

PP-28

BTK PROTEIN DETERMINATION BY FLOW CYTOMETRY IN PATIENTS WITH X-LA

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X linked agammaglobulinemia (X-LA), is caused as a result of mutations in the Bruton's tyrosine kinase (Btk) gene and characterized by the discontinuation in stages of maturing and differentiation of B lymphocytes. Eightyfive % of agammaglobulinemia (AG) cases have mutations in Btk gene rendering in absence of mature B and plasma cells and very low levels of serum immunoglobulin. After the demonstration of absence of B cell and BTK expression with immunophenotyping by flow cytometry (FCM) in cases of AG, Btk gene mutation analysis can be carried out for the molecular genetic diagnosis of X-LA. The aim of this study is to determinate BTK expression in monocytes of AG patients and their mothers by FCM before BTK gene mutation test with PCR. We analysed 11 AG male patients having low Ig levels and CD19 expression with recurrent infections. In order to prove B cell deficiency, B cell expression has been determined by FCM using anti-CD19 monoclonal antibodies (mAb). Btk expression in B cells and monocytes were determined by using intracytoplasmic staining method with anti-BTK mAb after surface staining with anti-CD45 and -CD14 (Figure 1). Following this, DNA samples of the cases were screened for Btk gene mutation with PCR (Table 1). Five of 11 patients with AG had no Btk expression in monocytes (Figure 2) but had mutations in Btk gene. Ten of 11 cases had very low expression of B cells (<1.59%). Five of them had normal Btk expression in monocytes with no mutation in Btk gene. AG cases with Btk gene mutation were diagnosed as X-LA. For the remaining 6 patients, bone marrow aspirate analysis is planned with FCM in order to obtain further data with respect to the type of B cell precursor and also mutation analysis in genes (like BLNK) responsible for B cell maturation.

Keywords: agammaglobulinemia, Bruton's, tyrosine kinase, flow cytometry, immunodeficiency

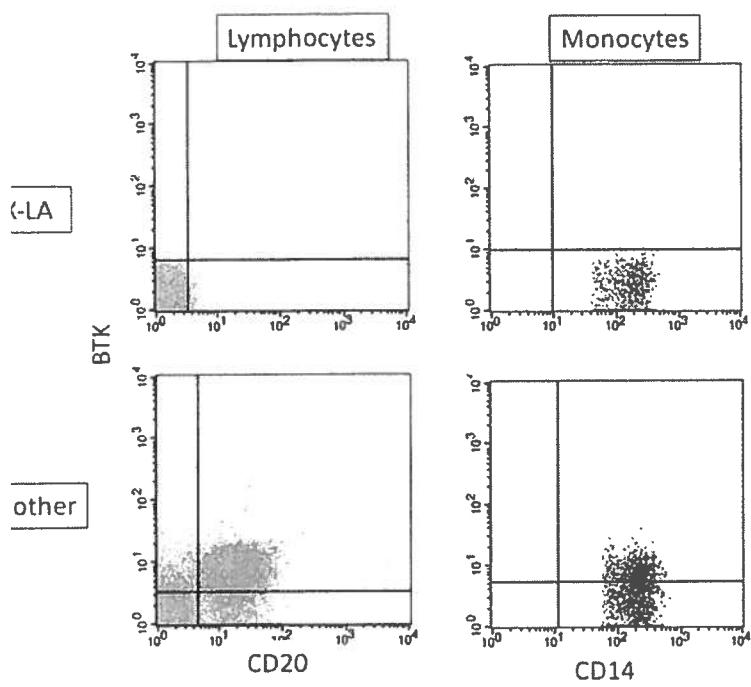


Figure 1: Btk expression in X-LA case is negative in monocytes

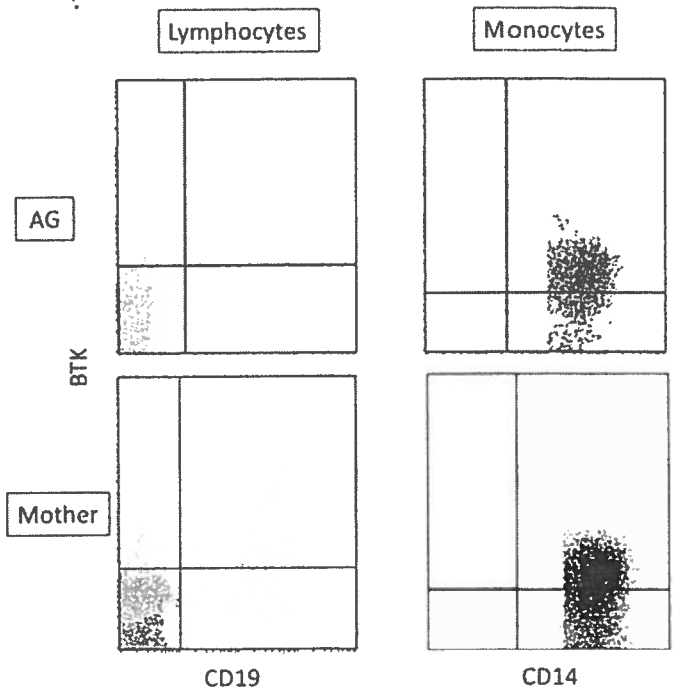


Figure 2: Btk expression in AG case is positive in monocytes

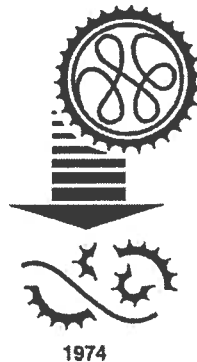
Case	B cell % (lymphocyte gate)	Btk (monocyte gate)	Btk mutation	Diagnosis
AG 1	0,00	Negative	Positive	X-LA
AG 2	0,03	Negative	Positive	X-LA
AG 3	11,22	Positive	Negative	
AG 4	0,05	Negative	Positive	X-LA
AG 5	0,00	Negative	Positive	X-LA
AG 6	0,00	Positive	Negative	
AG 7	0,05	Positive	Negative	
AG 8	1,59	Positive	Negative	
AG 9	0,00	Negative	Positive	X-LA
AG 10	1,59	Positive	Negative	
AG 11	0,48	Positive	Negative	

Table 1: AG cases analysed by FCM

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