The implications of the cloning of the XLA gene

The gene for Bruton’s X-linked agammaglobulinemia (XLA) was isolated earlier this year [1]. XLA is a rare primary immunodeficiency which was first reported in 1952 [2], and affects about one in 50,000 live male births.

Clinical features of XLA

Affected boys usually present during the first few years of life with recurrent, life-threatening bacterial infections, as well as some viral and parasitic infections. Although the peak in the age of onset is at six months, presumably when maternally acquired antibodies are diminishing, 40% of patients remain asymptomatic during their first year, and 21% present as late as between three and five years of age [3]. The principal features prior to diagnosis include sinusitis, otitis media, mastoiditis, pneumonia, cellulitis and boils, and diarrhoea/malabsorption. Treatment includes intensive courses of antibiotics when indicated, together with recurrent intravenous infusions of immunoglobulins which superseded intramuscular immunoglobulin injections in the 1980s [4]. Meningoencephalitis is one of the principal causes of death.

Immunology of XLA

The pathological defect in XLA is a deficiency of immunoglobulins, caused by a lack of circulating mature B cells, or plasma cells. Yet there is a normal number of their precursors, the pre-B cells, in bone marrow and gut-associated lymphoid tissue, indicating a block in the differentiation of B cell precursors into mature antibody-secreting B cells, which express surface-bound Ig [5].

Studies of X-chromosome inactivation (Lyonisation) patterns in XLA carrier females demonstrated that the XLA defect is intrinsic to the B cell lineage [6,7]. This was established by experiments on female carriers of XLA (who are immunologically normal), who were heterozygous for glucose-6-phosphate dehydrogenase. Their mature B cells expressed only one of G6PD’s isoenzymes, but their T cells and neutrophils expressed both isoenzymes [6]. B cells from female carriers all show inactivation of the X chromosome carrying the XLA defect, with the active chromosome being exclusively that carrying the normal XLA allele. This suggests that the XLA gene is essential for the development or survival of B cells, and there is therefore only one population of mature B cells with the active, normal X chromosome in a female carrier [6,7].

Mapping the XLA gene

Many X-linked genes have been cloned via cytogenetic abnormalities, such as translocations, but no such abnormality has been described in any XLA patient. Conventional linkage studies performed in a number of pedigrees mapped the gene to Xq22, and the polymorphic marker DXS178 showed no recombination with the disease [8-10]. Refinements to the genetic and physical maps of Xq22 [10,11] were helpful in identifying candidate genes [12]. Yeast artificial chromosome (YAC) clones contain large (100–1000kb) segments of human DNA which can readily be propagated in yeast, thus providing an excellent source of material from which to isolate genes within a large stretch of DNA. A YAC clone containing a segment of DNA including DXS178 was used to isolate candidate genes as complementary DNAs (cDNAs) via a novel method of gene-detection known as cDNA direct selection [13,14]. One candidate was found to be a novel member of the src family of genes, encoding a protein-tyrosine kinase expressed in B cells [1]. The final proof that this was the gene involved in XLA came when a variety of deletions and point mutations in the gene was detected in eight unrelated XLA patients.

Immediate clinical implications

Until now, prenatal diagnosis of XLA required a DNA linkage study [15]. The problems associated with linkage studies are well known, and include predictive errors caused by genetic recombination. Attempts at carrier detection have previously relied on exploiting the detection of non-random Lyonisation. Recombinant DNA probes were used which simultaneously
detect restriction fragment length polymorphisms and patterns of methylation (indicative of gene activity status). In order to define female carriers, it was necessary to demonstrate random X-inactivation in T cells and granulocytes, together with completely non-random X-inactivation in B cells [7,16].

The cloning of the XLA gene means that, once a precise mutation has been defined in an affected individual, accurate carrier detection and first trimester prenatal diagnosis become available for that particular family. But as each mutation defined so far has been unique, each family will need detailed and sometimes lengthy molecular studies before a customised test can be offered.

Gene therapy

Inherited immune defects are ideal candidates for gene therapy, because of the relative ease with which bone marrow cells can be obtained, treated and replaced in the patient. The first attempt at gene therapy in the UK is currently under way at Great Ormond Street, where a child with a related immunological disorder (ADA-deficient severe combined immunodeficiency) is being treated with a bone marrow infusion infected with a modified retrovirus carrying the healthy gene.

The idea that some human genetic diseases will become amenable to correction at the genetic level is now broadly accepted [17,18].

One advantage of somatic gene therapy is that it avoids problems relating to organ transplantation such as immune rejection and shortage of donors. It may also prove to be cost effective, as regular infusions of immunoglobulins are expensive. As yet, however, somatic gene therapy has not been used on a sufficient scale for definite conclusions to be drawn regarding its potential for human patients.

The ethical aspects of gene therapy have been considered by the Clothier Committee which reported to the UK government in 1992.

There are far more profound ethical and practical reservations about the prospect of germ-line therapy [18]. The main concern is that germ-line modification, even with the intent to cure human disease, could lead to unintended damage of the genetic material which would be perpetuated through subsequent generations.

There is general agreement that, at present, until the technology has improved and the potential risks have been minimised, only somatic gene therapy, and not germ-line therapy, should be pursued for patients with life-threatening conditions.

The XLA gene and cancer

The sequence of the XLA gene is similar to that of the src family of proto-oncogenes. Members of the src gene family (characterised by extensive homology with the kinase domain of the transforming gene of the Rous sarcoma virus [v-src]), encode intracellular (non-receptor) protein-tyrosine kinases which are bound to the plasma membrane. Protein-tyrosine kinases, of which the src family is but one sub-class, mediate cellular signalling pathways regulating cell growth, proliferation and differentiation. This is accomplished via phosphorylation of tyrosine residues on themselves (as a means of autoregulation), and on their protein substrates. Such events serve to activate or inactivate the enzymatic functions of a variety of proteins, and thus trigger a wide range of cellular responses. It is possible that the protein product of the XLA gene (dubbed atk for agammaglobulinaemia tyrosine kinase) could mediate the cellular events which result in pre-B cells becoming B cells, and may also participate in subsequent stages of B cell differentiation.

However, because of its similarities to the transforming src genes, will (or can) atk also contribute to neoplastic diseases when its normal function is disturbed? For this reason the study of the XLA gene and its protein product in certain types of B cell lymphomas and leukaemias will be an exciting area of research during the next few years.

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