SLAM-associated Protein Deficiency Causes Imbalanced Early Signal Transduction and Blocks Downstream Activation in T Cells from X-linked Lymphoproliferative Disease Patients*

Silvia Sanzone†‡‡, Maximilian Zeyda†‡‡, Marcus D. Saemann***, Maddalena Sonceini†‡‡, Wolfgang Holter†‡‡, Gerhard Fritsch†‡‡, Walter Knapp***, Fabio Candotti‡§§, Thomas M. Stulnig†¶¶, and Ornella Parolini†¶¶

From the †Centro Ricerche Parco Scientifico "E. Menini," Ospedale Poliambulanza, Brescia 25124, Italy, ‡‡Dipartimento di Scienze e Tecnologie Biomediche, Laboratorio Interdisciplinare Tecnologie Avanzate, 20090 Segrate, Milano, Italy, the †¶¶Division of Endocrinology and Metabolism, Department of Internal Medicine III, and the ‡§§Institute of Immunology, University of Vienna, A-1090 Vienna, Austria, the †¶¶Disorders of Immunity Section, Genetics and Molecular Biology Branch, NHGRI, National Institutes of Health, Bethesda, Maryland 20892, and the ¶¶¶CeMM-Center of Molecular Medicine of the Austrian Academy of Sciences, A-1090 Vienna, Austria

Deficiency of SAP (SLAM signaling lymphocyte activation molecule-associated protein) protein is associated with a severe immunodeficiency, the X-linked lymphoproliferative disease (XLP) characterized by an inappropriate immune reaction against Epstein-Barr virus infection often resulting in a fatal clinical course. Several studies demonstrated altered NK and T cell function in XLP patients; however, the mechanisms underlying XLP disease are still largely unknown. Here, we show that non-transformed T cell lines obtained from XLP patients were defective in several activation events such as IL-2 production, CD25 expression, and homotypic cell aggregation when cells were stimulated via T cell antigen receptor (TCR)-CD3 but not when early TCR-dependent events were bypassed by stimulation with phorbol 12-myristate 13-acetate/ionomycin. Analysis of proximal T cell signaling revealed imbalanced TCR-CD3-induced signaling in SAP-deficient T cells. Although phospholipase Cγ1 phosphorylation and calcium response were both enhanced in T cells from XLP patients, phosphorylation of VAV and downstream signal transduction events such as mitogen-activated protein kinase phosphorylation and IL-2 production were diminished. Importantly, reconstitution of SAP expression by retroviral-mediated gene transfer completely restored abnormal signaling events in T cell lines derived from XLP patients. In conclusion, SAP mutation or deletion in XLP patients causes profound defects in T cell activation, resulting in immune deficiency. Moreover, these data provide evidence that SAP functions as an essential integrator in early TCR signal transduction.

Mutations or deletions in the SH2D1A1 (SH2-containing adapter protein SH2 domain protein 1A) gene result in an immune disorder, the X-linked lymphoproliferative disease (XLP), which is usually associated with fatal outcome after EBV infection (reviewed in Ref. 1). The SH2D1A gene encodes SLAM-associated protein (SAP), which is primarily expressed in T and NK cells, in the reactive germinal centers and paracortical regions of lymphoid tissues (2, 3), in some Epstein-Barr virus (EBV)-positive B cell and Burkitt lymphoma cell lines, but not in purified CD 19+ B cells from peripheral blood of healthy controls (4, 5). SAP-deficient T and NK cells have been reported to be functionally impaired (6–8). Several studies have described a functional role of SAP in NK cell activation (6, 9, 10), but the role of this protein in T cell-mediated immune responses still remains largely enigmatic.

T cell activation requires cross-linking of the T cell receptor (TCR)+CD3 complex. Upon TCR-CD3 binding by major histocompatibility complex-presented antigen or CD3-specific antibodies, cascades of protein tyrosine phosphorylations are rapidly induced. Subsequently, the adapter protein LAT (linker for activation of T cells) recruits phospholipase C (PLC)γ1 to the cell membrane, enabling its activation by tyrosine phosphorylation. Activated PLCγ1 liberates inositol (1,4,5)-trisphosphate from the plasma membrane, mediating an increase in cytoplasmic calcium concentration, a major event for promoting downstream activation (11, 12). Furthermore, LAT mediates activation of the Ras/ERK pathway (13, 14), as well as tyrosine phosphorylation of the GTP exchange factor VAV (15). TCR-induced VAV-dependent cytoskeletal rearrangements are necessary for the maintenance of tyrosine phosphorylation and thus for effective signal transduction (16, 17). Moreover, T cell adhesion is controlled by VAV-mediated changes of the cytoskeleton that result in enhanced avidity of β1 or β2 integrins (18–20). Early signaling events, induced by TCR-CD3 plus

* This work was supported by the Austrian Science Foundation Grant P13973GEN (to O. P.) and Grant P13507-B01 (to T. M. S.), CCRI-Children’s Cancer Research Institute Vienna and Ministero dell’Istruzione, dell’Università e della Ricerca Italy Grant Fundo per gli Investimenti della Ricerca di Base 2002 (to O. P.), and by the CeMM - Center of Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria (to T. M. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† Both authors contributed equally to this work.
¶ To whom correspondence should be addressed: Ornella Parolini, Centro Ricoerc Parco Scientifico “E. Menini,” Ospedale Poliambulanza, Via Romiglia 4, 25124 Brescia, Italy. E-mail: ornella.parolini@tin.it or ornella.parolini@univie.ac.at.

This paper is available on line at http://www.jbc.org
costimulatory receptors such as CD28 (21), promote activation of mitogen-activated protein kinases (MAPK) such as ERK, JNK, and p38, which mediate the transactivation of critical transcription factors such as NF-κB (22, 23). In consequence, several genes encoding cytokines, including IL-2, and cytokine receptors are transcribed to drive the immune response.

Previous studies have suggested that SAP acts by binding of its SH2 domain to a consensus motif in the cytoplasmic tail of the cell surface proteins CD150 (SLAM signaling lymphocyte activation molecule), CD244 (B4), CD229 (Ly-9), CD84, and NK-T-B-antigen (NTA-B) (1, 24–27). However, recent work indicates also a direct role of SAP in cytokine-mediated signaling. Here, we report on retroviral-mediated CD4+ T cell lines from XLP patients were shown to exhibit elevated levels of early tyrosine phosphorylation of CD3ζ chain, Zap 70, and Chl, along with altered MAPK activation and cytokine production (28).

In this study, we show that non-transformed T cell lines derived from XLP patients are defective in several activation events, leading to impaired IL-2 production. Importantly, defects were bypassed by stimulation with PMA/ionomycin and corrected by retroviral-mediated expression of the SH2D1A gene. Analysis of proximal T cell signaling revealed that SAP deficiency causes an imbalanced signaling with enhanced calcium response but defects in other proximal signaling events, which could underlie inactiuate T cells downstream activation. In conclusion, these data indicate that SAP functions as an integrator in very early T cell signal transduction that is crucial for proper T cell activation.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—Antibodies were obtained as follows: CD3 (anti-CD3), Leu-28 (anti-CD28), anti-CD3ε, PE-labeled anti-CD25 and PE-labeled anti-IL-2 from PharMingen; anti-PLCγ, anti-phospho JNK and anti-JNK-1, anti-IB-α and anti-p38 from Santa Cruz Biotechnology (Santa Cruz, CA); anti-phospho-p38 from New England Biolabs (Beverly, MA); anti-VAV and horseradish peroxidase-conjugated anti-phosphotyrosine (4G10) from Upstate Biotechnology (Lake Placid, NY); anti-SAP from Exalpa Biologicals (Boston, MA); F(ab')2-fragments of goat anti-mouse IgG from Sigma; horseradish peroxidase-labeled goat anti-mouse IgG from Bio-Rad; horseradish peroxidase-labeled goat anti-rabbit IgG from Accurate (Westbury, NY).

**T Cell Line Establishment**—Two XLP patients and four healthy age-matched controls were included in the study. The two XLP patients (H.X., T.D.) have been characterized previously (29). They presented with the following SH2D1A mutations: patient H.X. revealed a g deletion at the invariable dinucleotide of the acceptor splice site of exon 3, resulting in a splicing defect that causes a frameshift with premature stop codon; patient T.D. presented a G to T nucleotide change at the translation initiation codon (ATG to ATT), leading to a methionine to isoleucine amino acid change. T cell lines were generated according to a previously described protocol (30). Briefly, 5 × 10⁵ peripheral blood lymphocytes were cultured in 24-well plates (Costar) with 2 × 10⁴ allogeneic irradiated (50,000 milligray) EBV-transformed B cells and 1 × 10⁵ allogeneic irradiated peripheral blood lymphocyte cell, in 1 ml of Yssel’s medium supplemented with 10% fetal calf serum, phytohemagglutinin (1 μg/ml, Sigma) and rhIL-2 (100 units/ml, kindly provided by Dr. Aversa, Novartis Institute, Vienna, Austria). Cells were maintained and expanded by periodic media changes and restimulation every second week. T cell lines were cultured in medium without rhIL-2 for 3 days before performing all functional assays reported here.

Periodic immunophenotyping revealed 100% CD3+ cells and normal percentages of CD4+ and CD8 (60 ± 15%; 40 ± 15%, respectively) in T cell lines from XLP patients and healthy controls. No significant variations were observed during cell expansion or after retroviral gene transfer.

**T Cell Proliferation Assay**—Fourteen to seventeen days after the last stimulation, 1 × 10⁵ cultured T cells were plated in U-bottom 96-well plates in 200 μl of RPMI supplemented with 10% fetal bovine serum in the presence of soluble anti-CD3 (1 μg/ml), alone or in combination with soluble anti-CD28 (7 μg/ml) and PMA (0.1 μM) plus ionomycin (1 μM). Three days later, cells were harvested for scintillation counting after pulsing with 1 μCi of [H]-labeled tritiated thymidine during the last 16 h of culture.

**SH2D1A Retroviral Vector Generation, Transfections, and Transductions**—The SH2D1A cDNA (31) and the MND-IRES-EGFP retroviral vector were generous gifts of Drs. C. S. Duckett (University of Michigan) and D. B. Kohn (Children’s Hospital Los Angeles), respectively. The full-length SH2D1A DNA was blunt-ended and ligated into the SpeI site of MND-IRES-EGFP (M-IE), upstream of the IRES sequence, thus generating MND-SH2D1A-IRES-EGFP (M-SAP-IE), a bicistronic retroviral vector expressing both SH2D1A and EGFP under the control of the myeloproliferative sarcoma virus LTR. M-SAP-IE and MND-IRES-EGFP plasmid DNAs (20 μg) were transfected into the ectropic packaging cells GP+ E-86 (32). EGFP-positive cells were FACS-sorted (FACS Vantage, BD Biosciences) and used to produce retroviral supernatant that was in turn used to transduce the gibbon-ape leukemia virus envelope packaging cell PG13 (33). EGFP-positive PG13 cells were FACS-sorted and used to generate retroviral supernatant that was used to transduce SAP-deficient and control T cells. Initial transduction efficiency, as evaluated by determination of EGFP fluorescence, varied from 5 to 10% for both the control (M-IE) and SH2D1A constructs (M-SAP-IE). Positive cells were sorted (FACS Vantage, BD Biosciences) and restimulated for two rounds resulting in a 100% EGFP-positive population. Retrovirus-mediated expression of SAP protein in XLP cell lines was proven by Western blot analysis (data not shown). All retroviral cell lines were maintained at 37°C, 5% CO2 in Dulbecco’s modified Eagles medium supplemented with 10% fetal bovine serum, 5 mM l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (all from Invitrogen).

**Expression of IL-2 and CD25, Homotypic Cell Aggregation**—T cells from controls and XLP patients (10⁶ cells/well) were plated in RPMI supplemented with serum in 24-well plates and stimulated with 1 μg/ml anti-CD3, alone or in combination with 7 μg/ml Leu-28 or with PMA (10 ng/ml) plus ionomycin (1.5 μM) at 37°C for 8 h in the presence of brefeldin-A (5 μg/ml). After washing with phosphate-buffered saline, the cells were fixed with 4% formaldehyde solution for 20 min, washed with saponin (0.1%), and incubated with PE-labeled rat anti-human IL-2 for 25 min at room temperature. The samples were analyzed using a FACScalibur (BD Biosciences). For detection of homotypic aggregation, cells were stimulated in the same way but without brefeldin-A for 24 h at 37°C. Photographs were taken, and cells were analyzed by standard flow cytomeric analysis using anti-CD25-PE.

**Protein Phosphorylation and Jnk Degradation**—Cells were washed with Hank’s balanced salt solution (Invitrogen) plus 10 μM Hepes, pH 7.4, and for induction of VAV and MAPK phosphorylation as well as Jnk degradation, preincubated on ice with 10 μg/ml Leu 28 for 15 min followed by 5 μg/ml anti-CD3 for 1–15 min at 37°C. Phosphorylation of PLCγ was induced by incubation with 10 μg/ml anti-CD3 cross-linked with 1 μg/ml F(ab)2-fragments of goat anti-mouse IgG for 2 min at 37°C. After stopping by addition of ice-cold RPMI medium, cells (5–10⁷ cells/ml) were lysed on ice for 30 min in Tris-buffered saline (pH 7.4) containing 1% Nonidet P-40 (Pierce), phosphatase (1 μM sodium orthovanadate, 10 μM NaF, 5 μM sodium pyrophosphate, 25 μM β-glycerophosphate, 5 μM EDTA), and protease inhibitors (10 μg/ml aprotonin; 5 μM iodoacetamide, 10 μg/ml leupeptin, 1 μM pepstatin, 0.4 μM Pefabloc). After removing nuclei by short centrifugation, samples were boiled in SDS sample buffer. Proteins were separated by reducing SDS-PAGE and blotted onto nitrocellulose membranes (Hybond ECL, Amersham Biosciences). Phosphorylation of ERK, JNK, and p38 was determined with phospho-specific antibodies. Subsequently, membranes were stripped and reprobed for non-phosphorylated antigen as a loading control. PLCγ and VAV tyrosine phosphorylation detected by anti-phosphotyrosine monoclonal antibody and subsequent stripping and reprobing of the membrane with anti-PLCγ and anti-VAV, respectively. Jnk degradation was calculated by subtracting the amount of Jnk in stimulated samples from that of unstimulated samples, which were set to 100%. Chemiluminescence was generated by BM chemiluminescence substrate (Roche Applied Science) and quantified on a Lumi-Imager (Roche Applied Science) as detailed elsewhere (34).

**Quantitation of Cytoplasmic Calcium Response**—Cells were labeled with the fluorescent Ca²⁺-indicator INDO-1 acetoxymethylster (Molecular Probes, Eugene, OR) by incubation of 2 × 10⁶ cells/ml in Hank’s balanced salt solution, 1% bovine serum albumin, and 2 μM INDO-1 at 37°C for 30 min. Samples (10 μM cells in 250 μM HEPES, pH 7.4) were then equilibrated to 37°C for 7 min. During flow cytometric measurement, cells were stimulated via CD3 by addition of 2 μg of anti-CD3. Measurement of cytoplasmic Ca²⁺ concentration by flow cytometry was performed on
a FACStar™ Plus (BD Biosciences) equipped with an argon laser (excitation, 353-363 nm; detection, 530 nm for calcium-free INDO-1, 395 nm for calcium-bound form) at 37 °C constant temperature as detailed (35). The ratio of the fluorescence intensities at both wavelengths was computed as a direct estimate of the cytoplasmic calcium concentration and was determined for 5 min following stimulation.

**Cell Adhesion**—T cell lines from controls and XLP patients (10⁵ cells/well) were plated on ice for 45 min in Iscove's modified Dulbecco's medium supplemented with 0.4% bovine serum albumin in 96-well flat bottom cell culture plates coated with fibronectin (20 μg/ml in phosphate-buffered saline). Cells were stimulated with different concentrations of anti-CD3 (from 2.5 to 0.15 μg/ml in serial dilution 1:2) or with PMA (100 ng/ml) for 15 min at 37 °C. After washing three times in phosphate-buffered saline, each well was photographed for counting adherent cells.

**Statistics**—Patient and control cells were always tested in parallel. Values of controls and patients were related to the mean of controls of the same experiments, which was set to 100%. Differences between patient and control cells were compared by two-tail unpaired Student's t test. For comparison of cells transduced with M-SAP-IE and M-IE, values of control vector-transduced cells were related to cells from the same donor reconstituted with M-SAP-IE, which was set to 100%. Differences between M-IE vector and M-SAP-IE-transduced cells were analyzed by one-group student's t test. Data are given as mean ± S.E.; p < 0.05 was considered statistically significant.

**RESULTS**

**T Cells from XLP Patients Show Reduced Proliferative Response and Defective IL-2 Production That Are Corrected by Retroviral Introduction of SH2D1A Gene**—We obtained non-immortalized T cell lines from two previously diagnosed XLP patients (29) and several healthy controls. XLP T cell lines showed a significantly diminished proliferative response both to CD3 as well as to CD3 plus CD28 antibody stimulation (Fig. 1A). In contrast, cell proliferation following PMA/ionomycin stimulation was comparable between patients and controls (Fig. 1A). Furthermore, both CD3 alone and CD3 plus CD28 stimulation lead to a markedly decreased IL-2 production in XLP T cell lines when compared with normal controls as demonstrated by intracellular FACS analysis (Fig. 1B) and enzyme-linked immunosorbent assay testing of culture supernatants (data not shown). Importantly, also IL-2 production was completely unaffected in XLP T cells when early TCR-dependent events were bypassed by stimulation with PMA/ionomycin (Fig. 1B). To test whether the functional defects observed in patient-derived cell lines were caused by alterations of the SH2D1A gene, retroviral-mediated gene transfer was applied to express a normal copy of the SH2D1A cDNA in these cells. As postulated, activation defects in XLP T cell lines were restored following gene transfer. Both T cell proliferation and IL-2 production were significantly more pronounced in patients' T cell lines reconstituted with the M-SAP-IE vector when compared with those transduced with the M-IE control (Fig. 2, A and B). Transduction of SH2D1A gene in T cell lines derived from normal controls did not influence their functional responses (data not shown).

**Impaired Activation of T Cells from XLP Patients**—Besides production of interleukins, proliferation of T cells is regulated by expression of the inducible IL-2Ra chain (CD25), which enables the formation of the high affinity IL-2 receptor complex (36). Expression of CD25 in response to CD3/CD28 stimulation was significantly decreased in T cell lines derived from XLP patients when compared with controls (Fig. 3A). As observed for IL-2 production and cell proliferation, stimulation with PMA/ionomycin induced CD25 expression in T cells from patients and controls to a similar extent (Fig. 3A).

As a further hallmark of successful T cell activation we investigated homotypic cell aggregation; Fig. 3B shows typical CD3 and PMA/ionomycin-induced clustering of T cell lines derived from XLP patients and normal controls. Homotypic aggregation was severely impaired in XLP T cell lines when induced via CD3 but was comparable with controls after stimulation with PMA/ionomycin.

To elucidate the particular signaling events affected by SAP deficiency that lead to the observed downstream effects, we analyzed signaling pathways in T cell lines of XLP patients. Induction of IL-2 transcription requires activation of MAPKs such as ERK-1, -2, JNK, and p38 whose kinase activities are regulated by phosphorylation (23). CD3/CD28-stimulated phosphorylation of all three MAPKs was markedly decreased in XLP when compared with control T cell lines (Fig. 4A), although reduction in ERK phosphorylation failed to reach statistical significance. In addition to reduced MAPK phosphorylation, CD3/CD28-stimulated degradation of IκB, which masks the NFκB nuclear localization signal in quiescent cells, was profoundly blocked in XLP T cell lines when compared with controls (Fig. 4B). In summary, all
investigated downstream T cell signaling events were markedly impaired in XLP patients.

**SAP Deficiency Causes Imbalanced Proximal T Cell Signaling**—To elucidate the mechanisms underlying diminished downstream signaling in XLP T cell lines, we analyzed very proximal T cell signaling events. Interestingly, CD3-stimulated tyrosine phosphorylation of PLCγ1/H9253 was markedly enhanced when compared with control cell lines (Fig. 5A). Also, calcium responses were significantly increased in XLP T cell lines following stimulation with low concentrations of anti-CD3 that induced only minimal responses in control cells (Fig. 5B). Furthermore, calcium responses in XLP cells reconstituted with the SH2D1A gene were markedly diminished when compared with XLP cells transduced with control vector, indicating that the enhanced CD3-induced responses are due to SAP deficiency (Fig. 5C).

In contrast to PLCγ, the tyrosine phosphorylation of VAV following CD3/CD28-stimulation was significantly decreased in XLP T cell lines when compared with controls (Fig. 6A). To analyze whether the blocked VAV phosphorylation had an impact on VAV-dependent downstream events, we analyzed CD3-mediated cell adhesion. Following stimulation with different concentrations of anti-CD3, T cell adhesion to fibronectin was significantly reduced in XLP patients when compared with controls (Fig. 6B), whereas no difference between patients and controls was observed after PMA stimulation. Similar to the cell proliferation and IL-2 production experiments shown earlier, this adhesion defect of patient cell lines could be corrected by transfection with M-SAP-IE but not with the control vector M-IE (Fig. 6C). These results clearly indicate that SAP deficiency causes an imbalance in early signaling events associated with enhanced PLCγ/calcium signaling but impaired VAV phosphorylation and consequently defective T cell adhesion.

**DISCUSSION**

By analyzing non-transformed T cell lines from XLP patients before and after retroviral-mediated SH2D1A gene reconstitution, we show here that defective TCR-induced IL-2 production and T cell proliferation are caused by SAP deficiency. Our results with non-transformed T cells are in accordance with the
Role of SAP in TCR Signaling

FIG. 4. Defective downstream T cell signal transduction in XLP patients. T cells from controls and patients (pat.) H.X. and T.D. were stimulated via CD3/CD28, and as shown in A, analyzed for phosphorylation of different MAPks using antibodies specific for their phosphorylated isoforms as indicated. The signal of the phospho-MAPk was related to the amount of the respective proteins detected on stripped membranes. Combined results from seven independent experiments, each with two patients and four controls are shown in diagrams and are expressed as percentage of the mean of stimulated controls ± S.E. Significance of differences between patient and controls cells are indicated (*, p < 0.1; *, p < 0.05; ***, p < 0.001). As shown in B, lysates were immunoblotted for IxB. Membranes were reprobed for CD3ζ as a quantity control. The diagram shows mean ± S.E. of calculated IxB degradation of four independent experiments, each with two patients and four controls, expressed as percentage of control (**, p < 0.01).

Previously reported defective IL-2 production in Herpes saimiri-transformed XLP T cell lines (28). However, detailed analysis of crucial proximal T cell signaling events including studies with SH2D1A-reconstituted XLP T cell lines revealed an integrating role of SAP in early T cell signaling that may underlie the defective T cell activation in XLP patients.

SAP consists almost exclusively of an SH2 domain, which allows binding to phosphotyrosine residues of other proteins, and a short carboxyl terminus (37, 38). Due to these characteristics, a variety of possible binding partners and thus functions of SAP in different signaling pathways appear conceivable. For instance, SAP interacts with the cytoplasmic tails of members of the SLAM receptor family and has been proposed to play a role in SLAM-mediated signaling by recruiting the tyrosine kinase FynT and by preventing access of the phosphatase SHP-2 to this pathway (25, 39, 40). SLAM-mediated events seem to act as costimulatory signals in T cells and modulate cytokine profiles during T cell activation (25, 30, 41). Thus, SAP was suggested to contribute to T cell activation by controlling SLAM signaling (25–27, 42). However, the results presented here, corroborated by data from another study (28), indicate a direct role of SAP in TCR-CD3-mediated signal transduction.

The fact that stimulation with PMA and ionomycin overcomes defects of SAP-deficient T cells observed after stimulation via TCR-CD3 strongly indicates that SAP is involved in very proximal T cell signaling. Because cell proliferation, IL-2 production, and cell adhesion were impaired in XLP T cells when stimulated via CD3 plus CD28 and CD3 alone (Figs. 1, 2, and 6), it is likely that impairment of downstream T cell activation by SAP deficiency is primarily caused by a defect in TCR signal transduction. However, impairment of CD28 signaling or other costimulatory pathways cannot be ruled out by these experiments. Interestingly, defective CD3-stimulated T cell proliferation in the murine SAP-deficient model could be overcome by costimulatory signals from total splenocytes (7), whereas CD28 co-stimulation did not alter defective T cell proliferation or IL-2 production, as induced by CD3 alone in our experiments with human cells. Moreover, T cells from the
SAP-deficient mice exhibit no defect in IL-2 production. The differences of our results with these mouse data could be due to the fact that XLP patients could have encountered more infections than SAP-deficient mice, and that could have further modified TCR signaling. However, our experiments with SAP reconstitution clearly reveal that the observed alterations in TCR signaling of patients’ cell lines are due to SAP deficiency. Notably, EBV infection is regarded to be the most potent trigger of the XLP phenotype in humans but does not affect SAP-deficient mice because mice lack the receptor for this virus.

The central adapter for transduction in proximal CD3 signaling, LAT (13), mediates PLCγ/calcium signaling and activation of SOS/Ras/Erk (43) as well as VAV. VAV activity results in cytoskeletal reorganization, integrin clustering, and cell adhesion (19, 44, 45). Furthermore, VAV is involved in ERK and JNK activation (46, 47) as well as degradation of IκB (48). Our results revealed that SAP deficiency causes a striking imbalance in activation of these pathways. Although the PLCγ-mediated calcium response is significantly enhanced, virtually all other TCR-CD3-activated pathways are blocked in XLP T cells, indicating an integrative role of SAP for activation of diverse LAT-binding signaling proteins. Interestingly, distinct tyrosine residues of LAT are responsible for binding various molecules and hence activation of different pathways (49–51). There were no alterations in overall LAT tyrosine phosphorylation between XLP T cell lines and controls in our experiments (data not shown). However, SAP deficiency could provoke selective alterations in the phosphorylation of distinct LAT tyrosine residues that may be responsible for the imbalanced signaling in T cells from XLP patients.

Although SAP deficiency underlies enhancement and reduction of proximal T cell signaling events, all analyzed downstream signaling events were impaired in XLP T cells as well as IL-2 production and proliferation. The reduction of TCR-induced CD25 expression (Fig. 3) that is required for formation of high affinity IL-2 receptors probably contributes to the severely blocked proliferative response of XLP patient-derived T cells (Fig. 1). Notably, bypassing membrane proximal T cell signaling events by stimulation with PMA and ionomycin overcomes the impairment of downstream signaling events in SAP-deficient cells. Therefore, we postulate that the observed imbalance in early T cell signaling in SAP-deficient cells underlies the block in downstream activation in T cells from XLP patients. The observed reduction of homotypic cell aggregation as well as adhesion to fibronectin may indicate an impairment of cytoskeletal rearrangements and avidity signaling, which are necessary for T cells to interact with antigen-presenting cells or to act as effector cells in target cell lysis (52). Thus, these molecular functional alterations may be directly responsible for the inability of T cells of XLP patients to control the growth of EBV-transformed B cells.

In conclusion, this study reveals a striking imbalance in early TCR-CD3-mediated signaling events and impaired downstream T cell activation in T cells derived from XLP patients, which can be completely restored by retroviral-mediated reconstitution of SH2D1A gene. Our data therefore indicate an integrative role of SAP in TCR-mediated signaling, which might be of crucial importance for defense against specific antigenic challenges such as EBV infection.

Acknowledgments—We are indebted to Drs. Colin Duckett and Donald B. Kohn for providing SH2D1A cDNA and the MND-X-IRESGFP vector, respectively, and to G. Jayashree Jagadeesh, Birgit Kagerbauer, Tanja Thurn and Dieter Printz for providing excellent technical assistance.

REFERENCES

1. Morra, M., Howie, D., Grande, M. S., Sayos, J., Wang, N., Wu, C., Engel, P., and Terhorst, C. (2001) Annu. Rev. Immunol. 19, 657–682
2. Nichols, K. E., Harken, D. P., Levine, S., Kraemer, M., Kolquist, K. A., Genovese, C. J., Keen, A., Hau, L., Snyder, E., Buckler, A. J., Wise, C., Ashley, J., Lovett, M., Valentine, M. B., Look, A. T., Gerald, W., Housman, D. E., and Haber, D. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13765–13770
3. Shapatska, L. M., Mikhapal, S. V., Berdova, A. G., Zelensky, O. M., Yun, T. J., Nichols, K. R., Clark, E. A., and Sidorenko, S. P. (2001) J. Immunol. 166, 5480–5487
4. Nagy, N., Corbi, C., Mattsson, K., Maeda, A., Gogolak, P., Sumeri, J., Lanyi, A., Szekely, L., Carbone, E., Klein, G., and Klein, E. (2000) Int. J. Cancer 88, 439–447
5. Pardini, O., Weinhausel, A., Kagerbauer, B., Sassmann, J., Holter, W., Gaden, H., Haas, O. A., and Knapp, W. (2003) Immunogenetics 55, 116–121
6. Pardini, S., Bettino, C., Falco, M., Augugliaro, R., Giliani, S., Franceschini, R., Ochs, H. D., Wolf, H., Bonnefiy, J. Y., Biassoni, R., Moretta, L., Notarangelo, L. D., and Moretta, A. (2000) J. Exp. Med. 192, 337–346
7. Czir, M. J., Kersh, J. N., Sijarees, L. A., Lanzer, G., Lewis, J., Yap, G., Chen, A., Shor, A., Buckett, C. S., Ahmed, R., and Schwartzberg, P. L. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 7449–7454
8. Wu, C., Nguyen, K. B., Pien, G. C., Wang, N., Gullo, C., Howie, D., Sosa, M. R.,...
Role of SAP in TCR Signaling

29599

Edwards, M. J., Borrow, P., Satokati, A. R., Sharpe, A. H., Biron, C. A., and Terhorst, C. (2001) Nat. Immunol. 2, 410–414

Tangye, S. G., Chernyak, H., Lanier, L. L., and Phillips, J. H. (2000) Mol. Immunol. 37, 495–501

Avouaf, A., and Tan, R. (2002) J. Biol. Chem. 277, 13331–13337

Clapham, D. E. (1995) Cell 80, 259–268

Berridge, M. J., Lipp, P., and Bootman, M. D. (2000) Nat. Rev. Mol. Cell Biol. 1, 11–21

Zhang, W. G., Sloan-Lancaster, J., Kitchen, J., Trible, R. P., and Samelson, L. E. (1998) Cell 92, 83–92

Zhang, W. G., Irvin, B. J., Trible, R. P., Abraham, R. T., and Samelson, L. E. (1999) Int. Immunol. 11, 943–950

Fischer, K. D., Tedford, K., and Penninger, J. M. (1998) Semin. Immunol. 10, 317–327

Valitutti, S., Dussling, M., Aktories, K., Gallati, H., and Lanzavecchia, A. (1995) J. Exp. Med. 181, 577–584

Krawczyk, C., and Penninger, J. M. (2001) J. Leukocyte Biol. 69, 317–330

van Kooij, Y., van Vliet, S. J., and Figdor, C. G. (1999) J. Biol. Chem. 274, 26869–26877

Griffiths, E. K., and Penninger, J. M. (2002) Curr. Opin. Immunol. 14, 317–322

Krawczyk, C., Oliveira-dos-Santos, A., Sasaki, T., Griffiths, E., Ohashi, P. S., Snapper, S. Alt, F., and Penninger, J. M. (2002) Immunity 16, 331–343

Schwarz, R. H. (1992) Cell 71, 1065–1068

Karim, M., and Hunter, T. (1995) Curr. Biol. 5, 747–757

Garrington, T. P., and Johnson, G. L. (1999) Curr. Opin. Cell Biol. 11, 211–218

Bottris, C., Falco, M., Pardini, S., Marchenko, E., Augugliaro, R., Sivori, S., Landi, E., Biassoni, R., Notarangelo, L. D., Moretta, L., and Moretta, A. (2001) J. Exp. Med. 194, 235–242

Latour, S., Gish, G., Helgason, C. D., Humphries, R. K., Pawson, T., and Veillette, A. (2001) Nat. Immunol. 2, 681–689

Saya, J., Martin, M., Chen, A., Simarro, M., Howie, D., Morra, M., Engel, P., and Terhorst, C. (2001) Blood 97, 3867–3874

Howie, D., Simarro, M., Saya, J., Guarino, M., Sanchez, J., and Terhorst, C. (2002) Blood 99, 957–965

Nakamura, H., Zarycki, J., Sullivan, J. L., and Jung, J. U. (2001) J. Immunol. 167, 2657–2665

Galofra, O., Kagerbauer, B., Simonich-Hummel, I., Ambrus, P., Jaeger, U., Mann, G., Haas, O. A., Morra, M., Gaden, H., Terhorst, C., Knapp, W., and Hölter, W. (2002) Ann. Hematol. 81, 441–447

Cocks, B. J., Chang, C. C., Carlhollde, J. M., Yssel, H., de Vries, J. E., and Aversa, G. (1995) Nature 376, 260–263

Lewis, J., Eisen, J. L., Nelson, D. L., Cohen, J. I., Nichols, K. E., Ochs, H. D., Notarangelo, L. D., and Duckett, C. S. (2001) Clin. Immunol. (Orlando) 100, 15–23

Markowitz, D., Goff, S., and Bank, A. (1988) Virology 167, 400–406

Miller, A. D., Garcia, J. V., von Suhr, N., Lynch, C. M., Wilson, C., and Eiden, M. V. (1991) J. Virol. 65, 2220–2224

Zeyda, M., Staffler, G., Horejsi, V., Waldhaeusl, W., and Stulnig, T. M. (2002) J. Biol. Chem. 277, 28418–28423

Stulnig, T. M., Berger, M., Sigmund, T., Stockinger, H., Horejsi, V., and Waldhaeusl, W. (1997) J. Biol. Chem. 272, 19242–19247

Taniguchi, T., and Minami, Y. (1998) Cell 78, 5–8

Buday, L. (1999) Biochim. Biophys. Acta 1422, 187–204

Veillette, A. (2002) Science’s STKE http://stke.sciencemag.org/cgi/content/full/20021210/pe9

Sayas, J., Wu, C., Morra, M., Wang, N., Zhang, X., Allen, D., van Schaik, S., Notarangelo, L., Geha, R., Roncarolo, M. G., Oettgen, H., De Vries, J. E., Aversa, G., and Terhorst, C. (1998) Nature 395, 462–469

Chan, B., Lanyi, A., Song, H. K., Griesbach, J., Simarro-Grande, M., Poy, F., Howie, D., Sümegi, J., Terhorst, C., and Eck, M. J. (2003) Nat. Cell Biol. 5, 155–160

Heinzel, G., Kraft, M. S., Derfuss, T., Pirzer, R., de Saint-Basile, G., Aversa, G., Fleckenstein, B., and Meinl, E. (2001) Eur. J. Immunol. 31, 2741–2750

Latour, S., Roncagalli, R., Chen, R., Bakhinovskia, M., Shi, X., Schwartzberg, P. L., Davidson, D., and Veillette, A. (2003) Nat. Cell Biol.

Fino, T. S., Kadlecek, T., Zhang, W., Samelson, L. E., and Weiss, A. (1998) Immunity 9, 617–626

Babcock Wardenburg, J., Pappo, R., Bu, J. Y., Mayer, B., Chernoff, J., Straus, D., and Chan, A. C. (1998) Immunity 9, 607–616

Villalba, M., Bi, K., Rodriguez, F., Tanaka, Y., Schoenberger, S., and Altman, A. (2001) J. Cell Biol. 155, 331–338

Villalba, M., Coudronniere, N., Deckert, M., Teixeiro, E., Mas, P., and Altman, A. (2000) Immunity 12, 151–160

Moehler, A., Dienz, O., Hehner, S. P., Droge, W., and Schmitz, M. L. (2001) J. Biol. Chem. 276, 20022–20028

Marini, B., Costanzo, A., Viola, A., Michel, F., Mangino, G., Acuto, O., Leverero, M., Piccolella, E., and Tuosto, L. (2002) Eur. J. Immunol. 32, 447–455

Aguado, E., Richelme, S., Nunez-Cruz, S., Miazek, A., Mura, A. M., Richelme, M., Gou, X. J., Sainot, H., He, H. T., Malissen, B., and Malissen, M. (2002) Science 296, 2036–2040

Perez-Villar, J. J., Whitney, G. S., Sitnick, M. T., Dunn, R. J., Venkatasekaran, S., O’Day, K., Schievve, G. L., Lin, T. A., and Kanner, S. B. (2002) Biochemistry 41, 10732–10740

Sommer, C. L., Park, C. S., Lee, J., Feng, C., Fuller, C. L., Grinberg, A., Hildebrand, J. A., Lacana, E., Menon, R. K., Shores, E. W., Samelson, L. E., and Love, P. M. (2002) Science 296, 2040–2043

van Kooyk, Y., and Figdor, C. G. (2000) Curr. Opin. Cell Biol. 12, 542–547

Downloaded from http://www.jbc.org/ by guest on July 25, 2018
SLAM-associated Protein Deficiency Causes Imbalanced Early Signal Transduction and Blocks Downstream Activation in T Cells from X-linked Lymphoproliferative Disease Patients
Silvia Sanzone, Maximilian Zeyda, Marcus D. Saemann, Maddalena Soncini, Wolfgang Holter, Gerhard Fritsch, Walter Knapp, Fabio Candotti, Thomas M. Stulnig and Ornella Parolini

J. Biol. Chem. 2003, 278:29593-29599.
doi: 10.1074/jbc.M300565200 originally published online May 23, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M300565200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 50 references, 18 of which can be accessed free at http://www.jbc.org/content/278/32/29593.full.html#ref-list-1