnick *et al.*⁴⁹ found 2 groups of patients with Down's syndrome with greater and lesser degrees of cellular immunodeficiency. The former may be more prone to become persistent HBsAg carriers (and to develop leukaemia?). Campion and Wangel¹⁰ attempted to define the relative importance of HBsAg carrier status and cell-mediated immune response in Down's syndrome. They demonstrated an association, but the correlation was not complete and it was thought unlikely that defective cell-mediated immunity could be the only reason for the HBsAg carrier state. Geographical factors, age and the size of the institution seem to influence the prevalence of HBsAg among groups of people with Down's syndrome.

An increased prevalence of antithyroid⁴⁸ and smooth muscle auto-antibodies¹⁰ has been found in the serum of individuals with Down's syndrome. According to Schaller⁴⁹ one could postulate that viruses play a major role in the aetiology and pathogenesis of human auto-immune diseases. Viruses appear to be capable of exerting significant effects on the host immune system, including depression of T-cell function and stimulation of B-cell function. Individuals with some underlying immune deficiency might be predisposed to harbour the viruses in the first place. This concept appears to fit in very well with what is known about auto-antibodies, depressed T-cell function and antiviral immunodeficiency in Down's syndrome.

CANCER RISK IN DOWN'S SYNDROME

Three surveillance mechanisms seem to protect normal individuals from cancer, according to a scheme proposed by Kersey and Good.³²

Target cells must be relatively resistant to transformation by exogenous or endogenous agents. The increased susceptibility to oncogenic SV40 virus transformation indicates an intracellular or first-component defect in patients with Down's syndrome.

Both cellular and humoral mediated immune responses are important in the subsequent control of a transformed cell. In addition to the deficiency of T cells, which has been mentioned, B-cell defects also exist in Down's syndrome. These second-component defects would allow uncontrolled proliferation of transformed target cells. Variation in the degree of T- and B-cell defects may partly explain why only some children with Down's syndrome develop leukaemia. Chronic antigenic stimulation, lack of immunoregulatory feedback mechanisms and undefined graft-versus-host reactions also feature in the scheme of Kersey and Good. The significance of these factors in Down's syndrome is unknown.

CONCLUSION

Present knowledge of genetics, immunology, virology, haematology and cytology makes it possible to construct a

unifying concept from the different factors which may cause leukaemia in individuals with Down's syndrome (Fig. 1).

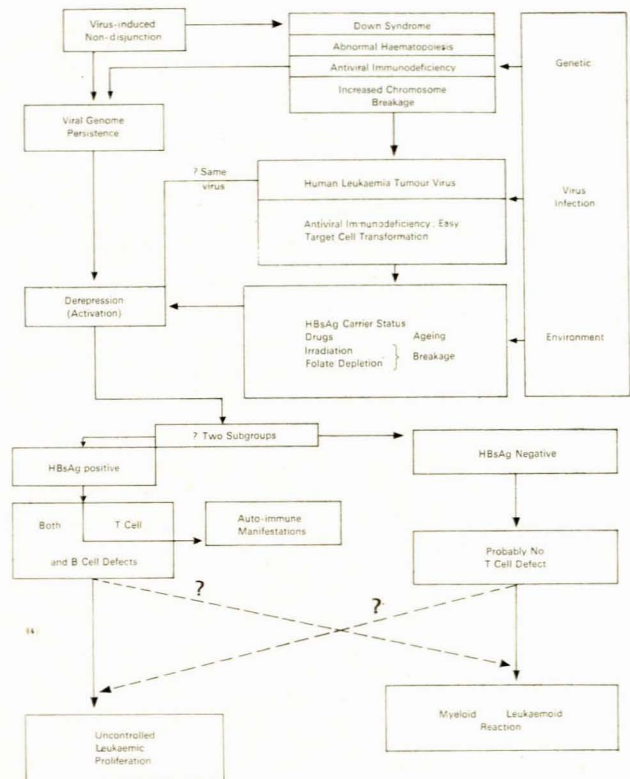


Fig. 1. Proposed multifactorial concept of leukaemogenesis in Down's syndrome.

We wish to repeat the idea expressed by Burkitt⁹ that cancer research is '... sometimes too "blinkered" in that it often tends to be focused only on malignant lesions to the exclusion of other conditions, the investigation of which might point the way to a better understanding of certain forms of cancer'.

In view of the special circumstances associated with Down's syndrome, the individuals with this condition provide a suitable model for the investigation of the complex interaction between genetics, viruses and the environment.

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