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Hot Topic

Aetiology of childhood leukaemia

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SUMMARY

The acute leukaemias account for about 30% of all malignancy seen in childhood across the Western world. A peak incidence of precursor B cell ALL has emerged as socio-economic conditions have improved in countries worldwide. From twin studies and the use of neonatal blood spots it has been possible to back track the first initiating genetic events within critical haemopoietic cells to foetal development in utero for most precursor B cell ALL and some cases of AML. These events may occur as part of normal foetal development. Whether other factors (environmental or constitutional) are involved to increase the chance of these first genetic changes happening is unclear. For some leukaemias (e.g. infant MLL positive ALL) the first event appears adequate to create a malignant clone but for the majority of ALL and AML further 'genetic' changes are required, probably postnatal. Many environmental factors have been proposed as causative for leukaemia but only ionising irradiation and certain chemicals, e.g. benzene and cytotoxics (alkylators and topoisomerase II inhibitors) have been confirmed and then principally for acute myeloid leukaemia. It appears increasingly likely that delayed, dysregulated responses to 'common' infectious agents play a major part in the conversion of pre-leukaemic clones into overt precursor B cell ALL, the most common form of childhood leukaemia. Constitutional polymorphic allelic variants in immune response genes (especially the HLA Class II proteins) and cytokines may play a role in determining the type of immune response.

High penetrance germ-line mutations are involved in only about 5% of childhood leukaemias (more in AML than ALL). There is little evidence to support any role of viral transformation in causation, unlike in animals. Other environmental factors for which some evidence exists include non-ionising electromagnetic radiation and electric fields, although their mode of action in leukaemogenesis remains unclear. There is no single cause for childhood leukaemia and for most individuals a combination of factors appears to be necessary; all involving gene–environment interactions. To date few clear preventative measures have emerged, except the complete avoidance of first trimester X-rays in pregnancy; a healthy diet with adequate oral folic acid intake both preconception and early in pregnancy; and the early exposure of children to other children outside the home to facilitate stimulation and maturation of the natural immune system. Here then are clear echoes of the "hygiene hypothesis" regarding the initiation of allergies, autoimmune disease and type I diabetes mellitus in children and young people.

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Introduction

The diagnosis of leukaemia has become increasingly more precise with morphology supplemented by immunological, cytogenetic and molecular markers of disease. Recently sophisticated gene expression profiling has enabled definition of subtypes with favourable and unfavourable prognosis. All of these methods demonstrate that childhood leukaemia is not a single homogeneous disease and that both acute lymphoblastic (ALL) and myeloid leu-

kaemia (AML) have definable subtypes with their own characteristics and potentially different aetiologies.^{1,2}

Within leukaemic cells, chromosomal translocations and the creation of fusion genes; deletions of chromosomal segments or of individual genes; duplication of segments or whole chromosomes; all define specific subtypes of leukaemia. Identification of fusion genes within malignant clones has enabled researchers investigating twin pregnancies and using neonatal blood spots to show that the initiating events for leukaemia (at least for most childhood ALL and some AML) occur during foetal development in utero.^{3–7} Such first events may inevitably lead to overt leukaemia as seen in infant leukaemia, but more often further "events" are required in order to convert a pre-leukaemic clone into overt leukaemia. It would appear that the developments of such translocations are quite frequently generated within normal foetal development.⁸

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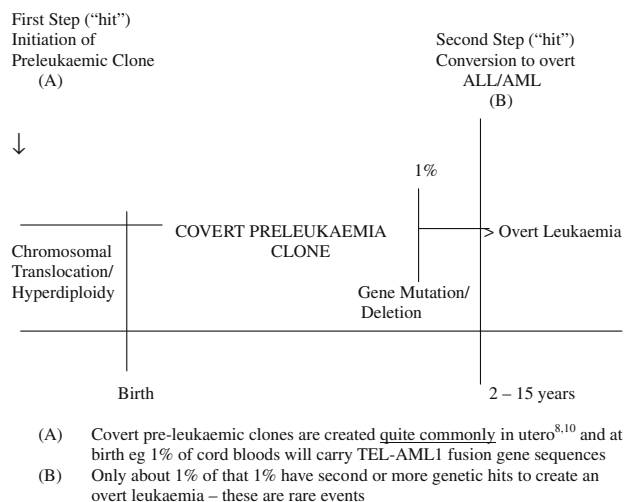


Fig. 1. A model illustrating the events required to produce overt leukaemia in childhood. Modified from Greaves MF. *Nat Rev Cancer* 2006;(6):193–203.

In precursor B cell ALL with TEL-AML fusion gene the second event leading onto overt leukaemia is often deletion of the other TEL gene. However, in leukaemic cells there are very frequently a series of genetic rearrangements, mutations, etc. which may influence cell survival and sensitivity to drug therapy. These may confuse the picture as to what changes are critical for development of the leukaemia.⁹

It is quite clear that no single cause for all cases and/or subtypes of childhood leukaemia exists.¹⁰ It is most likely that for any individual child who develops leukaemia, it occurs as a result of a combination of his/her constitutional genome plus the exposure to potential genotoxics and the ability to metabolise toxins or eradicate them and of course repair DNA damage appropriately. There is growing evidence that response to infection may play a critical role in the development of overt leukaemia. Excess cellular proliferation in response to infection may trigger the further genetic events.

Fig. 1 shows the model which we now accept is most likely to represent what happens in the progression from an initiating event through to overt leukaemia. For any implicated causative factor it is important for researchers to be clear as to what stage they feel the effect may be occurring.

Epidemiology of childhood leukaemia

Incidence

The epidemiology of cancer in childhood is well covered elsewhere in this volume but there are some specific features especially of acute lymphoblastic leukaemia which have provided clues to causation. As seen in Table 1¹¹ leukaemia incidence, especially ALL, shows a trend for lower rates in less socio-economically developed countries than in resource rich nations and even amongst different ethnic groups within countries. Some of these differences have been explained by the lack of true population based cancer registries in many countries (affecting ascertainment of cases), or the lack of medical resources and diagnostic facilities leading to diagnostic failure. Though these may be contributory factors, the higher rates of lymphoma seen in many countries with low leukaemia rates (e.g. Burkitt's lymphoma in sub Saharan Africa) suggest that there is a genuine worldwide variation in ALL incidences. The emergence of a peak incidence of ALL in those

Table 1
Incidence of childhood leukaemia – international comparisons^a (age standardised rates per million).

Registry	Leukaemia	ALL	Lymphomas
Denmark (1983–1991)	53	42.8	11.8
Australia (1982–1991)	49.9	39.9	13.3
Sweden (1983–1989)	48.7	40.1	12.9
USA SEER ^b			
White (1988–1992)	46.9	38	15.1
Black (1983–1992)	29.4	20.8	10.6
England and Wales (1981–1990)	40.8	32.8	11.2
Cali Colombia (1982–1992)	42.8	31.5	23.3
Japan (1980–1992)	38.5	22.6	10.6
Estonia (1980–1989)	37	19.9	14.8
Israel Jews (1980–1989)	25.7	18.6	20.9
Non Jews (1980–1989)	27.8	16.2	28.7
Bulgaria (1980–1989)	33.2	18.5	18.4
India Bombay (1980–1992)	25.4	16.0	11
Ibadan Nigeria (1985–1992)	8.3	2.6	27.1

^a Modified from Parkin DH, Kramarova GJ, Draper G, et al., editors. International incidence of childhood cancer, vol. II. Lyon: IARC Scientific Publications No. 144; 1998.

^b SEER – North American 'Surveillance Epidemiology and End Results' Program.

aged 2–6 years (median 4 years) with a somewhat higher male to female ratio (1.2:1) has been mapped in countries where socio-economic conditions have been improved.^{11,12}

For childhood acute myeloid leukaemia the sex ratio is close to parity, and rates are highest in infants. There is also some ethnic variation but nowhere near as marked as seen with ALL.

Incidence trends/clustering

Exploring temporal trends in incidence, space time clustering (the irregular grouping of cases of a disease simultaneously in space and time), and spatial clustering (irregular distribution of cases in space) have yielded interesting data for ALL. McNally and Eden¹³ reviewed that data which show a continuing significant increase of about 1% per year for precursor B cell ALL in resource rich countries.

Two European-wide studies showed space time clustering for leukaemia based on time and place of diagnosis and two (UK and Sweden) for time and place of birth and 1 UK study showed both.¹³ Little¹² had previously identified evidence for space time clustering for leukaemia in 13 studies but not in another 15. The significance of clustering related to place of birth implies an early environmental exposure (potentially in utero) while those related to place of diagnosis imply a later environmental exposure closer to diagnosis. Fig. 1 shows the relevance of these time points regarding any initiation of a pre-leukaemic clone in utero or a later postnatal events leading to overt leukaemia. In ALL, space time clustering around the time of diagnosis might be more relevant,¹⁴ if initial events do truly occur spontaneously.

Significant evidence of spatial clustering for leukaemia has been presented from studies in Europe, Hong Kong, Greece, New Zealand (in older children) but not from the USA or Sweden.^{12,13,15} Positive studies indicate potential involvement of environmental factors in causation. This data does not relate to small locality clustering which is more difficult to evaluate (see below).¹⁵

Seasonal variation

Seasonal variation in incidence has been reported in 9 studies and not in a further 2 as reviewed by McNally and Eden.¹³ Westerbeek et al. reported such variation for ALL but also Hodgkin lymphoma.¹⁶

All of this data provides some modest evidence for environmental factors influencing progression of a pre-leukaemic clone into overt leukaemia.

Ecological studies

Ecological studies have investigated variations in population mixing and density, social isolation and socio-economic status/deprivation. Kinlen¹⁷ proposed that the excess of childhood leukaemia and non-Hodgkin lymphoma reported in the communities around the villages of Seascale and Thurso in the UK (the sites of two nuclear processing plants) could not be due to the recorded exposure levels of radiation but rather were due to unusual population mixing from incoming workers/families in these previously quite isolated communities. He compared incidence in these two sites with a 'rural' new town in Glenrothes Scotland and found a similar, albeit transient, 3-fold rise there at the time of the influx of a substantial number of newcomers. He speculated that such clusters could be due to an unusual outcome of a common but relatively non-pathological infection arising in non-immune individuals. The extreme population mixing in previously isolated communities would make this possible. Kinlen and co-workers have followed up with a series of studies where unusual population mixing has occurred^{18–22} with similar results.

The measurement of less extreme population mixing is fraught with methodological difficulties and very variable results obtained. Childhood ALL was shown to be related to population mixing at a county district level by Stiller and Boyle²³ and by small census ward areas specifically for those aged 1–4 years by Stiller et al.²⁴ but not for younger or older children. This paper reported a lower incidence for peak ALL in more deprived areas of England and Wales.²⁴ They were able to study, within small geographical areas, the diversity of origin of incomers from national census data. Kinlen's studies had demonstrated the effects of major migration of children as well as adults whilst in Stiller et al.'s²⁴ study ALL incidence was highest where there was least diversity of origin of child migrants. In such areas the child population may tend to have impaired herd immunity through low exposure rates to child–child transmitted infections. In contrast the UK Childhood Cancer Study (1991–1996) which recruited 3838 children with cancer, 1736 with leukaemia and 7629 unaffected children²⁵ reported an increased risk of ALL with low diversity of origins of migrants in England, Scotland and Wales.²⁶ A similar finding had been reported by Parslow et al.²⁷ in a regional study. Adelman et al.²⁸ studying children with leukaemia aged 0–4 years in 200 counties across the United States and Hawaii showed an increased incidence of boys but not girls in counties where more than or equal to 50% of the population relocated. There was a correlation in their study with socio-economic deprivation but only for girls. There may be methodological reasons for the variable evidence from these studies and others which have been assessed in the reviews by Little¹² and McNally and Eden.¹³

Table 2
Summary of epidemiological evidence for causation of childhood leukaemia.

- ALL especially shows worldwide variation in incidence
- A peak age incidence emerges with socio-economic development
- That ALL peak occurs between 2 and 6 years with a small male preponderance
- No similar peak is seen in AML
- Some evidence exists for space time and spatial clustering – environmental factors
- Seasonality of ALL diagnosis is reported
- Population mixing (surrogate for infections/immunity) has some impact
- Social deprivation may lower risk of developing ALL in some communities

Table 3

Major putative factors for causation of childhood leukaemia.

- Genetic predisposition
- Genetic susceptibility
- Environmental factors
 - Ionising radiation
 - Non-ionising electromagnetic fields
 - Electric fields
 - Chemicals/cytotoxics
 - Parental smoking/alcohol consumption
 - Perinatal and reproductive factors
 - Infections/response to infection

At the same time Greaves had proposed a 'delayed infection' hypothesis for childhood leukaemia,²⁹ (particularly for peak incidence precursor B cell ALL). Infection was postulated to play a crucial role in promoting, through an abnormal immune response, the crucial second or postnatal genetic error or hit. This did not imply a direct viral transformation. This hypothesis appears to fit more closely the epidemiological evidence which has accrued from recent genetic-epidemiology studies (Table 2).

Potential causative factors

Table 3 summarises factors which have been considered as potentially causative although it is very likely that most children with leukaemia require a number of such factors to come together in the development of their leukaemia

Genetic predisposition

By predisposing genes, we mean those genes with high penetrance, mutated sequences which lead to an increased familial risk of cancer. There is a high relative risk of developing malignancy within the family but a low attributable risk for the population in general. Carriage of such germ-line mutations appears to require little additional environmental contribution to cause overt cancer or leukaemia. It is estimated that for leukaemia less than 5% of cases can be attributed to such genes.^{12,30} A higher percentage of predisposition may be seen in AML than in ALL.

Pedigrees of families with germ-line TP53 mutations do include cases of leukaemia (including ALL) but they tend to be in older children/young adults. Neurofibromatosis type 1 has been associated with both acute and chronic leukaemia and lymphoma. Ataxia telangiectasia is associated with ALL and lymphoma. The underlying AT may only be identified when extreme toxicity is induced by chemotherapy or radiotherapy. Mutation in the Fanconi anaemia genes predispose to a range of congenital abnormalities, aplastic anaemia and acute myeloid leukaemia with poor prognosis. Recently greater interest in this complex of DNA damage recognition and repair genes has occurred because of the identification of FANC D1 as BRCA2. Mutations in FANC D1 lead to a range of malignancies not just leukaemia and breast cancer. Hereditary immunodeficiency also predisposes to leukaemia, more often T cell ALL but not precursor B cell ALL.

The markedly increased risk for children with Down Syndrome (DS) to develop leukaemia has long been recognised. About 1 in 95 children with DS, develop leukaemia and this is most commonly of the AML-M7 phenotype with high chemo-sensitivity and toxicity. Children with DS also have an increased risk of developing ALL.³¹ The transient abnormal myelopoiesis seen in the neonatal period is reported in about 10% of patients but most recover, 25% however proceed to develop AML subsequently.

Most parents ask whether their child's leukaemia is infectious or whether it is genetic. It is important to emphasise that the work performed in twins does not signify an overall increased incidence

in either identical or non identical twins. When one twin acquires leukaemia the other twin has been of interest/anxiety since the first case report in 1882.³² Concordant leukaemia between twins has now been reported in over 70 cases. It is difficult to be definitive about actual risk but it seems to be greatest in the first 10 years of life with often some latency between one twin developing leukaemia and the other. Both appear to carry the same first genetic event which would be extremely rare by chance. It is assumed therefore that there has been a clonal transfer between the twins of a pre-leukaemic clone. The second genetic events may be different though the leukaemia reads true to either ALL or AML in both twins. Collectively the estimated concordance rate for both ALL and AML in identical twins is between 5% and 25%, but for infant ALL it is much higher. Concordance decreases with age but in the important peak time of B cell precursor ALL between 2 and 5 years it appears to be about 15%. A few triplet pregnancies have been studied. Maia et al.³³ reported in 2001 on a triplet pregnancy of whom two were monozygotic. They shared an identical acquired TEL-AML1 genomic fusion sequence indicative of a single cell of origin in one of the fetuses followed by dissemination of the clone to the other monozygotic twin via a shared placental circulation. Using their stored neonatal blood spots it was possible to identify the presence of those fusion sequences at birth. Of interest the blood spot from the third healthy dizygotic triplet was also fusion positive but only in a single segment. There has been no evidence of subsequent fusion gene presence in blood or marrow and no leukaemia developed in him. Both affected twins had a deletion of their normal non-rearranged TEL allele. By fluorescence in situ hybridisation this change was sub clonal in both twins and of interest TEL deletions were individually distinct in the twins. This confirms that those changes were independent and secondary events probably after birth. We do not know if the sequences within the dizygotic triplet were from contamination at the time of sampling or during storage but it is also possible that there was some placental transfer of cells, subsequently rejected immunologically.³³

Genetic susceptibility

By susceptibility genes we mean polymorphic variation in low penetrance genes which influence the way each individual handles environmental exposures, metabolism of chemicals or toxins, response to DNA damage, and handling of infections. Anyone carrying such variants will rarely present with a family history of cancer and have a low relative risk themselves of acquiring it. However, there may be a high attributable risk for all cancers related to these genes. Recent developments in gene expression profiling have enabled researchers to screen for constitutional up and down regulation of a wide range of potential candidate genes.³⁴ Some examples will demonstrate the issues.

Infant leukaemia – a unique story

At least 60% of infants under 1 year with AML and over 80% with ALL have an abnormality involving the MLL gene (11q23).^{35,36} This gene is also involved in treatment-related secondary myeloid leukaemia induced by topoisomerase II inhibitors. Ross et al.³⁷ reported an association between a maternal diet rich in naturally occurring topoisomerase II inhibitors and an increased risk of infant AML in infants (not ALL). Felix et al.³⁸ reported an excess risk for development of secondary AML associated with use of epidophyllotoxins in those who did not inherit the CYP3A4-V allele of P-450 CYP3A4 gene. This allele metabolises such chemicals to quinone metabolites. They did not find such a relationship with de novo leukaemia with MLL gene abnormalities. Another enzyme NAD(P)H: quinone oxoreductase (NQO1), unlike CYP3A4-V, is expressed in fetal liver. As part of the

UKCCS study,^{25,39} 36 infants with “MLL rearranged” leukaemia (30 ALL, 6 AML) and 100 unselected cord blood controls were assayed using PCR-RFLP for a polymorphism of the enzyme (a C-T change at NT 609) with low function. The infants with leukaemia were more likely to have low function (heterozygote C-T or homozygote TT) NQO1 than controls; (odds ratio 2.5, $p = 0.015$). The odds ratio for the 24/36 who had MLL-AF4 fusion genes was 8.12, $p = .00013$. This data suggests that a detoxification weakness might play a part in the development of this specific type of leukaemia. Strick et al.⁴⁰ did demonstrate that dietary bioflavonoids (topo 2 inhibitors) can induce cleavage of the MLL gene and speculated that they might contribute to infant leukaemia. Smith et al.⁴¹ confirmed the findings by Wiemels et al. as did two other studies. Wiemels et al. also studied³⁹ 50 patients with TEL-AML1 fusion genes and 29 with hyperdiploid ALL. There was no evidence of an excess of low function NQO1 variants in either group.

The controls in this study had a 17% ‘low function’ allele frequency, comparable to the rates found in control populations in Europe and North America (13–25%). Of interest the highest rate reported has been 40% in Asian populations where there is also a higher frequency of infant leukaemia.⁴² Not only do natural topoisomerase II inhibitors cross the placenta but maternal and foetal inability to detoxify them adequately may indeed be a factor in the development of infant leukaemia. Given the relatively common population frequency of low function NQO1 alleles and the ubiquity of quinine containing substances other factors including DNA damage and repair genes may be involved in reducing or increasing the risk that any individual infant might develop MLL involved ALL or AML.

Folate metabolic pathways

Folate deficiency and alterations in folate metabolism resulting from polymorphic variants of the enzyme methylenetetrahydrofolate reductase (MTHFR) have been associated with neural tube defects, heart disease and some cancers. There are two common low function variants of MTHFR; a T variant at nucleotide 677 (MTHFR C677T) and a C variant at nucleotide 1298 (A1298C). C677T has been linked to vascular disease and neural tube defects but appears to afford some protection against colon cancer.⁴³ It is thought that this is due to increased fidelity of DNA synthesis (it increases the availability of methyl groups for the conversion of uracil to thymidine). If there is a deficiency of thymidine, excess uracil incorporation into DNA leads to strand breaks and chromosomal translocations and deletions. Low function MTHFR has previously been reported to be protective for adult ALL.⁴⁴ It was postulated that childhood leukaemia characterised by double strand DNA breaks, translocations and production of gene fusion products (e.g. precursor B cell ALL with TEL-AML1 or infant leukaemia with MLL-AF4, etc.) by means other than V(D)J recombinase would be a good model to test for such low function MTHFR variants.

Wiemels et al.⁴⁵ studied 37 infants with MLL translocations, 78 with TEL-AML1 ALL, and 138 with hyperdiploid ALL. 200 newborn healthy controls were included. A marked protective effect against development of MLL + ALL was found with carriage of the C677T variant (OR 0.36, $p = 0.017$) but not with A1298C alleles. For hyperdiploid ALL there was protection with both C677T (homozygote TT, OR 0.49, 95% CI 0.2–1.17) and A1298C (homozygote TT, OR 0.26, CI 0.07–0.81). There was no such association for TEL-AML1 ALL. Folate deficiency may play a part in some forms of ALL but each subtype has different aetiological pathways. This study requires further confirmation. Forty per cent of the population do carry two variant allelic copies of MTHFR so that the attributable risk for childhood leukaemia could potentially be quite high. There is clearly a need for adequate dietary folate and supplementation in early pregnancy and even preconception in young women. However there is some

Table 4

Summary of genetic factors.

- Germ-line mutations account for not more than 5% of childhood leukaemia (TP53, NF1, AT, etc.)
- Down Syndrome increases risk of ALL and AML considerably
- Twins do not overall carry increased risk for leukaemia but early in life there is high twin–twin concordance if one develops
- Polymorphic variants of metabolic, immune response and repair genes may play a major role (e.g. NQO1, MTHFR, HLA Class II)
- Gene and protein expression profiling is likely to identify many more susceptibility genes

evidence from animal and human studies which suggest that high levels of folate may promote tumour growth in preneoplastic lesions.^{46,47} Table 4 summarises putative genetic factors.

Environmental factors

Ionising radiation (see Table 5)

Introduction

That ionising radiation causes leukaemia is in no doubt. Evidence is based on the atomic bomb survivor studies who received up to 200 mSv in an acute dosage and of course from those fetuses who received X-rays in utero with much lower radiation dosages.⁴⁸ Currently in the UK, background ionising radiation (natural radon and gamma radiation, plus from man made sources) yields exposures of approximately 2–3 mSv per year. Not all individuals are equally exposed depending on where they live, what type of house they live in and whether they have required radiological imaging or are occupationally exposed to radiation sources. Individuals are variable in their sensitivity/resistance to the effects of radiation.

Background radiation

In the UKCCS study,²⁵ in addition to parental interviews (covering social, occupational and medical histories) household radon concentrations were measured over a 6 month period in any house in which a child with cancer or leukaemia (total 2226 cases) had lived for the time of diagnosis and for 6 months before (one detector in the bedroom, one in the main living area). Control houses (3773) were also monitored.⁴⁹ No evidence of higher radon levels were found in the houses of patients with either leukaemia or solid tumours compared with the control homes. Detailed analysis did suggest that control homes were more likely to have features such as double glazing and central heating which lead to higher radon levels and case families were more likely to have living rooms above ground level (which decreases radon levels). Analysis by standard deprivation scales did not identify these home characteristics.

In a second study component 2, thermoluminescent dosimeters (gamma detectors) were set in the case and control houses. No evidence of any significant difference in radiation levels was found between case and control homes even when adjusted for deprivation.⁵⁰

Most other studies have agreed with these findings for leukaemia although Axelson et al. reported an increased risk (odds ratio 1.4, 95% CI 1.1–1.9) from gamma radiation and leukaemia in homes built from uranium-containing shale concrete.⁵¹ Kaletsch et al.⁵² had reported a non-significant elevated risk for solid tumour risk and for gamma radiation levels. Despite much anxiety all studies which have investigated nuclear reactor accidents, weapons testing fallout and residential proximity to nuclear plants have provided little definitive evidence for direct causation of leukaemia. This includes the Chernobyl accident which did significantly increase thyroid cancer in young people in Belarus and Ukraine but not leukaemia.

Table 5

Ionising radiation.

- Ionising radiation does cause leukaemia if exposure is high and/or the host vulnerable (foetus/DNA damage recognition/repair syndromes)
- Background irradiation does not account for the vast majority of childhood leukaemia
- Parental occupational/diagnostic exposure is controversial/mechanisms are not clear
- Avoidance:
 - X-rays in first trimester
 - Care with selection of imaging in childhood

Parental occupational exposures

A population based case-control study from Germany reported that maternal exposure to radiation sources increased risk of developing lymphomas in offspring and also that fathers working in the nuclear industry had children with an elevated risk of developing leukaemia (odds ratio 1.8, 95% CI 0.71–4.58). However, this risk was not statistically significant.⁵³ Several other case-control studies have shown inconsistent results and the mechanism whereby such a risk could be transferred remains controversial, including the possibility of germ-line mutations during spermatogenesis. The evidence for leukaemia causation is not strong.

Parental diagnostic exposures

The original reports of risk to the foetus in utero have been alluded to earlier^{48,54} but thankfully the risk has been very significantly lessened by a very drastic reduction in any maternal X-rays during pregnancy especially in the first trimester. Of interest Meinert et al.⁵³ in their case-control study reported an increased risk for leukaemia (OR 1.33, 95% CI 1.10–1.61) after paternal prenatal X-ray investigations. At least three other larger studies have not found such a correlation.

Childhood diagnostic exposures

Infante-Rivard⁵⁵ reported the risk of leukaemia development to be increased with previous diagnostic X-ray investigation (more than 2) for a child and also emphasised the possibility of a relationship with DNA damage and repair mechanisms. To date other studies have not confirmed this finding. Radiation protection authorities are carefully watching exposures related to a clinical trend to increasingly use CT scanning. If alternative imaging with lower radiation risk is possible, it should be used in infancy and childhood.

Non-ionising electromagnetic fields (extremely low frequency and radio frequency)

There is no doubt that there is some effect of non-ionising electromagnetic radiation (EMF) arising from adjacency to high voltage electricity supply lines, internal household wiring configurations and from electrical appliances. At face value this would be a good candidate for an environmental effect arising from urbanisation and increasing socio-economic advances. Individual studies have produced variable results^{56,57} but in a meta-analysis of nine case-control studies performed, Ahlbom et al.⁵⁸ found no evidence of increase in leukaemia related to exposure levels under 0–4 micro-Tesla (μ T) but above these levels there was a doubling of risk. Some of the effect was considered to be related to bias in the acquisition of controls. In most case-control studies volunteer controls tend to have a higher socio-economic status than cases. 0.4 μ T is a Weak magnetic field (about 1% of the World's magnetic field which affects us all). In the UK study⁵⁷ which contributed to this review only 17 individual homes (8 cases, 9 controls) had higher exposures than 0.4 μ T were measured. Overall in the pooled review of

9 studies, 99.2% of children had exposures below 0.4 μT .⁵⁸ All of these studies measured EMF using portable meters in houses where the child had lived at diagnosis. Greenland et al.⁵⁹ conducted a similar pooled analysis of magnetic fields and the findings agreed with Ahlbom et al.⁵⁸ Researchers are trying to explain how such exposures could cause leukaemia biologically. Laboratory cell culture studies show very variable effects with inhibition and stimulation of proliferation under different circumstances. Just like irradiation the vast majority of children are not exposed to the levels of EMF shown to have such an adverse effect from any study to date. No clear and plausible biological mechanism for the effect has been yet identified.⁶⁰

One area of recent interest relates to the ever increasing use of mobile telephones (more than 3 billion users worldwide) including by very young children and of course the radio masts needed for them to function. Radio frequency (RF) EMF from handheld phones is concentrated in the part of the head closest to the handset and its antenna. This includes the temporal lobe of the brain and parotid gland. The phone masts emit radio frequency EMF also but at lower levels to the whole body. For children any exposure will be long term. However, if texting is used rather than the earpiece there may be some extremely low frequency EMF to the hands. To date, there is no clear evidence of adverse impact of phone usage in terms of leukaemia causation. There remains doubt about CNS tumorigenesis. In the past there have been similar concerns and investigation into radio and TV transmitters. Currently it is not thought that any emissions from any of these sources are linked causally to the vast majority of leukaemia cases. Methodological issues remain regarding accurate and appropriate ways to measure EMF from any source.⁶⁰

Electric fields

Feychting and Ahlbom used distance of a home from a particular power line as a surrogate for measurement of EMF, with built in calculations on load conditions, height and distance between conductor, direct current and other nearby power lines. Interest has been sparked in the effects of exposure to power frequency electric fields themselves.⁶¹ In the UKCCS study⁶² electric field measurements were made at 473 case homes (leukaemia and solid tumours) and 453 control homes (matched by age, sex, and geographical location). No evidence of any significant difference in field exposures between controls and cases for any tumour especially leukaemia was found.⁶² This was a retrospective post diagnosis study. Others have shown variable results. In 2005, Draper et al.⁶³ reported on a large cohort (29,081) of registry defined children with cancer (9700 with leukaemia) and matched controls (by sex, date of birth and domicile at birth). They used the Electricity National Grid Records in England and Wales to determine distance from homes at birth to the nearest high voltage overhead power line. They reported that there was a significant trend ($p < 0.01$) for increased risk of developing leukaemia but not other cancers in relation to the reciprocal of distance from the power line to over 600 m from the home. This was a much further distance than any previous studies had reported for any effect. Publication of the report stimulated tremendous interest and controversy regarding; methodological aspects, lack of actual measurement of electric or EMF fields and as to causation of the effect. There was general agreement that it was unlikely to be due to EMF however.⁶⁴ Alternative theories were proposed including chance⁶⁵; air pollution from vehicle traffic since power lines were usually parallel or crossed roads and train lines; herbicides used to clear around pylons; possible health effects down wind of corona ion emissions on air pollutants produced by electric fields^{66,67} and disruption of melatonin (by radiation from the power line) an anti-toxicant and natural anti-cancer agent. Draper et al.⁶³ did not propose any

biological mechanisms themselves. However, the importance of this study is that it involved assessments at birth addresses implying, if real, any effect was prior to birth and hence at potential initiation of pre-leukaemic changes and that 4% of children in England and Wales live within 600 m of such a power line.⁶⁴ However, Draper et al. did conclude that their finding could be due to chance but if real could only account for about 1% of childhood leukaemia.

Summary

EMF from any source and electric fields, if they really have an effect, would account for only a small percentage of cases, not the majority. Plausible biological mechanisms have not yet been successfully demonstrated for either. For EMF timing/mode of effect is thought to be most likely at a proliferative stage of a leukaemia clone, and at an initiating stage for electric fields. It should be stressed that neither can explain most cases of childhood leukaemia. Mobile phones appear to have some sequelae but not probably in leukaemia causation and of course as a relatively modern invention cannot explain the emergence of the peak of ALL over the last 60+ years.

Chemicals/cytotoxics

Exposures to chemicals with the potential to cause cancer can occur via the placenta in utero, e.g. naturally occurring topoisomerase II inhibitors^{36–41} or postnatally. The range of implicated carcinogenic exposures include pesticides, hydrocarbons especially benzene, solvents, metal dust, car and train exhaust fumes, industrial pollutants, wood dust, insulation materials, contaminated water and hair dye.

Meinert et al.⁶⁸ reported a small increased risk of leukaemia (OR 1.5, 95% CI 1.0–2.2), from early childhood exposure to pesticides. Shu et al.⁶⁹ reported a risk of parental exposure to hydrocarbons (OR 1.8, CI 1.3–2.5) although the mechanism whereby the damage could be transmitted remains unclear. Schuz et al.⁷⁰ reported on a number of case-control studies regarding self report of histories of parental exposures. Solvents were the principal risk identified (OR 2.00, 95% CI 1.2–3.3) although other studies have not found this or have reported a relationship only with CNS tumour formation. Benzene has long been implicated as a potential leukaemogenic agent⁷¹ and both Feychting et al.⁷² (Sweden) and Crosignani et al.⁷³ (Italy) have reported elevated relative risks and odds ratios, respectively. The relationship appears to be predominantly with AML. Of course the major source of exposure to the general population is from motor vehicle exhausts. Feychting et al.⁷² felt that their reported increase for all cancers related to very high levels of nitrogen dioxide in vehicle exhausts.

There have been two case-control studies specifically investigating contaminated water supplies. Infante-Rivard et al.⁷⁴ reported from Canada on an increased risk for leukaemia associated with high levels of trihalomethanes in a water supply. Costas et al. looked at leukaemia incidence in Woburn Massachusetts USA related to zinc contamination of drinking water.⁷⁵ In the UKCCS²⁵ no evidence has been identified from the extensive parental questionnaire of any parental occupation and any excess risk for leukaemia. Pregnancy and postnatal risks from diet and some chemicals will be discussed in a later section.

Summary

It is difficult to interpret some of the small cohort or case-control studies on individual pollutants but the evidence to date does not suggest that parental exposure or indeed early childhood

exposures to chemicals or pollutants can explain the majority of childhood leukaemias.

Parental smoking/alcohol consumption

Clearly smoking is the single most well documented and prevalent carcinogen. Could it play a part in childhood carcinogenesis? Tobacco derived products have been identified in human placenta, fetal blood, newborn urine and in the breast milk of maternal smokers. A number of case-control studies have shown weak associations between smoking during pregnancy and cancer in offspring.^{76–78} All of these studies also showed more consistent evidence (but with relative risks mostly just above one) for paternal preconceptional smoking. This is presumed to involve germ cell damage and/or mutations during spermatogenesis. Thornton and Lee⁷⁹ reviewed the evidence for both paternal and maternal smoking having a causative role in childhood cancer.

In the UKCCS study,²⁵ Pang et al.⁸⁰ analysed the self reported smoking habits of mothers and fathers of all 3838 children with cancer and 7629 control children. There was no significant evidence for parental smoking (fathers or mothers) being a risk factor for childhood leukaemia in their study. In this and other studies there may be a risk from maternal and paternal smoking for other tumour formation (e.g. NHL, CNS tumours, hepatoblastoma⁸⁰ and neuroblastoma).

Many of the studies reporting on smoking have also analysed alcohol consumption^{76,77} independently and together since smoking and alcohol consumption are clearly correlated. Infante-Rivard et al.⁸¹ showed a reduced risk of ALL in mothers consuming at least moderate alcohol in pregnancy. They did emphasise an observation of interaction between parental alcohol consumption, polymorphic variants of metabolising genes and risk of ALL. Severson et al.⁷⁶ did report a link between maternal alcohol consumption and AML.

Summary

Smoking is clearly not advised during pregnancy because of other foetal risks but the evidence of risk for offspring developing leukaemia is weak. That heavy smoking may cause germ-line mutations during spermatogenesis is an interesting observation worthy of further investigation but the evidence is not strong for a role in leukaemogenesis. The interaction between alcohol consumption and smoking preconception and during pregnancy are well documented. Whether these have an additive role in carcinogenesis is as yet unclear. If at all maternal alcohol consumption should be modest during pregnancy. Genetic susceptibility may play a role in influencing the effects of tobacco products and alcohol in carcinogenesis.

Perinatal and reproductive factors

From the time when Alice Stewart et al.⁵⁴ reported on the dangers of abdominal X-rays in pregnancy there has been considerable interest in the effects of a range of potential transplacental carcinogens alongside studies on foetal growth and development. Radiation remains the only confirmed in utero cause of childhood leukaemia.^{9,48,54} However, since the identification of at least initiation of pre-leukaemic clones in most, if not all, infant leukaemia,⁴ precursor B cell ALL,^{5,35,83} some T cell ALL⁸² and AML^{6,35} greater endeavour has been made to investigate whether any of the putative aetiological factors of irradiation, EMF/electric fields, chemicals/drugs, alcohol and tobacco, etc. could be linked to these early events. The issue of diet containing high levels of naturally occurring topoisomerase II inhibitors alongside genetically influenced metabolic deficiency has already been mentioned,^{37–42} as

has the issue of relative or absolute folate deficiency.⁴⁵ In each previous section there has been an attempt to focus on whether individual factors might be principally active at initiation or later stages of leukaemogenesis.

Some interesting additional factors were identified in the UK Childhood Cancer Control Study⁸⁴ when previously recorded maternal and obstetric histories were analysed for 1485 case families (child with haematological malignancy) and 4864 control families. Associations were identified for severe hyperemesis (OR 3.6, 95% CI 1.3–10.1) with all leukaemias; polyhydramnios with myeloid leukaemia (OR 4.0, 95% CI 1.5–10.3) and maternal anaemia (Hb < 10 g) and pre-eclampsia with non-Hodgkin lymphoma (OR 2.6 and 1.7, respectively). Babies who developed leukaemia and their older siblings had heavier mean birth weights. Mothers of a child with B cell precursor ALL were more likely to have had a previous molar (hydatidiform mole) pregnancy (OR 5.2, 95% CI 1.9–14.7). Naturally this study also demonstrated the expected excess of AML and ALL in children with Down Syndrome.

As yet we do not know what these findings really mean and how they correlate with other previously observed potential factors including older maternal age at conception, nulliparity, history of previous foetal death and low fertility. However, many studies have been equivocal for these connections and even contradictory. The UKCCS did not find an effect of older maternal age, number of previous pregnancies or live births for leukaemia.⁸⁴ Most researchers do not find greater maternal age is related to ALL or AML apart from Down Syndrome.^{85–89} UKCCS found no excess in first born infants⁸⁴ (see below: in the infection section) and evidence is now divided with more studies reporting no effect of birth order compared with those which do find a correlation (evidence reviewed in Ref. [84]).

At present it is difficult to explain the correlation between severe pre-eclampsia/eclampsia and NHL and that between severe hyperemesis requiring hospitalisation and all haematological malignancies. It maybe merely a chance finding related to the small numbers involved given the rarity of these severe conditions.

Polyhydramnios is linked with well recognised malformations but the associations in the study were between AML, maternal anaemia and polyhydramnios without the affected child having any physical abnormalities. The association between maternal anaemia and childhood leukaemia has been demonstrated previously⁹⁰ in ALL and AML and of course maybe related to variations of folate metabolism and/or actual deficiency.⁴⁵ Of interest Thompson et al. reported on a protective effect against development of ALL with supplementation of iron and folate during pregnancy.⁹¹

Summary

Currently it is impossible to provide biological mechanisms for most of these observations. The data does emphasise how rapid foetal growth is and the first trimester represents a hazardous time for the developing foetus. Perhaps the miracle is how high the rate of normality is and the low rate of malignancy seen. The fact that 1% of normal newborn infants have been identified to have a TEL-AML1 fusion gene (apparently functional in frame, in a correct cell type and clonal) generated by double strand DNA breakage and error prone for non-homologous end-joining repair^{7,8,14} confirms the risks. However, only 1% of these 1% appear to proceed to develop leukaemia so (except in MLL positive infancy leukaemia) repair, correction and eradication must occur. Jensen et al. reported that a healthy diet of vegetables, fruit, protein and related nutrients provided during preconception and pregnancy might afford protection from leukaemia.⁹²

Immediate postpartum events

It would be inappropriate here not to comment on the issue of neonatal vitamin K usage to prevent haemorrhagic disease of the newborn and an earlier report of an adverse effect of its use leading to an excess of leukaemia.⁹³ Subsequent to that study two further case-control studies^{94,95} reported some evidence in favour of an effect. However, the larger subsequent UKCCS⁹⁶ reported no evidence of a linkage and Roman et al. followed that with an analysis of all individual patient data from six case-control studies.⁹⁷ This review showed no overall evidence of an increased incidence of leukaemia with vitamin K administration (iv or oral). A follow up study by Ansell et al.⁹⁸ reviewed the practice of vitamin K administration across hospitals in the UK and demonstrated that in a majority of the centres it was impossible to confirm or not whether, on an individual basis, vitamin K had been given or not. Reliance upon hospital policies for evidence as to whether the vitamin had been administered or not and subsequent analysis of data was clearly unreliable as evidence of whether a neonate had received vitamin K. It is important to realise that even in Golding et al. study⁹³ there is a greater risk from haemorrhage, (potentially life threatening) than the risk of developing leukaemia from a single IM injection of vitamin K. If the oral preparation of vitamin K is used, however, repeat dosing is required to prevent the risk of haemorrhage particularly in vulnerable infants.

Infections and response to infection

A series of papers well reviewed by Greaves¹⁰ from early in the 20th century speculated that infection was the cause of leukaemia, or even, as is now hypothesised as a reaction to infection rather than as a contagious disease.⁹⁹ The subsequent identification of the viral link to Burkitt lymphoma and Hodgkin lymphoma in childhood especially (Epstein-Barr virus), the human T cell lymphotropic virus I (HTLV1) as the causative agent in adult T cell leukaemia/lymphoma and HHV8 (herpes virus) in primary effusion lymphoma reactivated interest in an infectious link for childhood leukaemia. After all there was good evidence of a viral causation in chickens, cats and cattle. As alluded to earlier in 1988 Kinlen¹⁷ and Greaves²⁹ both proposed hypotheses as to an infection link, respectively, either through population mixing and effects on herd immunity to a common viral or other infective agent¹⁷ or that infection played a crucial role in the promotion of a pre-leukaemic clone's transformation into overt leukaemia.²⁹ In Greaves' hypothesis infection through an immune response would play a crucial role in creating the postnatal genetic errors seen in childhood ALL especially. This concept has close similarities to the emerging ideas of the 'hygiene' hypothesis for childhood allergies^{100–103} and autoimmune diseases.¹⁰⁴ In countries with improving socio-economic status one of the first features which emerges is better hygiene and health care with reduction of early life infection risks and of improving perinatal mortality. The downside of such improvement is that the immune system has historically been primed by such early infection in order to develop the necessary responses to infections and reach a state of maturity for the body to protect itself against future infections. Greaves hypothesised that absence of such an early immune challenge and priming with 'delayed' exposure to infection might well predispose to aberrant, excessive or pathological responses to infection. Such responses either via proliferation or apoptotic stress would produce the necessary second or more 'genetic' hits. Since he and others clearly demonstrated that the first hits and therefore pre-leukaemic clones^{3–8,82} were present from foetal life, the ideas attracted considerable support in parallel with further development of the hygiene hypothesis. An increasing body of evidence linking a

similar explanation for atopy,^{100–102} type 1 diabetes mellitus¹⁰³ and multiple sclerosis was emerging.¹⁰⁴ Leo Kinlen's hypothesis predicted a lack of immunity to a common or a number of common infections whilst Mel Greaves' hypothesis postulated abnormal and delayed response to infections rather than the infection itself. If leukaemia was due to a direct viral transformation you might expect to see evidence of genome inclusion of a virus(es) or some evidence of infection consistently present as you do see in Burkitt lymphoma, Hodgkin lymphoma and in animal leukaemia.

Screening for viral sequences within leukaemia blood or marrow and in neonatal blood spots has been performed for a number of putative viruses (polyoma viruses JC and BK,^{105–107} parvovirus,¹⁰⁸ human herpes viruses,^{109,110} bovine virus¹¹¹ and TT virus¹¹²). Any positive leukaemia samples would suggest recent postnatal infection whilst positive neonatal blood spots would likely to be negative unless the infection was a prenatal event. The only positive results reported are for the DNA virus TT in relapse but not in initial diagnostic samples.¹¹² Recently a method called representative difference analysis has been used to look at samples for non-endogenous DNA sequences but to date that has not yielded any significant findings. An interesting but unexplained finding of evidence for recent parvovirus infection in a series of newly diagnosed ALL and AML patients has been reported.¹¹³ However, to date there is little evidence for direct viral transformation in childhood ALL or AML. Yet all the evidence from space time clustering, spatial clustering, seasonality and ecology studies outlined earlier in this paper points to the likelihood of an environmental agent(s) and most probably an 'infection' component in the aetiology of at least precursor B cell ALL. The evidence is outlined in greater detail in the review by McNally and Eden.¹³

Maternal infections (either all infections or of specific viruses including EBV, varicella, flu) and influenza during pregnancy have been reported from case-control studies to increase the risk of leukaemia^{85,114,115,117,118} but others have found no such connection¹¹⁶ (reviewed in McNally and Eden¹³). Seven out of 9 recent case-control studies on childhood infections have shown a protective effect for infection in early childhood (e.g. for roseola, EBV, and HHV-6) and 3 have found an increased risk especially from infections in the first year of life.¹¹⁹ All were reviewed in Ref. [13]. Historical case-control studies essentially were negative. All of this evidence is conflicting and unhelpful in really advancing our understanding. Methodological problems of how infections are documented, recalled and verified probably explain a lot of the contradictory results.

Smith et al.¹²⁰ attempted to relate childhood ALL to hygiene conditions by assessing patterns of hepatitis A virus (HAV) infections which have been used as a measure of improving hygiene. The agent is acquired via the faecal-oral route. They correlated changes in HAV infection with changes in incidence and mortality rates from leukaemia and found that a fall in HAV rates preceded increases in childhood leukaemia rates in White American and Japanese children. Their hypothesis was that improved hygiene might increase the frequency of an in utero (or early in infancy) transmission of another primary infection with an associated lack of maternal protective antibody. They felt that a genome transforming infection was most likely. The lack of evidence for viral genome inclusions, although not totally exclusive, makes this less likely as a plausible process.

Vaccinations

The evidence of risk or prevention from childhood vaccinations is also not conclusive. Four case-control studies reported since 1987 showed protective effects of conjugate haemophilus influenza B (HIB), BCG and measles vaccinations (with odds

ratios/relative risks ranging from 0.1 to 0.57) and one showed a non-significant effect for Hib vaccine especially when given at 3 months rather than at 2 years.^{121–124} Dockerty et al.¹¹⁹ in a single study reported a non-significant decreased risk with “any” vaccination but an increased risk with measles vaccine. One other study showed an increased incidence of ALL following measles, mumps and rubella vaccines.¹³

Breast feeding

Breast feeding which is considered to be a surrogate for immune protection but also for exposure to infection and foreign antigens through repeated close maternal contact, was examined in the UK Childhood Cancer Study.¹²⁵ For leukaemia the risk was marginally reduced if a child was “ever breast fed” (OR 0.89, CI 0.8–1.0, $p = 0.06$). The investigators reviewed 15 other case-control studies and when all the data was collated a protective odds ratio of 0.86 (0.81–0.92) for “ever breast fed” was found with duration of feeding beyond 6 months marginally improving the protective effect (OR 0.78, 95% CI 0.71–0.85). Perrillat et al.¹²⁶ also reported a protective effect against ALL development with breast feeding and a greater effect of duration of feeding beyond 12 months (OR 0.5, 95% CI 0.1–1.25). Three other recent studies showed no association, no matter how long the baby was fed. Whether the protective effect is real cannot be really gauged from these case-control studies particularly as the controls in all of the case-control studies tend to be skewed towards higher socio-economic groups. Breast feeding rates show a similar bias towards the least deprived which may be an insurmountable obstacle to deriving unbiased evidence on the protection afforded or not from breast feeding in any case-control study.

Day care attendance

Perhaps the most compelling evidence regarding early infection exposure being protective against development of leukaemia comes from a series of case-control studies looking at day care attendance, as evidence of early life exposure to other children and their infections. McNally and Eden reviewed 10 of these studies in 2004¹³ and Urayama et al. have updated and extended the review to 14 studies in 2008.¹²⁷ None show an adverse effect of day care attendance with any increase in ALL and the majority show a protective effect. The two largest studies, the UKCCS and the North California Childhood Leukaemia Study reported similar levels of protection. In the UKCCS when compared with families reporting no regular activity outside the home protective odds ratios for any contact, regular contact, and formal day care were 0.73, 0.62 and 0.48, respectively (p -value for trend < 0.001). Attendance in the first 3 months gave an odds ratio of 0.52 (95% CI 0.33–0.84, $p = 0.007$).¹²⁸

The North Californian study originally reported in 2002⁸⁹ showed a reduction of risk with a duration of attendance effect. It has been updated with a larger sample size.¹²⁹ This confirmed their original evidence of protection but only for non-Hispanic white children and not for Hispanics. The reduced risk for white non-Hispanics showed a dose-response relationship for ALL (OR 0.6, 95% CI 0.28–1.27) and c-ALL (OR 0.28, 95% CI 0.08–0.95) for those with the highest level of day care and hence child-hours exposure ($\geq 15,000$ child-hours) compared with non attenders at day care.¹²⁹ There was a protective effect in the study if children only attended in the first year of life. Even if no protection was afforded to Hispanic children there was no increased risk of ALL or c-ALL for them. The Hispanic children were shown to start day care at a later age, attend for a shorter period of time, attend for less hours/week but to have more children living in their house-

holds. Exposure to infections would occur at home but not with the same diversity as experienced by day care attendance. Their study is continuing. Of interest attendance at day care nurseries has been reported to also reduce allergies and type 1 diabetes.^{130,131}

Another final piece of epidemiological data regarding the immune system was identified in the UKCCS study¹³² (in which 720 children with ALL, 538 with c-ALL), 101 with AML and 1337 controls (age, sex and region matched) had their primary care records analysed for evidence of allergies. Over 1/3 of cases and controls had some history of allergies. A prior history of eczema was associated with a 30% reduction in development of ALL and precursor B cell ALL in particular. A somewhat higher association (40% reduction) was found for hay fever but not for asthma. No association between allergies and AML were found albeit on a smaller sample. Parental interview reporting appeared to correlate poorly with primary care records, which makes it essential in future studies to precisely confirm, by medical records the diagnosis of eczema and other allergies. Since the original proposals of the “hygiene hypothesis”¹³³ a number of studies have reported on the relationship between infections and allergy. Just as Greaves proposed for ALL (especially precursor B cell ALL) early life infection exposure leads to maturation of the immune system and pushes it towards a Th1 phenotype and away from a Th2 phenotype. This shift should protect against diseases generated by Th2 biased immune dysfunction, such as allergies. There has been speculation that only certain infections like measles and tuberculosis^{100,134} protect against allergy and speculatively also the vaccinations against these infections. In contrast some other infections, e.g. respiratory viral infections may precipitate allergic disease.¹⁰² It would appear that both the timing of infection and the chronicity of the infectious exposure as well as vaccination history might all interact in the relationship between allergy and ALL. Ultimately the response to infection, development of allergy and of ALL will depend on each host's immune responses mediated through T cell subsets, cytokine signalling and the antigen presenting capacity of human leucocyte antigen Class II alleles (determined by a high degree of polymorphism), chemokines and their receptors.

Immune response genes

Taylor et al.¹³⁵ first reported evidence of an HLA-DQA1-DQB1 haplotype which influenced susceptibility to childhood ALL, interestingly specifically in boys. Within the UKCCS (2002) they proceeded to expand their work and clearly identified that carriage of the DPB1*0201 allele (and other HLA Class II DPB alleles with the same peptide binding pockets) increased susceptibility to develop precursor B cell ALL (relative risk 1.76) and T ALL (RR 1.93).¹³⁶

They showed that other alleles, e.g. DPB1*0101 might afford protection against ALL. These alleles of course influence the immune response to foreign antigens. Other HLA Class II antigens including the DR complex may also be implicated in ALL aetiology.¹³⁷ Studies are now progressing to look at the role of a range of cytokines especially Interleukin 12, interferon gamma and tumour necrosis factor as they have been in the other conditions lumped together under the “hygiene hypothesis”.

Greaves has postulated¹⁰ that an existing prenatal pre-leukaemic clone containing, e.g. TEL-AML fusion gene or hyperdiploidy, maybe converted to a malignant clone by a dysregulated T cell response to infection, triggering a potent inflammation response mediated by a cytokine cascade which may also inhibit normal haematopoiesis. It may produce apoptosis in normal myeloid precursors (the possible explanation for an aplastic prophase to ALL seen in 1–2% of cases).^{138,139} The pre-leukaemic clone may be

promoted to proliferate in this milieu¹⁴⁰ and have a selective advantage. Secondary genetic events would occur as a result of rapid proliferation and/or oxidative stress in the middle of an inflammatory response. Greaves and his team¹⁴¹ have developed a system for the expression of TEL-AML in a cellular context and are testing the biochemical basis of any selective advantage for pre-leukaemia clones. They identified that TEL-AML1 expression appears to slow cell growth and leads to over 200 genes being altered, mostly down regulated. Studies will be possible using in vivo mice models if the cytokine pathways involved can be defined by gene expression profiling particularly in cases of hypoplastic presentation of leukaemia. Although relatively rare these patients are usually presumed to have leukaemia or aplastic anaemia and are subjected to full marrow investigations anyway. If an abnormal clone is identified it affords a real opportunity to explore what is driving the aplasia and the subsequent emergence of the leukaemia clone.

Conclusions

There is very strong evidence that for the majority of childhood acute leukaemia the first genetic event occurs prenatally in the foetus. For infant leukaemia with MLL gene rearrangements, that 'hit' appears adequate to drive a leukaemic clone. The factors which predispose to the rare infant leukaemia include maternal diet rich in naturally occurring topoisomerase II inhibitors but also a potential defect in the mother and foetus to adequately metabolise the agents. For childhood peak precursor B cell ALL we do not know how the pre-leukaemia clones (TEL-AML1, hyperdiploidy, etc.) are initiated. It may be a common and chance phenomenon as described by Mori et al.⁸ but whether ionising or non-ionising radiation or chemicals play any part is still very uncertain. Reduction of risk may occur with a healthy maternal diet and preconception/early pregnancy folate and iron supplementation. Only 1% of pre-leukaemia clones convert to overt leukaemia and the most plausible explanation currently available is of an abnormal dysregulated response to infection as proposed and investigated by Greaves.¹⁰ Whether any of the other putative leukaemogenic factors play any part in these secondary events is as yet unclear but if they do, statistically they account for a minority of cases. Genetically determined responses certainly do provide a degree of individual susceptibility, e.g. to produce an abnormal immune response which may drive the secondary events to convert pre-leukaemic clones into overt ALL.

Conflict of interest statement

None declared.

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