Immunogenetics and the aetiology of childhood leukaemia

Cancer in children accounts for only 0.5% of all human neoplasms, but it is responsible for about 10% of deaths before the age of 15 years. The single most common childhood cancer is acute leukaemia which comprises around 30% of all cases. Each year approximately 400 children develop acute leukaemia in the UK, the risk being spread fairly equally over the increasingly diverse ethnic mix in the UK childhood population. Although most leukaemias occur in the 1–9 year age group, there is a well-defined peak between 2 and 5 years which consists mainly of the common form of pre-B acute lymphoblastic leukaemia (c-ALL). In 10% of childhood leukaemia that occurs in infants under 1 year of age, the outlook is poor. In older children, including those with c-ALL, survival overall is now quite good, though prognosis depends on leukaemia subtype.

Against the background of progressive refinements in the diagnosis and treatment, the cause or causes of childhood leukaemia remain unknown. However, recent thinking has been strongly influenced by evidence that most adult cancers are caused by environmental agents. Since the number of haemopoietic progenitors and their sensitivity to environmental damage probably exceeds that in adults, the notion of an environmental causation in childhood leukaemia seems perfectly reasonable. However, the rarity and diagnostic heterogeneity of childhood leukaemia has made it difficult to undertake the large scale epidemiological studies required to pinpoint the role of environmental factors. Much of the support for an environmental aetiology has therefore had to rely on anecdotal cases of acute exposure and clusters of leukaemia associated with suspected but not proved hazards. In the majority of cases of childhood leukaemia, no evidence of an environmental cause has yet been found.

Despite this uncertainty, speculation and unsubstantiated hypotheses about the role of environmental factors such as ionising radiation have fuelled public anxiety about the causes of childhood leukaemia. Some families have found it necessary to resort to litigation in an attempt to obtain damages from the nuclear industry who they suspect to be responsible. In this climate of apprehension, it is clear that matters will not be settled until the aetiology of childhood leukaemia is clearly understood. This issue is of such national concern that the cancer charities and the nuclear industry in the UK are sponsoring a comprehensive nationwide epidemiological study that is designed to find the causes of childhood cancer and leukaemia.

The UK Childhood Cancer Study (UKCCS) has now been in progress for two years, and will continue for a further three years. It is described in more detail later, but one aspect is mentioned here. This is the hypothesis that the genetic background of a child contributes to the risk of developing leukaemia. Heredity is known to be more important in the aetiology of childhood than in adult cancer, and some children with rare inherited or congenital disorders causing genetic instability are predisposed to the development of leukaemia. However, it is not yet clear whether hereditary factors contribute to 'sporadic' childhood leukaemia.

The development of molecular genetic techniques now makes it possible to analyse the role of heredity in non-mendelian disorders such as leukaemia. By using these methods to identify the genes which increase the risk of leukaemia, it may soon be possible to define the role of environmental factors with more certainty than at present.

In this article I shall consider some of the background to recent attempts to determine the aetiology of childhood leukaemia. I shall discuss evidence that seems to suggest that childhood leukaemia has an infectious aetiology, and how molecular genetics may help to resolve this question.

Epidemiology and aetiology

In the mid-1800s infant mortality, mainly from infectious diseases, was about 40% of live births and leukaemia as a separate disease entity was treated with some degree of scepticism. As deaths from infections such as pneumonia declined in the early 1900s, mortality from childhood leukaemia appeared to increase, giving rise to the idea that reduced resistance to infections and an increased risk of leukaemia were in some way associated.

Although this theory has been disputed it suggests that children susceptible to fatal infection a century ago might in some way resemble those now at greatest risk of developing leukaemia. As there is ample evidence that susceptibility to infection is genetically determined, it is pertinent to inquire whether the same and possibly additional genes that predispose to infection also determine the risk of developing leukaemia.

Improvements in chemotherapy in the 1960s resulted in a dramatic improvement in survival in childhood leukaemia. Remission rates approaching 90%, and disease free survivals of 50% after five years are now considered remarkable. However, this achievement has not been
without problems. Children cured of cancer are now presenting with late effects of treatment, including second malignancies. Interest has therefore been turning to the factors responsible for changes in the incidence of the disease, both recently and in the past. The mortality rate in childhood leukemia was increasing by 4-5% per year in children under 10 years between 1911 and 195919 until mortality became a less reliable index of incidence because more children were surviving.20 However, incidence figures show that childhood leukemia continued to increase up to 1.5-fold in boys between 0-4 years in the UK between 1968-7631 and in the USA between 1935-79.22

Much of this increase can be attributed to ALL, which comprises about 80% of childhood leukaemias. It remains to be determined whether the phenotypic and genetic heterogeneity of ALL revealed by immunophenotyping23 and high resolution cytogenetics24 is a reflection of different interactions between heredity and environment. In disorders of genetic stability such as ataxia-telangiectasia, Fanconi's anaemia, Bloom's syndrome, and Down's syndrome25 the constitutional genetic contribution appears to determine within limits, the type of leukemia that develops. Moreover, in rare cases of familial leukemia, there is often concordance for type suggesting a role for hereditary factors.

It is unlikely that variations in age standardised rates of childhood leukemia worldwide (11-59/million)26 are due solely to differences in geographical (environmental) location. Some contribution from ethnic (hereditary) background must be involved, though this is presumably modified by socioeconomic factors,27,28 parental occupation,29 and 'lifestyle' factors. It is not difficult to imagine that the genetic makeup of a child is important in influencing responses to these multiple environmental factors.

The change in the incidence of childhood leukemia this century in the developed Western countries has parallels with trends in other diseases. Although this has been attributed to changing lifestyles, particularly diet,30 the major area of concern in childhood leukemia is the increasing use and exposure to DNA damaging agents. These agents are seen generally as having the potential for causing an increase in cancer and genetic disorders.31 However, direct evidence that environmental hazards are the cause of the increased incidence of childhood leukemia is remarkably limited.32 None the less, this issue continues to dominate public concerns, and none more so than the role of ionising radiation. Paradoxically, the concern may reflect the fact that ionising radiation exposure is more comprehensively monitored in the population than other important hazards such as infection.33

The Seascale cluster
This situation is vividly illustrated by the Seascale leukemia cluster. Epidemiologists searching for environmental clues have focused specifically on leukemia clusters and, not surprisingly, the Seascale leukemia cluster has received much attention because of its proximity to the Sellafield (formerly Windscale) nuclear plant. The Seascale cluster first came to light in the 1983 Yorkshire Television programme Windscale: the nuclear laundry. This identified a 10-fold excess of childhood leukemia in the village of Seascale in Cumbria, which was confirmed in several subsequent studies.34 However, levels of radioactive emission from Sellafield appeared to be less than predicted to cause leukemia35 and in any case plausible alternative explanations for the Seascale cluster were proposed.36

When the Seascale cases were reappraised in the context of paternal occupations,37 an association was found with paternal preconceptional exposure to relatively high levels of radiation in Sellafield workers. This was interpreted as suggesting that damage induced by ionising radiation in paternal germ cells (the 'Gardner hypothesis') could cause childhood leukemia. A vigorous public and scientific debate on this issue ensued,38,39 though this has had as much as much as the future of the nuclear industry and radiation protection issues, as with the aetiology of childhood leukemia.

Although there is still a lack of direct evidence to support the Gardner hypothesis, there is one important genetic implication of this speculation. If childhood leukemia can indeed be caused by germinal mutation, then such mutations almost certainly occur naturally. If they do, they ought to be responsible for a proportion of 'sporadic' childhood leukemias. Until this can be verified by direct molecular analysis, germinal mutation as an aetiological factor in childhood leukemia will remain firmly in the realm of speculation.

The UKCCS
To any observer concerned with preventive approaches it must be clear that there are so many contentious issues about the causes of childhood leukemia that only a concerted effort will resolve the problems. For this reason the UKCCS has been set up to examine the aetiology of childhood cancer and leukemia in the UK. The study is being carried out by a group of research workers under the chairmanship of the eminent cancer epidemiologist Sir Richard Doll, with funding provided by the UK Coordinating Committee on Cancer Research.

The UKCCS is a case-control study that aims to identify all cases of childhood cancer and leukemia in the UK over a five year period. It will test five hypotheses concerning histories of exposure in children who develop cancer and leukemia, and their parents, to a variety of actual or suspected DNA damaging, cancer-causing agents. These include ionising radiation including radon gas, electromagnetic fields, infections, genotoxic chemicals, occupational hazards, and lifestyle factors. In terms of its size, complexity, and objectives, the UKCCS is unique. What makes it additionally important is the systematic collection of case and family blood samples that will enable detailed studies of diagnostic subtypes, infections, agents, and susceptibility genes to be carried out. With DNA samples from perhaps 1000 children with leukemia, the molecular analysis of susceptibility genes will have the statistical power lacking in smaller studies.

The Gravess hypothesis
An important aspect of the UKCCS will be a careful re-examination of the role of infection in childhood leukemia. Infection has long been suspected to play a part in the causation of childhood leukemia, but this has been particularly difficult to prove. One reason is that no specific agent has been implicated; another is the high frequency of infectious complications at presentation as a result of generalised immunodeficiency in children with leukemia.

Recently, Gravess has taken a new look at the role of infection in ALL.17 He suggests that the cause of childhood ALL lies in the unique developmental biology of the lymphoid system, and specifically in the process of immunoglobulin gene diversification which is required to synthesise antibodies to deal with foreign pathogens. The immunoglobulin genes in normal pre-B lymphoid cells go through an ordered process of somatic recombination which results in the juxtaposition of V(D)J gene segments

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to form an antibody (V gene) coding sequence. This is hypermutated to give the high level sequence variation required to produce antibodies. Greaves proposes that an error in this recombination mechanism could occasionally lead to a premalignant phenotype. This probably involves one of the enzymes responsible for cutting, splicing, repairing, or hypermutating immunoglobulin genes. Whether these mutations are spontaneous, as originally envisaged by Greaves and Chan, or inducible by environmental factors, as seems possible, remains to be determined. However, it is fairly certain that the enzymes responsible are active at a specific stage of fetal lymphoid development, probably in utero. Any DNA damaging agent which is capable of causing mutations would therefore need to gain access to the fetus, and in this situation materno-fetal relations via the placenta could be a crucial factor in childhood leukaemia.

Greaves also envisages that progression to neoplasia requires at least one further mutation. In order to explain the sharp age focused peak of childhood ALL, he suggests that postnatal infection causes proliferative stress which exerts the clone of preneoplastic, self renewing progenitor B cells, and thereby increases the chance of second and subsequent mutations progressing to neoplasia. It is an intriguing possibility that the first mutation may occur in a gene which itself is responsible for maintaining genomic stability, and that loss of this stability may actually promote the development of further mutations in pre-B cells under the stress of proliferation.

The main difficulty with the Greaves hypothesis is that the infectious agent has not yet been identified. As Greaves points out, this need not be specific as long as it induces the required proliferative response. Although there has been speculation about viral involvement in childhood leukaemia, it is difficult to see how a single type of virus could bring about the massive expansion of a neoplastic pre-B cell clone required to generate additional mutations. Not surprisingly, attempts to attribute childhood leukaemia to specific virus infection have so far come to nothing, and associations between childhood leukaemia and infection have not been convincing.

None the less, there is epidemiological support from Kinlen and colleagues for an infectious aetiology through the increased frequency of childhood leukaemia in new towns, and in association with population mixing and commingling. This seems to suggest that patterns of exposure, transmission, and pre-existing immunity are important. This makes it possible that there are plausible alternatives which would repay careful study. One of these is the class of antigens which can directly activate T cells, the so-called bacterial (or viral) superantigens. These are exemplified, for instance, by staphylococcal enterotoxin and could be polyclonally mitogenic for premalignant pre-B cells. It would be instructive to carry out a study of infectious complications in infants to search for situations where superantigens gain access to the immune system. A role for superantigens derived from enteric infection in childhood leukaemia has interesting similarities with the dietary aetiology of other 'modern' diseases.

Genetic susceptibility to infection
As already suggested, it is possible to envisage that a child most at risk of developing leukaemia is one who inherits gene(s) which confer increased susceptibility to infection. Although susceptibility genes are neither sufficient nor necessary to cause disease, they may contribute significantly to increasing the risk. Susceptibility genes may be the normal allelic variants of genes involved in resistance to infection or they may be autosomal recessive, asymptomatic gene mutations: the A–T mutation is an example.

The risk of developing leukaemia may involve a number of susceptibility genes and exposure to a particular environmental agent at a specific stage of development. The risk of leukaemia may be determined semiquantitatively by a 'threshold' with variable contributions from genetic and environmental factors. For instance, a child carrying one copy of the A–T mutation has in theory a lower threshold to leukaemia than a child who is not a carrier.

In the Greaves hypothesis, infection acts as a promoter rather than initiator of leukaemogenic mutations. This contrasts with the view of viral leukaemogenesis in which the virus induces transformation by insertion mutation. Haemopoietic progenitors are particularly susceptible to virus infection, and it would seem reasonable that T cell surveillance has evolved to protect them against viral oncogenesis. Children who develop leukaemia might thus be expected to have an immune system which is in some way defective in the recognition of a virus infection. The HLA genes are involved in controlling immune responses to virus infection through their highly polymorphic class I and II (HLA-1, HLA-2) gene products which bind and present processed viral peptides to T cells. The response of T cells to these peptides is to recognise and destroy any cell carrying the virus. However, the efficiency of viral peptide binding depends on the sequence of a given HLA allele, and this could, in the context of infant exposure to a potentially leukaemogenic virus, result in the virus escaping from protective immunity.

There is certainly evidence that virally induced leukaemia in man and mouse is influenced by HLA (H-2) type, but early studies of HLA in human acute leukaemia yielded disappointing results. However, the design problems encountered in these studies have now been overcome and HLA typing techniques have been refined to the point where most HLA alleles can be detected at the DNA sequence level. Moreover, structural predictions can be made about the possible effect of a given allelic type on peptide binding (and hence immune recognition). Therefore, associations which were originally found between ALL and HLA-C or DP locus alleles can now be tested using high resolution analysis. This approach will be used in the UKCCS to identify children with leukaemia who carry the same HLA allele more frequently than controls. Results of this pilot study have shown that children with common ALL are at least twice as likely to have the HLA-DPB1*0201 allele as unaffected children (G M Taylor, manuscript in preparation). The aim in future will be to establish why this or other HLA alleles confer increased susceptibility to childhood leukaemia by determining how these alleles interact with infectious agents. Using this 'reverse immunogenetic' approach it may even be possible to pinpoint the type of agent involved.

Concluding remarks
The development of preventive measures in childhood leukaemia is unlikely to be a realistic goal until the causes are known. Preventive strategies that depend on the monitoring of exposures to environmental agents will be prohibitively expensive unless the agent concerned and those at highest risk can be identified.

The role of the environment in the aetiology of childhood leukaemia has to be taken seriously as long as uncertainties about ionising radiation and other agents persist. Studies of the mechanisms which initiate genetic change leading to leukaemia will be a productive area of research.
research if they are able to define the somatic (stem cell) and germinal (inherited) contributions to childhood leukemia. The application of molecular genetics to detect genes common to children who develop leukemia may help to identify those most at risk and also pinpoint the causative agent(s). For this the UKCCS will be crucial both in obtaining samples and providing precise diagnostic and epidemiological background data for the analysis of genetic susceptibility.

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I thank the Kay Kendall Leukaemia Fund, Leukaemia Research Fund, and the NHSE for supporting this work, and numerous clinical and scientific colleagues for advice and suggestions. The views and opinions expressed in this article are my own, and should not be construed as the official policy of the UKCCS or any funding body.

Addendum

Shortly after the completion of this article, judgment was given in the High Court in a legal action brought on behalf of the families of two children whose fathers had worked at Sellafield, against British Nuclear Fuels plc (BNFL), the operators of the plant. One of the children died at the age of 11 months with ALL, and the other developed non-Hodgkin's lymphoma at the age of 23 years that was successfully treated. The action was brought in the context of the study by Gardner and colleagues that the excess of childhood leukemia among the residents of Seascale was due to high levels of paternal preconception irradiation.37

The fathers of both children cited in the High Court case had been exposed to high levels of paternal preconception irradiation before the conception of their affected children, and the plaintiffs sought to show that the two children had developed their lymphoid malignancies as a result of this. Neither family was resident in Seascale during this relevant period and the two affected children were not part of the Seascale cluster studied by Gardner et al.

In many ways the case is unique, as much reliance was placed on the scientific evidence and plausibility of the theory of paternal preconception irradiation. For this reason expert scientific testimony was required, and distinguished scientists gave evidence either for the plaintiffs or defendants on matters of the epidemiology, diagnosis, and aetiology of childhood leukemia, one of the authors was one of three outside the study concerned genetic aspects of leukemia, and specifically whether leukemia or lymphoma can occur as an inherited disease in the absence of other genetic abnormalities (non-syndrome leukemia).

Such evidence would of course lend weight to the notion that paternal preconception irradiation alone, or operating with a cofactor such as a virus, could give rise to leukemia in the offspring of an exposed parent. While allowing that there may be undiscovered mechanisms of hereditary involvement in leukemia, the judge was not convinced on the balance of probabilities that the two cases before him succumbed to leukemia and lymphoma as a result of paternal preconception irradiation incurred during work at Sellafield, and he found in favour of the defendants, BNFL.

Subsequent to the High Court judgment a paper by Parker et al cited in the trial at the prepublication stage has appeared.47 This deals with the distribution of Sellafield workers with paternal preconception irradiation doses in the same range as those associated with the Seascale leukemia cluster, and shows convincingly that there are too few cases born to fathers living outside Seascale to confirm the paternal preconception irradiation hypothesis.

The second publication is the report of a study by the Health and Safety Executive (HSE) into the occupational histories of the fathers cited in the study of Gardner et al and a retrospective case-control study of childhood cancer among the children of Sellafield workers.48 The HSE concludes that there is indeed an increased risk of leukemia in the children of Sellafield workers resident in Seascale compared with elsewhere, but that the association with paternal preconception irradiation based on cumulative dose records is weak for the workforce as a whole, and heavily biased by the Seascale cases. Taken with the paper by Parker et al,47 the Seascale cluster appears to be unique. Either it is due to paternal preconception irradiation and some unidentified confounding factor confined to Seascale, or that it is due to chance.

In bearing mind that case clustering can be heavily influenced by the genetic background of the resident population, there is little justification for investigating the role of heredity in leukemia on a population basis. It is worth reflecting that in the 30 or so years over which the nine Seascale cases appeared49 as many as 16 500 cases of childhood leukemia and lymphoma, most of unknown aetiology, have occurred in the UK.

The concerted national effort currently being put into the UKCCS may yield important results which could provide insights into the cause of the Sellafield cluster.

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Arch Dis Child 1994 70: 77-81
doi: 10.1136/adc.70.2.77

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