The X-linked lymphoproliferative syndrome: molecular and cellular basis of the disease

drs Morra, M.

Citation for published version (APA):
The X-linked lymphoproliferative (XLP) disease is a human congenital immunodeficiency primarily characterized by an inappropriate response to the Epstein Barr virus (EBV) infection. XLP was defined as separate clinical entity for the first time almost thirty years ago by David Purtilo. Three main phenotypes are seen in XLP patients: fatal infectious mononucleosis (FIM), malignant B-cell lymphomas and dysgammaglobulinemia. An XLP patient can develop more than one phenotype, particularly after exposure to EBV. Most patients with XLP die by the age of 40 and more than 70% of patients die before the age of ten. In most cases affected young boys develop a fulminant infectious mononucleosis with features of a hemophagocytic syndrome. This illness is usually fatal, due to a large polyclonal proliferation of T and B cells, leading to marked liver necrosis and bone marrow failure. Although EBV infection is usually the triggering event for the acute lymphoproliferative illness, this is not necessarily the case for the dysgammaglobulinemia and the lymphomas.

The immune defects responsible for XLP are poorly characterized in the human. Contrary to the orchestrated immune response to EBV infection seen in normal individuals, XLP patients exhibit a deregulated response, characterized by an excessive accumulation of CD8 T cells, NK cells and macrophages, and by an inability to mount an appropriate antibody response against EBV. Over the past few years a number of break-through discoveries have progressively led to the identification of some of the molecular and cellular underpinnings responsible for the XLP pathogenesis. These findings have not only made possible an XLP molecular diagnosis, but also shed light on new families of lymphocyte co-receptors and intracellular adapters. This Thesis describes a molecular and functional characterization of the XLP pathogenesis. In 1998 the XLP gene was cloned. The identification of the gene stemmed from two different approaches, namely a classical positional cloning effort and the isolation of the XLP gene product, which associates with a lymphocyte surface marker. In Cox Terhorst’s Laboratory the XLP gene was
cloned serendipitously, while focusing the studies on the characterization of biochemical pathways induced by engagement of a surface glycoprotein termed SLAM (Signaling Lymphocytes-Activation Molecule or CD150), a lymphocyte co-receptor effective at inducing IFN-γ production by T cells upon α-SLAM antibody binding. A cDNA encoding a novel SLAM-associated protein (SAP) was isolated in a yeast two-hybrid system by virtue of its specific binding to the cytoplasmic tail of SLAM. SAP, also called SH2D1A, is a 128 amino acid protein consisting of an SH2 domain and a 24 amino acid C-terminal tail, prevalently expressed in T and NK cells.

Based on these findings, we went on to biochemically characterize the interaction between SAP and its co-receptor SLAM (Chapter 2). In other work not included in this Thesis we showed that SAP interacts via its SH2-domain with a motif (T-I-Y-x-x-V) present in the cytoplasmic tail of SLAM-related cell surface receptors CD84, CD229/Ly-9 and CD244/2B4, which are selectively expressed on different lymphocyte sub-populations. Moreover in a structural study we have previously shown that the interaction between SAP and Tyr281 of SLAM uses a unique three-pronged modality of binding that occurs independently of phosphorylation. A number of XLP patient missense mutations identified by our and other groups, and spanning the entire SAP SH2 domain were then chosen to further dissect this interaction (Chapter 2). Results defined two sets of single amino acid substitutions: i) mutants with a marked decreased in protein half-life (e.g. Y7C, S28R, Q99P, P101L, V102G and X129R), or ii) mutants with structural changes that differently affect the interaction with SLAM receptors. In the second group, mutations that disrupt the interaction between SAP SH2 domain hydrophobic cleft and Val +3 of its binding motif (e.g. T68I) and mutations that interfere with the SAP phosphotyrosine-binding pocket (e.g. C42W) abrogate SAP binding to all SLAM receptors. A mutation in SAP able to interfere with Thr -2 of the CD150 binding motif (mutant T53I) was found and severely impaired non-phosphotyrosine interactions whereas preserving unaffected the binding of SAP to phosphorylated SLAM. Mutant T53I, however, did not bind to CD229 and CD224, suggesting that SAP control several
critical signal transduction pathways in T and NK cells. No correlation was seen between the different types of mutation and XLP patients clinical presentation thus indicating that additional unidentified genetic or environmental factors must play a strong role in XLP disease manifestations.

To deepen our structural and biochemical understanding of SAP we next focused on a previously reported mouse cDNA, termed EAT-2, which encodes a 132 amino acids single SH2-domain protein homologous to SAP with unknown functions (Chapter 3). The human homologue of mouse EAT-2 was cloned and its gene was located on chromosome 1q23 near the locus of SLAM and related genes. EAT-2 and SAP exon/intron organization analysis suggested their origin by duplication from a common ancestor gene. Because EAT-2 is prevalently expressed in B-lymphocytes and macrophages we propose it may represent the SAP equivalent in Antigen Presenting Cells (APCs) for it binds to CD84, SLAM/CD150, CD244, and CD229 through its SH2 domain. We also show that the structure of a complex of EAT-2 with a phospho-Tyr peptide (pTyr281) derived from the SLAM cytoplasmic tail is very similar to that of SAP with the same peptide. This explains the high affinity of EAT-2 for the pTyr motif in the cytoplasmic tail of SLAM, but unlike SAP, EAT-2 does not bind to non-phosphorylated SLAM. Because EAT-2 over-expression increases SLAM-receptors tyrosine phosphorylation and interferes with the recruitment of the tyrosine phosphatase SHP-2, EAT-2 may act as a blocker in analogy to SAP. We speculate that SAP and EAT-2 are introduced into the immune synapse via SLAM and CD229, CD84 or CD244 to ensure their presence at the T cell/APC interface. It is likely that following T cell receptor (TCR) or B cell receptor (BCR) triggering SLAM receptors are rapidly tyrosine phosphorylated, thus recruiting EAT-2 and SAP. In this fashion, SAP and EAT-2 could function indirectly to prolong phosphorylation of important substrates during TCR triggering. Thus, EAT-2 and SAP are free "floating" SH2-domains that define a new class of proteins, which play a role either in T cells or in APCs. Because patients characterized by a chronic infection by EBV and XLP-like patients with a negative family history tested

195
negative for SAP mutations, EAT-2 may be involved in the pathogenesis of XLP-related diseases, lymphomas and dysgammaglobulinemia.

Having established molecular basis through which SAP and EAT-2 adapters signal in lymphocytes and examined structural defects related to XLP patients, we focused on the functional definition of B-cellular events key for the XLP pathogenesis. As mentioned earlier, XLP has three major clinical phenotypes. Not only XLP patients suffer from chronic infections because of defective immunoglobulin production: the definition of XLP B cell abnormalities is central in the understanding of EBV-related lymphomagenesis. Our hypothesis was that SAP, in addition to T and NK cell function, is a key regulator of B cell functions and immunoglobulin production. The existence of a strong link between SAP and immunoglobulin production was clearly demonstrated when two unrelated families of patients previously diagnosed as affected by Common Variable Immuno Deficiency (CVID) were found to have mutations in the SAP gene (Chapter 4). In one family with progressive immunoglobulin deficiencies, three brothers presented with recurrent respiratory infections, while female family members showed only elevated serum IgA levels. In the second family, two brothers had B-lymphocytopenia and immunoglobulin deficiencies. Besides allowing genetic counseling and selection of a more aggressive therapy (such as bone marrow transplantation) for SAP-deficient CVID patients, these data clearly linked defects in the SAP gene to B cell abnormalities.

In order to determine the cellular basis of the B cell defect, SAP−/− mice were used (Chapter 5). Previous reports have indicated that SAP−/− mice T cells are impaired in their ability to differentiate in Th2 cells. Moreover, SAP−/− mice infected with the lymphocytic choriomeningitis virus (LCMV) or the Toxoplasma Gondii have greatly increased number of activated T cells producing IFN-γ in the spleen and liver compared to wt mice. To further dissect T and B cell dependent defects, SAP-deficient mice humoral responses were studied and specific antibody responses analyzed. In this chapter we show that primary and secondary IgM and IgG1, IgG2a, IgG2b and IgG3 responses to KLH and Ovalbumin are severely impaired in SAP-
deficient mice whereas T-Independent responses are preserved. In keeping with a
class-switching defect, Germinal Centers were absent in the spleen of the mutant
mice. Naive and TCR Tg SAP-deficient CD4 cells showed a severe impairment in
IL-4 production whereas SAP^/-^ B cells have reduced CD23 expression.
Dysgammaglobulinemia with low concentration of total IgG1 and IgE, and increased
IgG2a is also present in the serum of unimmunized SAP^/-^ mice. Transfer of primed
SAP-deficient T and B cells into irradiated wr mice demonstrated that combined
CD4 defects and inefficient generation of memory B cells contributes to the aberrant
antibody responses. Primary CD4 and B cell defects were also evident after transfer
of non-primed SAP-deficient cells into RAG2^/-^ recipients. Thus, the inefficient
primary antibody production is compatible with an early defect in T-Dependent B
cell help. Dysgammaglobulinemia and generalized Ig class-switching defects of
SAP^/-^ mice are also in line with observations in XLP patients. Our findings support
the notion that complex B and Th2 cell defects are responsible for the severe
alteration of humoral responses observed in the XLP disease. EBV exploits the
normal pathways of B cell differentiation so that the EBV-infected B blast can
become a resting memory cell. It is appealing to speculate that a failure in generating
B cell memory in absence of SAP would lead to a failure of infected cells to
differentiate into the memory compartment, resulting in stable expression of growth-
promoting genes and a concomitant risk of tumors development like in the XLP.

The results presented in this Thesis have contributed to further defining structural
and functional mechanisms involved in the pathogenesis of XLP. We hope that our
results will contribute to development of specific therapies for patients affected by
XLP and related diseases such as lymphomas and the CVID. Moreover, because the
SAP and SLAM families of genes are likely to regulate immune responses at
multiple levels, these results will help to advance our understanding on how the
immune system works in normal and pathological situations.