The molecular biology of chronic myeloid leukaemia

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INTRODUCTION
Chronic myeloid leukaemia (CML) is an acquired haemopoietic malignancy which manifests as an expansion of the pool of mature myeloid elements in the blood and bone marrow. This clonal disorder has a high incidence in individuals exposed to the atomic bomb blast in Hiroshima and Nagasaki. Contrary to an earlier belief the colony-forming unit–granulocyte macrophage (CFU–GM) pool in CML is smaller in size and retains normal sensitivity to growth factors such as the granulocyte macrophage-colony stimulating factor (GM–CSF). The response to inhibitory substances such as prostaglandin E, lactoferrins, etc. has been variable. The colony stimulating activity (CSA) is elevated in most patients with CML and this seems to be the result rather than the cause of CML. Recent evidence favours the theory that maturation arrest in the relatively mature progenitors results in an expanded pool of relatively mature myeloid cells; this is termed discordant maturation. The stimulus for the asynchrony is not known.

PHILADELPHIA CHROMOSOME
Our understanding of CML has improved with advances in genetics and molecular biology. In 1960 a minute chromosome was found in the myeloid cells of 2 patients with CML and this was called the Philadelphia chromosome (Ph1). In 1970 the Ph1 chromosome was confirmed to be chromosome 22 and three years later its origin was identified to be a translocation between chromosome 22 and 9 referred to as t(9;22). High resolution banding techniques have demonstrated the precise breakpoints on the two chromosomes: band q34.1 on chromosome 9 and band q11.21 on chromosome 22. Recent studies using molecular probes have confirmed the reciprocal nature of this translocation. However, the Ph1 chromosome is absent in 5% to 10% of patients with CML. In some cases this absence may be the result of its loss during sample processing or because the clinical features of CML precede the development of the Ph1 chromosome but both these situations are rare.

THE ONCOGENE CONCEPT
During the period when cytogenetic studies on patients with CML were performed, molecular studies in a group of transforming RNA tumour viruses known as retroviruses led to the identification of transforming genes within the retroviral genome which were called viral oncogenes (v-onc). Further studies revealed that v-onc were derived from homologous genes present in the normal human DNA; the latter have been termed cellular oncogenes (c-onc). The two fields of investigations merged when two of the c-onc in man were found at or near the chromosome bands involved in forming the t(9;22) translocation typical of CML. In 1982, it was discovered that the normal human homologue of the transforming gene of the Abelson murine leukaemia virus (c-abl) was located at chromosome 9 at band q34. Another group localized the human homologues of the transforming gene of the Simian sarcoma virus (c-sis) at bands q123-13.1 of chromosome 22. Each chromosome band may contain 1 to 5000-kb of DNA including several hundred genes; it was therefore important to determine if c-abl and c-sis were directly involved in the t(9;22) translocation. This was confirmed by demonstrating the translocation of c-abl from chromosome 9 to the Ph1 chromosome and the reciprocal translocation of c-sis from chromosome 22 to chromosome 9 in patients with CML.

MOLECULAR EVENTS
It is important to understand the molecular events related to the translocation of c-abl. The breakpoint on chromosome 9 occurs at a variable distance 5' (upstream) of the first known exon of the c-abl gene; it can occur over a range of 50 to 200-kb. Most of the c-abl gene is translocated to chromosome 22. The break on chromosome 22 occurs at a relatively restricted region of 5.8-kb; this region is called the breakpoint cluster region (bcr). The breakpoint on chromosome 22 occurs only in Ph1 positive CML which suggests that this abnormality is acquired and is specific for CML. Subsequent studies focused on the possible role of c-sis and c-abl in CML. It was found that c-sis was not expressed in CML cells and was unlikely to play a pivotal role in the pathogenesis of CML. Normal cells contain
two major c-abl homologous transcripts of 6-kb and 7-kb mRNA. Cells from patients with CML contain a novel 8-kb or 9-kb c-abl transcript both in the chronic and the blast phase. The 8-kb c-abl homologous transcript appears to be directly related to the Ph1 negative CML. There are various hypotheses for the formation of the 8-kb or 9-kb c-abl transcript, the most attractive being that these are fusion transcripts containing genetic information from chromosome 22 and 9. Recent data indicate that the 8-kb transcript is a hybrid RNA containing sequences from chromosome 22 (c-abl) as well as from chromosome 22 (bcr). Conceptually this implies that the t(9;22) translocation leads to the formation of a new gene by gene fusion containing genetic information from both the c-abl and bcr genes.

The normal c-abl and bcr related protein in the human cells is a 145 kd protein which has either a very low or absent tyrosine kinase activity. The identity of the normal bcr proteins is still doubtful. The bcr gene manifests with three transcripts—7, 4.5 and 4-kb. The predicted amino acid sequence of cDNA suggests that bcr mRNA encodes a protein 142 kd which is not associated with the cell membrane. In patients with CML both in the chronic and the blastic phase, a protein of the nature of 210 kd has been detected; it possesses potent tyrosine kinase activity. PTK activity is a feature of retroviral transforming proteins related to the v-abl in the Abelson murine leukemia virus. Both the 210 kd human protein and the 160 kd v-abl related protein show biochemical similarities. The 210 kd c-abl related protein constitutes a fused protein composed of bcr gene sequences at the amino (N) terminus and c-abl related sequences at the carboxy terminus; there are also some additional peptides at the N-terminus. Protein 210 kd is found both in Ph1 positive and negative but bcr positive patients. Presently PTK activity related to the p210 protein is considered to be important in the pathogenesis of CML.

It has been proposed that the altered tyrosine kinase activity of the p210 protein or some other biochemical alteration associated with the t(9;22) translocation, causes a disturbance in the transmission of regulatory messages through the transduction pathways with the result that the maturation sequence of the leukemic stem cells is distorted manifesting as nuclear or cytoplasmic asynchrony. This has three important consequences:

1. The earliest committed CML progenitors have reduced proliferative capacity,
2. The lag in the nuclear maturation enables the mature progenitors to undergo more divisions compared to their normal counterparts resulting in expansion of the total leukemic population, and
3. The mature cells have an increased life span.

OTHER ONCOGENES
The role of oncogenes other than c-abl in the pathogenesis of CML is not clear. The concept that c-sis is not involved is based on the findings that:

1. c-sis can be translocated to any one of several chromosomes,
2. there is no evidence of DNA rearrangement within c-sis, and
3. c-sis related RNA is not detectable in CML.

Yet the possibility of c-sis expression by a few cells cannot be ruled out. Recent data indicate that the beta chain of the c-sis gene product has considerable homology with platelet-derived growth factor, a glycoprotein produced by megakaryocytes and mesenchymal cells that stimulates collagen production by fibroblasts and accounts for the myelofibrosis seen in some cases of CML.

Other oncogenes involved in CML include N-ras, c-ras and c-myc. N-ras, a normal cellular gene closely related to the transforming gene of the Harvey and Kirsten murine sarcoma viruses has been found active in a CML cell line. Transforming genes related to the c-ras family have been identified in a few cases of CML; in others amplification of c-myc has been observed.

The current consensus is that a diagnosis of CML can be considered only in the presence of a bcr rearrangement and the p210 kd protein. If bcr rearrangement is not detected by conventional probes, additional probes should be employed since deletion mutation at or near the bcr/abl recombination point is not rare in CML and the position of deletion is variable. The exact reason for translocations between chromosome 9 and 22 is not known. A recent study shows that these two chromosomes are temporally close; once the translocation takes place, the same pattern is carried on in all the subsequent generations of cells. CML after a variable duration in the chronic phase terminates in the blastic phase. Factors governing the duration of the chronic phase are not well known but a recent study has shown that this was longer in patients with a breakpoint in fragment 2 of the bcr.

REFERENCES
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Immunology and immunogenetics of diabetes mellitus

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INTRODUCTION

Diabetes mellitus (DM), a disease of metabolism and blood vessels, has a strong immunological basis in both its aetiology and its complications. The immune system contributes to these and is also itself affected by the disease. The immunology and immunogenetics of DM may be discussed under the following headings:

1. The immunology of IDDM
2. The immune response to insulin and its sequelae
3. Diabetes affecting the immune system
4. Immunogenetics of diabetes mellitus
5. Gene therapy in IDDM

IMMUNOLOGY OF IDDM

Major advances over the last decade have led to a better understanding of the immunology of diabetes particularly the immunopathogenesis of type I DM. The interaction