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The X-linked immunodeficiency defect in the mouse is corrected by expression of human *Bruton's tyrosine kinase* from a yeast artificial chromosome transgene

Mutations in the gene for Bruton's tyrosine kinase result in the B cell differentiation defects X-linked agammaglobulinemia in man and X-linked immunodeficiency in mice. Here we describ the generation of two yeast artificial chromosome (YAC)-transgenic mouse strains in which high-level expression of human *Btk* is provided by endogenous regulatory cis-acting elements that are present on a 340-kb transgene, Yc340-h*Btk*. The expression pattern of the transgenic human *Btk* was found to parallel that of the endogenous murine gene. When the Yc340-h*Btk*-transgenic mice were mated onto a *Btk*-deficient background, the *xid* B cell defects were fully corrected: conventional and $CD5^+$ B-1 B cells were present in normal numbers, serum IgM and IgG3 levels as well as responses to T cell-independent type II antigens were in the normal ranges. *In vivo* competition experiments in $Btk^{+/-}$ female mice demonstrated that in the conventional B cell population the Yc340-h*Btk* transgene could fully compensate the absence of expression of endogenous murine *Btk*. We conclude that in the YAC-transgenic mice *Btk* is appropriately expressed in the context of native regulatory sequences.

1 Introduction

Bruton's tyrosine kinase (Btk) is a non-receptor protein tyrosine kinase that is mutated in X-linked agammaglobulinemia (XLA) in man and X-linked immunodeficiency in the mouse [1–4]. XLA is characterized by severe and recurrent bacterial infections. Affected males have very low serum levels of all Ig classes. In the periphery, surface Ig^+ B cell numbers are severely decreased and plasma cells are virtually missing. Because in BM of XLA patients pre-B cells are present, the disease is manifested as an arrest in differentiation of pre-B cells to later B cell stages (for review see [5]). Although *Btk*-deficient mice exhibit a less severe B cell deficiency, the first selective disadvantage of *Btk*-deficient cells was also found at the transition from small pre-B to immature B cells in the BM [6]. Both CBA/N mice carrying an Arg_{28} pleckstrin homology (PH) domain mutation, and mice with targeted disruptions of *Btk* in their germ line display the x-linked immunodeficiency (*xid*) phenotype. The disorder is characterized by a decrease of peripheral B cell numbers, specifically of mature surface $IgM^{low}IgD^{high}$ cells, an absence of peritoneal $CD5^+$ B-1 B cells, low levels of serum IgM and IgG3 and severely impaired responses to T cell-independent type II (TI-II) antigens [6–8].

The *Btk* gene encodes a 659-amino acid protein that contains a single kinase domain, the *src* homology domains SH2 and SH3, and an N-terminal region with a PH domain and a unique proline-rich Tec homology (TH) domain [1–5]. Several molecules, including *Src* family kinases, protein kinase C, $\beta\gamma$ subunits of heterotrimeric G proteins, and the 120-kDa protein encoded by the c-*cbl* proto-oncogene have been shown to interact with the individual domains of *Btk*, mainly by studies *in vitro* (reviewed in [9]). *Btk* has been implicated in signaling events induced by cross-linking of the surface Ig receptor, IL-5, IL-6, CD38 and CD40 in B cells and FcεRI in mast cells and basophils [5, 9].

The expression pattern of the *Btk* gene was investigated in mice and man using cultured cell lines [10, 11], as well as by analysis of β-galactosidase activity *in vivo* in mice with a targeted in-frame insertion of a *lacZ* reporter in the *Btk* gene [6]. *Btk* is expressed throughout B cell development, from the earliest identifiable pro-B cell stage ($B220^+CD43^+HSA^-Ig^-$; 12) up to mature B cell stages. At the transition from mature B cells to plasma cells, expression is down-regulated. *Btk* is not expressed in the T cell lineage. Although *Btk* is also expressed in myeloid cells, it is not required for myeloid differentiation, since myeloid cells are not affected in XLA or in *xid* [5].

The tissue-specific and differentiation stage-specific *Btk* expression may – at least in part – be accomplished by the *Btk* promoter region, which contains several binding sites for the transcription factors Sp1 and PU.1 [13, 14]. Although transient transfection experiments implicate PU.1 as a major regulator for *Btk* expression in B cells and myeloid cells, other elements may well be required, especially because *Btk* expression is not abolished in fetal liver of PU.1-deficient mice [14].

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Abbreviations: BCR: B cell receptor **(h)Btk:** (Human) Bruton's tyrosine kinase **TD:** Thymus-dependent **TI-II:** Thymus-independent type II **XLA:** X-linked agammaglobulinemia **xid:** X-linked immunodeficiency

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