Journal of Immunological Methods 362 (2010) 1–9

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Review

Using flow cytometry to screen patients for X-linked lymphoproliferative disease due to SAP deficiency and XIAP deficiency

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ARTICLE INFO

Article history:
Received 10 March 2010
Received in revised form 29 July 2010
Accepted 18 August 2010
Available online 9 September 2010

Keywords:
X-linked lymphoproliferative disease
Hemophagocytic lymphohistiocytosis
XIAP
SAP
BIRC4
SH2D1A

ABSTRACT

X-linked lymphoproliferative disease is a rare congenital immunodeficiency that is most often caused by mutations in *SH2D1A*, the gene encoding signaling lymphocyte activation molecule (SLAM)-associated protein (SAP). XLP caused by SAP deficiency is most often characterized by fulminant mononucleosis/EBV- associated hemophagocytic lymphohistiocytosis (HLH), lymphoma, and dysgammaglobulinemia. XLP has also been found to be caused by mutations in *BIRC4*, the gene encoding X-linked inhibitor of apoptosis (XIAP). Patients with XIAP deficiency often present with HLH or recurrent HLH, which may or may not be associated with EBV. XLP is prematurely lethal in the majority of cases.

While genetic sequencing can provide a genetic diagnosis of XLP, a more rapid means of diagnosis of XLP is desirable. Rapid diagnosis is especially important in the setting of HLH, as this may hasten the initiation of life-saving medical treatments and expedite preparations for allogeneic hematopoietic cell transplantation (HCT).

Flow cytometry offers a means to quickly screen patients for XLP. Flow cytometry can be used to measure lymphocyte SAP or XIAP protein expression, and can also be used to observe lymphocyte phenotypes and functional defects that are unique to XLP. This review will give a brief overview of the clinical manifestations and molecular basis of SAP deficiency and XIAP deficiency, and will focus on the use of flow cytometry for diagnosis of XLP.

Contents

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doi:10.1016/j.jim.2010.08.010

1. Introduction

X-linked lymphoproliferative disease (XLP; Mendelian Inheritance in Man [MIM] 308240) is a rare congenital immunodeficiency that was first described in 1975 (Purtilo et al., 1975). It is a disease characterized most often by fulminant mononucleosis/EBV-associated hemophagocytic lymphohistiocytosis (HLH), lymphoma, and dysgammaglobulinemia (Seemayer et al., 1995). XLP is prematurely lethal in the majority of cases, often due to EBV-associated HLH (Seemayer et al., 1995). The prevalence of XLP has been estimated at 2–3 per million males, (Purtilo and Grierson, 1991) though the frequency may be under-reported due to a variety of reasons including failure to recognize the disorder.

While clinical criteria exist for the diagnosis of patients with XLP, a genetic diagnosis was not possible until 1998 when it was discovered that the majority of cases of XLP are caused by mutations in *SH2D1A*, the gene encoding signaling lymphocyte activation molecule (SLAM)-associated protein (SAP) (Coffey et al., 1998; Nichols et al., 1998; Sayos et al., 1998). More recently, in 2006, mutations in *BIRC4* (encoding X-linked inhibitor of apoptosis, XIAP) were discovered in a minority of patients with XLP phenotypes (Rigaud et al., 2006). Because of these discoveries, a definitive genetic diagnosis is now possible in many patients with XLP phenotypes. Unfortunately, genetic studies often require several weeks to be completed. A rapid means of diagnosis of XLP and related disorders is desirable, especially in the setting of severe mononucleosis/hemophagocytic lymphohistiocytosis (HLH), as a clear diagnosis may hasten the initiation of life-saving medical treatments, as well as expedite preparations for allogeneic hematopoietic cell transplantation (HCT). The ability to use flow cytometry to quickly measure lymphocyte SAP or XIAP protein expression, or to observe lymphocyte phenotypes and functional defects that are unique to XLP, can facilitate a rapid diagnosis. These studies can also aid in the interpretation of genetic results when new or unreported sequence variants are encountered.

This review will give a brief overview of the clinical manifestations and molecular basis of SAP deficiency and XIAP deficiency, and will highlight the immunologic abnormalities that are unique to these disorders which can be exploited for use in patient screening with flow cytometry.

2. Clinical manifestations and molecular basis of SAP deficiency and XIAP deficiency

2.1. SAP deficiency

XLP is most often caused by deficiency of SLAM-associated protein (SAP) due to mutations in the *SH2D1A* gene found on chromosome Xq24-25 (Coffey et al., 1998; Nichols et al., 1998; Sayos et al., 1998). SAP is a 128-amino acid protein involved in the intracellular signaling of the SLAM (signaling lymphocyte activation molecule) family of receptors (Ma et al., 2007). XLP due to SAP deficiency usually presents in childhood or adolescence, and clinical manifestations include fulminant infectious mononucleosis/EBV-associated HLH (in ~60% of cases), lymphoproliferative disease including malignant lymphoma (~30%), hypo-/dysgammaglobulinemia (~30%), and aplastic anemia (3%) (Seemayer et al., 1995). Lymphomas are typically of B cell origin (non-Hodgkin's) and often occur in extra-nodal sites, particularly the ileocecal region (Harrington et al., 1987). Some patients with hypo-/dysgammaglobulinemia may be initially diagnosed as having common variable immune deficiency (Soresina et al., 2002; Aghamohammadi et al., 2003). Lymphocytic vasculitis, macrophage activation syndrome (an HLH variant), interstitial pneumonitis, and encephalitis have also been observed (Dutz et al., 2001; Kanegane et al., 2005; Snow et al., 2009; Talaat et al., 2009).

Loss of functional SAP causes several defects in lymphocyte function. In brief, SAP is necessary for normal T cell-dependent humoral immune responses, NK cell and CD8+ T cell cytotoxicity, and development of invariant natural killer T (iNKT) cells (Ma et al., 2007). More recently, SAP was found to be necessary for sustained T cell:B cell interactions that ensure proper germinal center formation and B cell help (Qi et al., 2008; Cannons et al., 2010). Moreover, SAP is also required for T cell restimulation-induced cell death (RICD), a self-regulatory mechanism of apoptosis critical for T cell homeostasis (Nagy et al., 2009; Snow et al., 2009). Although Epstein-Barr virus (EBV) has been historically identified as a triggering event for infectious mononucleosis and associated hemophagocytic lymphohistiocytosis (HLH), not all disease manifestations are associated with EBV, consistent with the presence of intrinsic lymphocyte defects.

2.2. XIAP deficiency

Deficiency of X-linked inhibitor of apoptosis (XIAP), caused by *BIRC4* gene mutations, was discovered to be associated with XLP phenotypes in 2006 (Rigaud et al., 2006). In contrast to SAP deficiency, over 90% of patients with XIAP deficiency develop hemophagocytic lymphohistiocytosis, with or without association with EBV, and recurrent HLH is common (Rigaud et al., 2006; Marsh et al., 2010; Zhao et al., 2010). A minority of patents may display hypogammaglobulinemia, and no cases of lymphoma have been observed in patients with XIAP deficiency to date.

XIAP is an inhibitor of apoptosis (IAP) family member, consisting of 3 baculovirus IAP repeat (BIR) domains and a C-terminal RING (really interesting new gene) domain. XIAP is best known for its caspase-inhibitory and anti-apoptotic properties, and BIR2 and its N-terminal linker region inhibit caspase-3 and caspase-7, while BIR3 inhibits caspase-9 (Chai et al., 2001; Huang et al., 2001; Shiozaki et al., 2003; Scott et al., 2005). The BIR regions of XIAP can also interact with non-caspase proteins such as RIP2 and TAB1. These and other XIAP interactions mediate signaling pathways involving Nuclear Factor-kappa B (NF-κB), c-Jun N-terminal kinase (JNK), NOD1 and NOD2, and the bone morphogenetic protein (BMP) receptors (Sanna et al., 1998; Yamaguchi et al., 1999; Lewis et al., 2004; Kaur et al., 2005; Lu et al., 2007; Krieg et al., 2009). The RING domain possesses an E3-ubiquitination function (Yang et al., 2000; Galban and Duckett, 2010). Exactly why deficiency of XIAP leads to the development of HLH is not currently understood.