X-linked severe combined immunodeficiency

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Severe combined immunodeficiency is one of the most common causes of primary immunodeficiencies in humans. Molecular biological techniques have allowed new, therapeutically useful treatments for these diseases to be introduced into clinical practice. This review will focus on the molecular basis and new treatments for X-linked severe combined immunodeficiency.

Infants born with any form of severe combined immunodeficiency (SCID) present as a paediatric medical emergency. The predominant problem lies in abnormal T lymphocyte development and function and, as a result of this, also abnormal B lymphocyte activity. The children can be recognized at birth by the detection of lymphopenia in the differential blood count (levels less than 2000–11 000/mm^3 should alert the clinician). If not noticed at birth, the child presents in the first few months of life with failure to thrive and recurrent infections, of which diarrhoea is often a common symptom. Viral and fungal infections are particularly problematic and are often fatal, reflecting the importance of T cell function in defending the body from these pathogens. The child lacks tonsils and lymph nodes while the thymus is atrophic. Unless treated death is inevitable during infancy (Buckley, 2000).

XSCID presents as above and, as it is X-linked, is virtually exclusively a male disease. Affected children are found to have extremely low peripheral T cell counts and although B cell numbers are normal or even increased they are functionally defective, reflected by low serum immunoglobulin levels. The XSCID gene product is believed to be important in terminal differentiation of B cells as shown by patterns of non-random X chromosomal inactivation in female carriers. The mature cells in these female carriers contain the wild-type X chromosome, indicating that cells with the defective X chromosome were eliminated and could not mature. This fact is often used to identify female carriers in a family at risk of XSCID.

The natural killer (NK) cell is also affected in XSCID although to a variable degree depending on the actual genetic cause of the XSCID. Most commonly the phenotype is T cell negative, B cell positive and NK cells negative (T-B+NK-).

CYTOKINE SIGNALLING

Over the last decade substantial progress has been made in identifying the cause of the majority of cases of XSCID in humans at a molecular level. In order to understand this the basis of cytokine signalling in the immune system must first be explained and a prototype model of interleukin-2 (IL-2) will be examined. IL-2 is a single polypeptide of molecular weight 15.5 kDa which is 133 amino acid residues long. Genetic investigation has shown there is only a single IL-2 gene locus, found on chromosome 4 in humans. It is a potent immunomodulator and has an important role in both the activation and maintenance of an immune response and in lymphocyte development. IL-2 activates numerous key cells in the immune system including helper T cells, cytotoxic T cells, B lymphocytes, NK cells, tumour-infiltrating lymphocytes and macrophage-monocyte cells.

IL-2 acts via a cell surface receptor made up of three separate components. The first to be identified was that of the α subunit of the receptor – a 55 kDa glycosylated integral membrane protein, also known as CD25. The two other components of the receptor have been identified and are called the β and γ chains (Nelson and Willerford, 1998). The individual chains have low affinities for IL-2 but when combined they act as a high affinity complex. The α subunit on its own represents the low affinity state, the βγ complex has an intermediate affinity but the high affinity complex contains the αβγ chains and is the active receptor. It is believed that the ligand binds to the α and β subunits first and

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then there is heterodimerization of the αβγ chains to activate the intracellular downstream signalling mechanisms (*Figure 1*).

The α subunit gene has been identified and is located on chromosome 10 in the human. The protein product of this gene is a 251 amino acid chain but the intracellular domain is too short to act as an important site for signal transduction. The β chain is a 70–75 kDa protein which is located on chromosome 22. This is an integral membrane protein and is constitutively expressed on resting lymphocytes, monocytes/macrophages and neutrophils. It is upregulated upon T cell activation.

The most recent chain to be identified is the γ chain. This is again an integral membrane protein and is 347 amino acids in size, giving a molecular weight of 64 kDa. The genetic locus is on the X chromosome. It has a 86 amino acid intracellular domain which is vital for IL-2 signalling and is constitutively expressed on lymphocytes, monocytes and neutrophils (Takeshita et al, 1992).

Both the β and γ chains are related to the cytokine receptor superfamily type 1 unlike the α subunit. These receptors are characterized by the possession of similar structural motifs – for example the conserved four cysteine residues at the N terminus.

The signal transduction from the IL-2 receptor is complex and involves a number of different mechanisms as the actual αβγ complex itself has no intrinsic enzyme activity. Perhaps one of the most important is through the Janus tyrosine kinase family and the Stat (signal transducer and activator of transcription) molecules.

The Janus kinases were first identified in the interferon system and are important cellular kinases which phosphorylate key tyrosine residues. Jak1 and Jak3 are involved in the IL-2 system – Jak1 is constitutively bound to the β subunit whereas Jak3 is bound initially with low affinity but after cellular activation with high affinity to the γ chain. After heterodimerization of the αβγ units Jak3 also interacts with the β unit and this heterodimerization activates the kinases. This leads to phosphorylation of key proteins including the β and γ subunits of the receptor.

This phosphorylation allows the Stat molecules Stat 3, Stat 5a and Stat 5b to dock on the receptor and themselves become phosphorylated. Once this is complete the Stats undergo tetramerization and translocate to the nucleus where they can bind to promotor sequences on key genes and upregulate the production of their protein (Leonard and O'Shea, 1998). As already noted the γ chain is needed for the kinase binding and, as explained below, this downstream signalling mechanism is seen to be a common feature with other cytokines.

Other important tyrosine kinase pathways are through the Src family and phosphatidyl inositol-3-kinase. The p56lck was the first kinase identified to be associated with the IL-2 receptor complex. The phosphatidyl inositol-3-kinase system has important downstream targets including p70 S6 kinase which has been identified as one of the targets of rapamycin, a new immunosuppressant agent used in transplantation (Kuo et al, 1992).

MOLECULAR BASIS OF XSCID

It had previously been shown (de Saint Basile et al, 1987) that the genetic locus for the XSCID, called SCIDX1, was located on the X chromosome somewhere between Xq11 and Xq13. As mentioned above the genetic locus for the γ chain had been mapped to the X chromosome and Noguchi et al (1993) used linkage markers to show that this gene co-localized to the SCIDX1 gene.

The same group showed that DNA sequencing of the γ gene in three patients with XSCID identified point mutations in all three leading to the formation of premature stop codons, while in normal patients no such mutations were seen. Further study has revealed numerous different types of mutations in the γ chain gene. Known XSCID mutations include single point mutations, deletions, insertions, frameshift mutations and splice-junction abnormalities (Leonard,

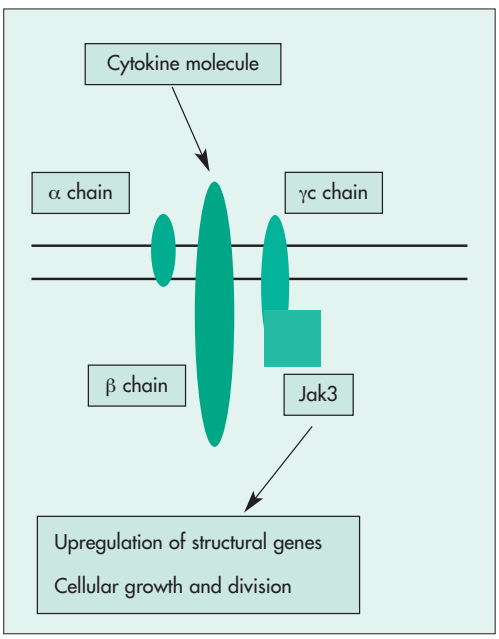

Figure 1. Outline of cytokine signalling. Defects in the γc chain leads to failure to bind either with Janus tyrosine kinase (Jak3) or with the ligand. This leads to X-linked severe combined immunodeficiency.

1996). The heterogeneity of the mutations will make prenatal detection problematic and it will only probably be useful in families at risk of XSCID in whom the genetic mutation is already known.

The development of IL-2 knockout mice in which the IL-2 gene is removed from murine germ cells by homologous recombination started the investigation which led to the discovery that the γ chain is shared by at least six other cytokine receptor systems. Schorle et al (1991) showed that IL-2 knockout mice had relatively normal immune systems with normal responses to in-vivo infection with vaccinia and varicella viruses. Weinberg and Parkman (1991) also identified a child who had a mild form of SCID as a result of a defect in the production of IL-2 messenger RNA leading to loss of IL-2 production. These cases illustrated that the severe forms of XSCID could not be just the result of interruption of the IL-2 system.

The sharing of the γ chain between other cytokines has lead it to be re-named as the common γ chain, γc. So far it is known to be used by IL-2, IL-4, IL-7, IL-9, IL-15 and more recently IL-21 (Asao et al, 2001). IL-15 is known to be very important in the development of NK cells and like IL-2 has a multi-subunit receptor structure made up of the γc and the IL-2b subunit along with a unique IL-15a subunit which confers specificity. Mutations in the γc chain therefore explain the NK cell defects seen in XSCID and this has been confirmed by the development of IL-15 receptor and IL-15 knockout mice (Kennedy et al, 2000). Moreover the fact that IL-2 and IL-15 are so similar and overlap in function may explain why the phenotype in IL-2 deficient patients is so minimal. This redundancy of function is a recurring theme in the cytokine system.

The profound T cell abnormality in XSCID is caused by the defective IL-7 signalling. This has been shown to be essential for T lymphocyte development by analysis of the phenotype of mice made IL-7 deficient by genetic manipulation. Buckley et al (1997), in looking at a group of SCID patients, noticed that three had a T-B+NK+ phenotype and failed to show any abnormality in the γc or Jak3 gene. Instead analysis demonstrated mutations in the IL-7a receptor gene on chromosome 5. This clearly demonstrated the selective T cell dependence on IL-7 signalling.

Furthermore the position of the mutation is important. Some mutations in the part of the exon coding for the extracellular binding region lead to failure of cytokine binding whereas intracellular region defects abrogates the coupling with downstream signalling molecules. Activation of the γc chain in the cytokine receptor leads to the activation of Jak3 kinase. This is critical for coupling of ligand binding to the receptor and intracellular signalling. Indeed knockout mice for the Jak3 gene produced an almost identical picture when compared to γc knockout mice.

Recently patients with Jak3 gene mutations have been identified and have a clinically and immunologically indistinguishable disease to XSCID. These patients have an autosomal recessive disease, however, as the Jak3 kinase gene is on chromosome 5, but it underlies the importance of Jak3 in γc-mediated signalling. Shortening of the γc cytoplasmic domain by XSCID mutations therefore prevents proper association with Jak3 and failure to mediate downstream signalling. A single amino acid change in the γc chain has also been shown to lead to reduced association with Jak3 and lead to a milder form of XSCID, indicating that the degree of immune compromise relates inversely to the amount of Jak3 binding (Leonard, 1996).

TREATMENT

The treatment of XSCID falls into two strategies: bone marrow transplantation (BMT) or gene therapy.

Bone marrow transplantation

In 1968 the first child with XSCID was successfully treated using a bone marrow transplant from his HLA-identical sister (Gatti et al, 1968). The child's immune system was reconstituted by this and he is still alive today. Since then many more patients with SCID have been treated with BMT. A number of features makes XSCID an appealing target for BMT. The patients lack T cells and so are unable to reject allografts. This means that HLA-identical transplants and haploidentical transplants (in which only one HLA is shared between the donor and recipient) are much more likely to engraft successfully and survive. It also means that no myeloablative conditioning with chemotherapy is required pre-transplantation since the T cells are already absent. Finally, through development of techniques for treating the bone marrow graft, donor T cells can be removed thus greatly reducing the chance of graft *vs* host disease occurring. Graft *vs* host disease is believed to be one of the main predictive factors for assessing whether a graft will survive or not.

The efficacy of BMT in this disease was clearly shown by Buckley and her group in Duke University (1999). They followed up 89 infants diagnosed with SCID who received BMT. Of this group 43 had XSCID, the others had SCID secondary to Jak3 deficiency, IL-7α subunit mutation, adenosine deaminase deficiency and unknown causes. Twelve of the 89 infants received HLA-identical BMT, with the rest receiving haploidentical BMT all of which were pre-treated to remove donor T cells. There was 100% survival for the HLA-identical grafts and 81% of the haploidentical grafts at the end of follow-up (median period of 5.6 years). Nine patients from the XSCID group died and the cause of deaths were principally viral infections.

This study showed several important points. The T cells from the donors engrafted well and the patients' peripheral T cells were essentially those of the donors. However, the time taken from transplant to the appearance of functional T cells differed between the types of graft, being on average 2 weeks in the HLA-identical group and 3–4 months in the other group, during which time the child is still at risk of opportunistic infections. Furthermore, in the XSCID group especially, the B cell engraftment did not take so well and although the numbers of B cells were normal or elevated the functional defect persisted. This resulted in over 50% requiring regular immunoglobulin infusions. The group also demonstrated that the earlier the BMT is given the better and they had a 95% survival rate if performed before 3.5 months of age. This obviously requires the diagnosis to be made as soon after birth as possible.

Other BMT strategies have used in-utero BMT in families who usually have a history of the disease and this allows antenatal diagnosis. The idea is that the graft will be better tolerated in an immature fetal immune system and that the B cells may also graft in better. However, the outcome of neonatal BMT has been shown to be better than that of in-utero BMT (Kane et al, 2001).

Gene therapy

Since the advent of molecular biology, the ability to replace a defective gene with a wild type has promised much but has so far delivered little. However, gene therapy has been successfully carried out in patients with XSCID. In 2000, two patients were reported who had received gene therapy with the γc gene (Cavazzana-Calvo et al, 2000). In 2002, the same group reported five patients who had undergone this treatment (Hacein-Bey-Abina et al, 2002). Using a defective Moloney retroviral vector containing the wild type γc gene, stem cells (CD34+) from patients' bone marrow were isolated and then transfected with the virus. Integration of the viral genome complete with the γc gene occurred and the cells were then transfused back into the patients.

The results were startling. Four infants demonstrated evidence of a functional immune system following treatment while in the fifth patient the treatment was not successful and he required urgent BMT. All four patients are now living at home in the normal environment after 2.5-year follow up and have successfully dealt with infections after leaving hospital. Moreover the gene therapy seems to have improved the B cell function and the patients do not require any supplemental immunoglobulin infusions.

Unfortunately, and only very recently, this group has detected a problem in one of their first patients treated with the retroviral gene therapy (Buckley, 2002). At routine follow up this child has been found to have a leukaemia-like picture with a very high white cell count. The cells have been genetically analysed and found to be of T-cell origin but containing the transgene inserted into a potential oncogene called LMO-2, which has been implicated, in acute lymphoblastic leukaemia. Although not conclusively proven yet, this finding raises concerns regarding the possible side effect of insertional mutagenesis with gene transfer. Further analysis will be required to elucidate this further.

CONCLUSIONS

XSCID is the most common cause of the SCID group of immunodeficiencies. The molecular basis of this condition lies in mutations of the γc receptor which plays a critical part of many cytokine receptors. These cytokines are essential for the development and orchestration of the day-to-day function of the immune system and failure in the signalling process leads to defects in the T, B and NK cellular population.

The initial management of this fatal condition was using BMT and this remains an effective and life-saving treatment. However, it has some drawbacks such as the failure to improve the B cell function. Genetic manipulation of bone marrow stem cells using retroviral vectors equipped with the normal γc gene has now proved to be a valuable and efficient treatment for this condition and will no doubt be used more frequently both in the management of the primary immuno-

deficiency diseases and other genetic diseases. However, further studies are needed before gene therapy can be regarded as a perfect and harmless treatment. HM

Conflict of interest: none.

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KEY POINTS

- X-linked severe combined immunodeficiency is caused by genetic mutations in the common-gamma chain of many cytokine receptors. The common-gamma chain is critical for intracellular signalling from cytokine binding.
- X-linked severe combined immunodeficiency is a fatal disease because of the loss of T and B cell function.
- Bone marrow transplantation offers a good treatment with good success rates especially in HLA-identical grafts done early on in life.
- Gene therapy has recently been shown to allow replacement of defective common-gamma gene with wild type resulting in the reconstitution of normal immunity in patients.

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