B Lymphocyte Memory in X-Linked Lymphoproliferative Disease (XLP)

Ana Coraglia*, Liliana Belmonte, Cecilia Parodi, Mariela Baston, Patricia Bare, Beatriz Ruibal-Ares, N. Galassi and Maria M.E. de Bracco

Instituto de Investigaciones Hematológicas Dr. Mariano R. Castex (IIHEMA), Academia Nacional de Medicina, Buenos Aires, Argentina

Abstract: X-linked lymphoproliferative disease (XLP) is a severe immunodeficiency characterized by hypogammaglobulinemia, fulminant infectious mononucleosis, and/or lymphoma associated to mutations of the *SH2D1A* gene, encoding SAP (signaling lymphocytic activation molecule-associated protein). The initial encounter with Epstein Barr virus (EBV) triggers a massive response that leads to a fatal outcome in around 50% of the XLP individuals. Most surviving patients develop hypogammaglobulinemia and eventually B cell lymphoma. B lymphocyte development seems to be normal, but there is a marked reduction of memory B cells (CD27+ B lymphocytes). In addition, Th1 cell mediated immune responses predominate over Th2 responses. Hypogammaglobulinemia and failure to develop a long term humoral immune response can be explained because both the germinal center (GC) reaction and GC formation in secondary lymphoid organs are greatly impaired both in human XLP and in experimental SAP deficiency. Non switched memory B cells (IgM+, IgD+, CD27+ B lymphocytes) persist in XLP patients, suggesting that in spite of the lack of a GC reaction, some subgroups of memory B lymphocytes can play a role in immune homeostasis in these patients. In addition, their persistence in the presence of EBV infection, could perhaps be associated to late occurrence of extranodal B-cell lymphoma, which is another pathological condition associated to the absence of SAP in humans.

Keywords: B cell memory, XLP, SAP, humoral response, CD27, long term memory.

INTRODUCTION

X-linked lymphoproliferative (XLP) disease is a rare complex disorder characterized by severe immune dysregulation that is exacerbated by Epstein Barr virus (EBV) infection, which many times leads to fatal infectious mononucleosis [1]. It was initially described by DT Purtilo as an immunodeficiency affecting 1 in 500,000 to 1 million males [2]. EBV is the cause of the clinical presentation in around 90 % of the cases. XLP patients are not as susceptible as other T or B cell deficient individuals to viral infections known to play a major immunopathogenic role (herpes simplex, varicella zoster or cytomegalovirus). Hypogammaglobulinemia or dysgammaglobulinemia frequently occur in XLP patients who survive acute EBV infection, and they may in time develop B cell lymphomas (Fig. 1) [3]. Because EBV may initiate disease progression in the majority of the cases, the defense mechanisms against EBV in XLP individuals have been carefully compared to those of non deficient controls. It is thought that initially, SAP-deficient NK and NKT cells fail to control EBV infection, leading to massive EBV expansion. Later on, a dysregulated immune response results in uncontrolled expansion of activated T lymphocytes and cells of the monocyte/macrophage lineage that cause tissue damage through the release of inflammatory cytokines and cytotoxic mediators. However, EBV is not the exclusive trigger of disease manifestations in XLP. Genetic studies have demonstrated that XLP is associated to mutations affecting *SH2D1A/SAP/DHSP*, a gene encoding a 128 amino acid protein comprised largely of an SH2 domain: the signal lymphocyte-activation molecule (SLAM)-associated molecule (SAP) [4]. SAP is expressed in T cells, NK cells, NKT cells and in some B cell populations. SAP participates in signal transduction by mediating protein/protein binding to conserved tyrosine-containing motifs in the intracellular domains of SLAM (CD150) and related family members [5]. It also recruits the Src family kinase Fyn, resulting in receptor tyrosine phosphorylation and binding of several down stream proteins [6]. Animal models of SAP deficiency have been useful to provide insight into the pathophysiology of XLP [7], although in these mice knockout models the triggering viral stimulus for disease is not EBV and they do not develop lymphoma (a relatively common clinical manifestation of XLP disease), limiting some of the interpretations regarding human pathology [8].

In this review we shall focus on the mechanisms that lead to impaired B cell memory generation underlying humoral immunodeficiency in XLP patients.

B CELL MEMORY

Immunological memory is a highly effective mechanism that ensures quick protection against infection by environmental microorganisms. B cell memory relies on two cellular compartments: plasma cells giving rise to antibodies (effector memory) and memory B cells representing central memory precursors capable of generating the plasma cell compartment through a combination of antigen-dependent and antigen-independent mechanisms. This view must be completed by incorporating other important functions that can be performed by "central" memory B cells as antigen (Ag) presentation, T and dendritic cell (DC) regulation and cytokine and chemokine production [9]. In primary immune responses, naïve B lymphocytes migrating into secondary

*Address correspondence to this author at the IIHEMA, Academia Nacional de Medicina, P. de Melo 3081, 1425 - Buenos Aires, Argentina; Tel/Fax: 54-11-4805 0712; E-mail: mebracco@hematologia.anm.edu.ar

1573-3955/10 $55.00+.00

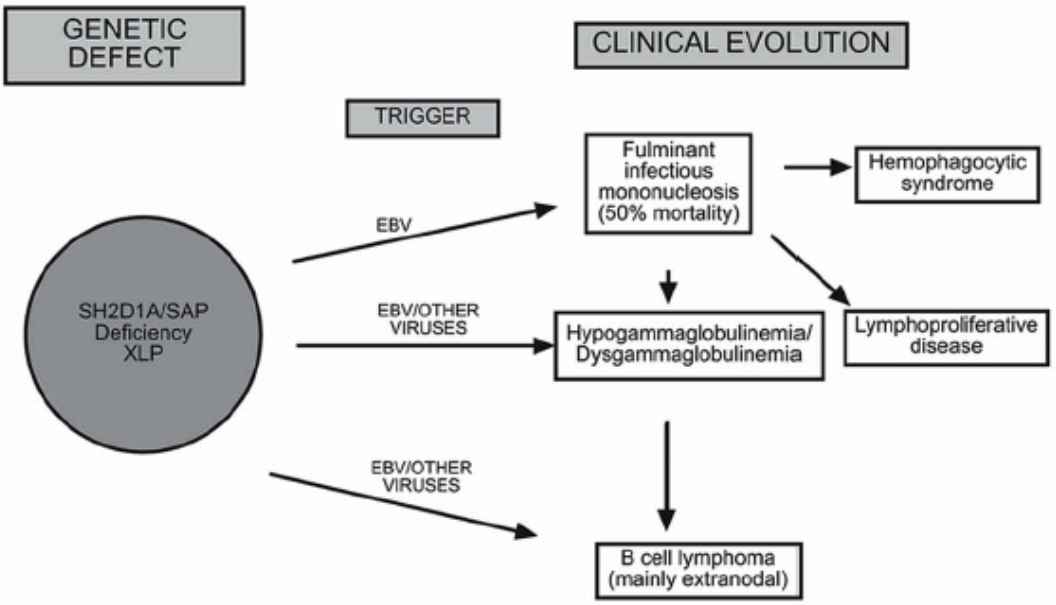

Fig. (1). Immunopathology of XLP disease. Mutations in SH2D1A/SAP lead to SAP functional deficiency in XLP individuals. Epstein Barr Virus (EBV) (or other viral infections) can trigger the clinical manifestations of this genetic deficiency; 50% of the patients die after the onset of fulminant infectious mononucleosis caused by EBV. Surviving XLP patients develop dysgamma globulinemia / hypogamma globulinemia, and in time extranodal B cell lymphoma may occur.

lymphoid organs encounter Ag in the extra-follicular regions. Ag-receptor signaling leads B cells to an activated state in which they can present Ag peptides to Ag-primed T cells. CD4+ T cells are important players in the generation of B cell memory, providing stimulatory ligands (CD40L, OX40, inducible co-stimulator ICOS) and cytokines that determine the fate of B lymphocytes (IL-4, IL-10, IL-21). Defects in the complicate mechanisms of T-B cell cooperation impair the correct set up of an effective humoral immune response. Help provided by T lymphocytes to B lymphocytes is fundamental to allow the production of high-affinity memory B cells and long-lived plasma cells specific for foreign antigens. B cells can also be activated in a T-cell independent manner by bacterial capsular polysaccharides and by microorganism-derived Toll-like-receptor ligands (TLR). These T-cell independent responses generally result in a rapid antibody response to pathogens through the generation of short-lived, low-affinity extrafollicular plasma cells. T-cell help occurs in secondary tissues providing the suitable microenvironment for cell interaction. B cells that can either become short-lived plasma cells or migrate into the follicles, where in cooperation with specialized CD4+ Tcells and DC, they form the essential structures for T cell-dependent responses. These are the germinal centers (GC) that develop within the B cell follicles of lymph nodes, spleen, tonsils and the Peyer's patches of mucosal-associated lymphoid tissue [10] (Fig. **2**). Thus, GC arise following antigenic stimulation providing the milieu for B cell proliferation, somatic hypermutation and class-switch-recombination of immunoglobulins. Proliferation is required for the selection of antigen reactive clones with increased antigen affinity and different immunoglobulin isotypes that are functionally adapted to confront different classes of foreign antigens or pathogens [11].

FOLLICULAR T CELLS AND B CELL DIFFERENTIATION

It is now recognized that a subset of CD4+ T cells with follicular tropism (follicular T cells, T_{FH}) are essential for the formation of the GC reaction that gives rise to a fully differentiated humoral immune response with high affinity, class switched antbodies and long term memory B cells necessary to maintain an efficient humoral response and replenish the long-lived plasma effector cell compartment that will be required to face successfully renewed encounters with foreign antigens [12]. T_{FH} constitute a subgroup of non polarized CD4+ T lymphocytes that express the chemokine receptor CXCR5. DC priming of T cells in the T-cell zone results in upregulation of CXCR5 expression and downregulation of CCR7 expression. DC-derived IL-12 is involved in this reaction [13, 14]. This allows cell migration to the T-cell-follicle boundary in response to the chemokine CXCL13, an ideal location for T-B interaction. It has been suggested that primed CD4+ T lymphocytes with high CXCR5 expression, differentiate into different subgroups of effector T cells according to the cytokine milieu, giving rise to T lymphocytes with different function and transcription

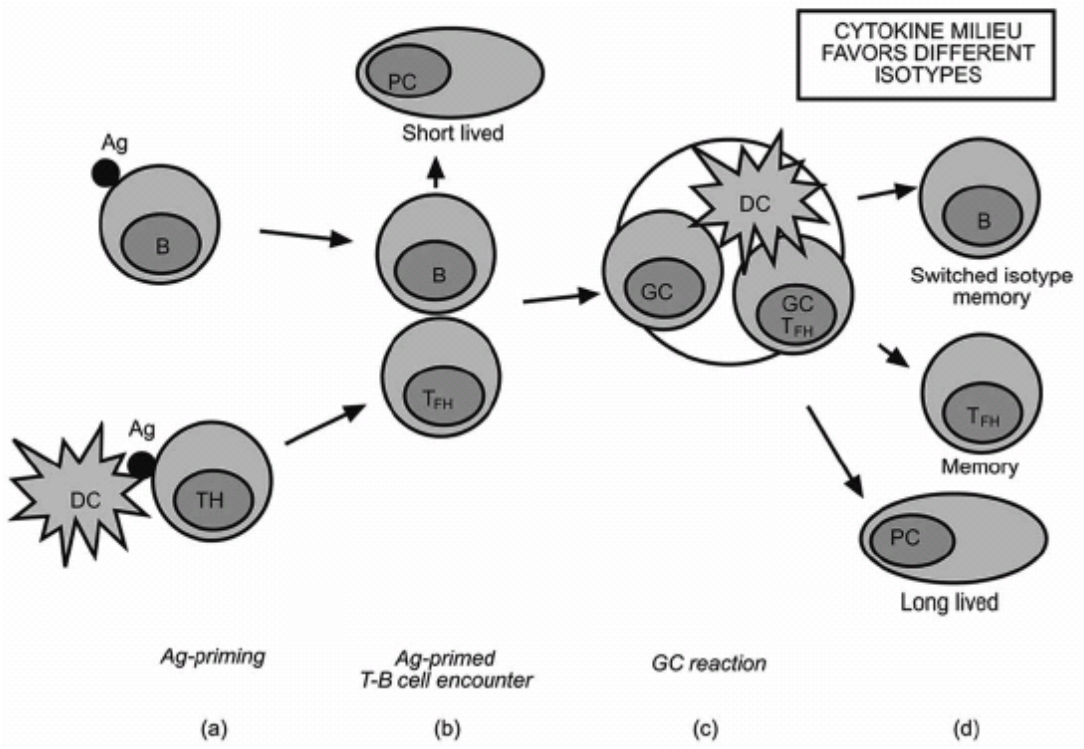

Fig. (2). B cell memory. a) Priming: naïve B cells and naïve T helper (TH) cells encounter antigen (Ag); b) Ag primed B and follicular T cells (T_{FH}) interact before the germinal center (GC) reaction (pre-GC); short-lived plasma cells (PC) evolve or B and T_{FH} initiate the GC reaction (c) that involves immunoglobulin (Ig) isotype switching and affinity maturation, leading to long-lived memory cells; d) post-GC B cells follow the path to memory B cells, T_{FH} give rise to memory T_{FH} and some Bcells follow the path to long-lived PC. The cytokine milieu favors differentiation into cells with distinct switched Ig isotypes.

factors. T_{FH} interact with B cells at different levels, and this reaction is crucial in order to ensure the complete assembly of a diversified, high affinity humoral immune response (Fig. 3) [12, 15, 16]. T_{FH} secrete IL-21 (as NKT and Th17 cells), and this cytokine is central for B cell help. Other cytokines and costimulatory molecules produced by T_{FH} as IL10 and ICOS are also important in B cell help. In this regard it is noteworthy that ICOS deficiency leads to a condition in humans resembling XLP in the severe reduction of CXCR5+ CD4+ germinal center T cells and lack of GC formation in secondary lymphoid organs [17]. CXCR5+ T_{FH} may also express CD57 in humans, and this fact has been used to distinguish tonsillar CD4+ T cells from polarized Th1 and Th2 cells [18]. While CD57+ CXCR5+ CD4 T cells present in the bloodstream are thought to represent GC T_{FH}, capable of providing B cell help [19], it has been recently suggested that helper activity is independent of CD57 expression and more related to ICOS expression [20]. It has been proposed that Bcl-6, a transcription factor whose expression is regulated by IL-6 and Il-21, plays a central role in T_{FH} cell generation [21]. Bcl-6 deficient T cells fail to develop into T $_{FH}$ and GC generation is abolished [22]. Bcl-6 acts on B lymphocytes by inhibiting terminal differentiation of GC B cells into plasma cells or memory B cells, and on T cells by repressing the transcription factor GATA-3 that is involved in Th2 differentiation [22]. It has been shown that the action of Bcl-6 is opposed to that of Blimp-1, a transcription factor that is down regulated in T_{FH} [23] In order to generate a GC reaction leading to affinity maturation and B cell memory, T_{FH} must establish a stable contact with B lymphocytes. When SAP is absent, this does not occur and the GC reaction fails, B cells do not acquire sufficient help from SAP-deficient T cells and SAP-deficient T cells are not recruited and retained into the GC. In Sap-/- mice it was shown that initial T-DC interactions were not altered, suggesting that impaired T-B interactions were responsible for failure of the GC reaction [24]. SAP regulates the function of T_{FH} but apparently SAP-Fyn interactions are not required in this [25]. Heterogeneity of T $_{FH}$ is associated to their role as helper T cells at different levels: 1) during first contact with antigen-primed B cells at the pre-GC level, where they control commitment to antibody isotype and entry into the short lived plasma cell pathway; 2) after entry into GC, where they control GC B cell selection and entry into the long-lived compartment. Finally their persistence in the lymph nodes as resting memory T $_{FH}$ will favor memory B cell expansion and rapid PC differentiation upon re-encountering antigen (Fig. 4) [26].

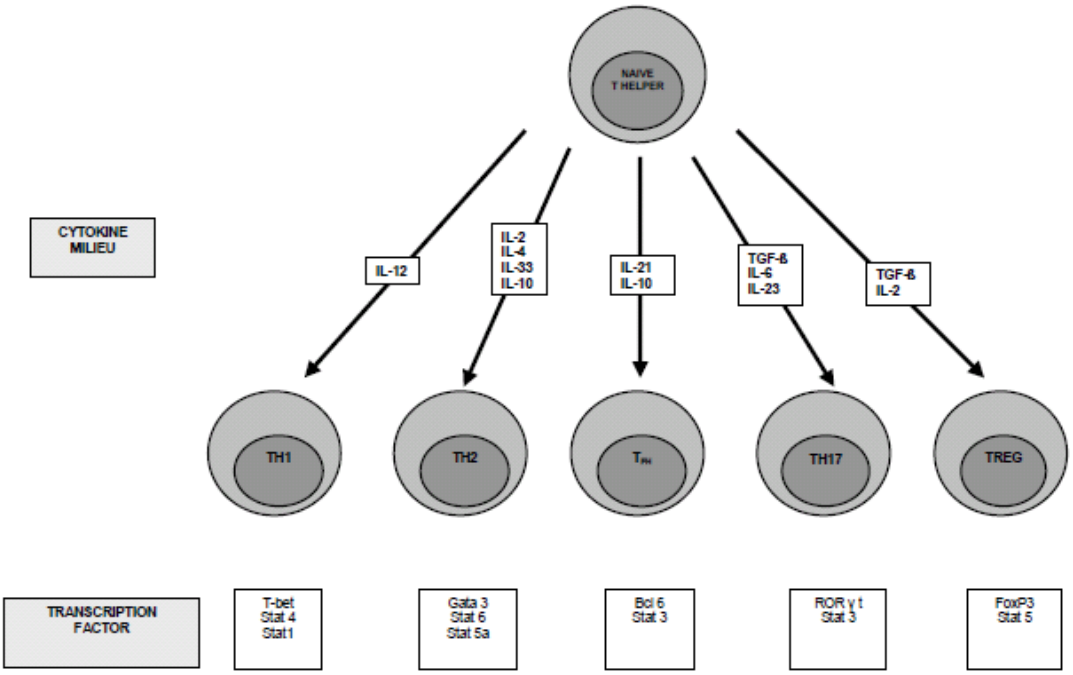

Fig. (3). Differentiation into distinct classes of effector TH cells. According to the cytokine milieu, TH cells differentiate into cells with diverse function, mediated by different transcription factors.

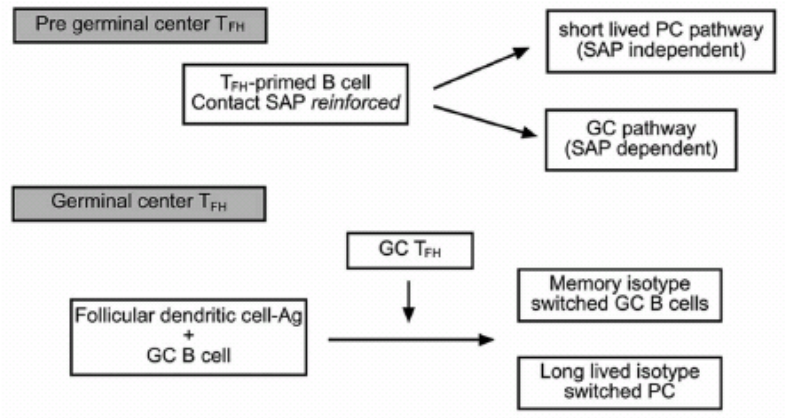

Fig. (4). Follicular T helper cells (T_{FH}) intervene at different points of B cell differentiation.

B CELL MEMORY IN XLP

A striking feature in both humans and mice with mutations in *SH2D1A* is a deficiency in the generation of B cell memory that leads to hypogammaglobulinemia [1]. In human XLP, the number of circulating B lymphocytes expressing CD27 (memory B cells) is low [27, 28]. Interestingly, this deficiency seems to be the result of T cell defects rather than B cell defects. In the *Sap*-/- knockout mouse model, it can be corrected by supplying *Sap*+/+ T cells to the deficient animals [8]. In *Sap*-/- mice, however, it was suggested that lack of SAP controlled both T and B cell activities [29]. In humans, it was observed that CD4+T cells from XLP patients were impaired in their B cell helping

ability, coincidently with decreased IL-10 secretion and ICOS expression [28]. On the other hand, the few purified B lymphocytes that were isolated from XLP could undergo somatic hypermutation and secrete Ig *in vitro* [30], indicating that the main defect in the generation of B cell memory in XLP was related to T cells rather than to B cells. Results from SAP-deficient mice and from XLP patients indicate that there is a perturbation in the balance of cytokines, with predominance of Th1 cytokines over Th2 cytokines [7], although SAP's regulation of cytokine production appears to be independent of SAP's participation in the generation of long term B cell memory in T-dependent responses [31]. Because Th2 cytokines are known to be important in the generation of a correct humoral immune response, we investigated if the normal ratio of Th1/Th2 lymphocytes was preserved in XLP. T lymphocytes associated to Th1 responses (CXCR3+ T cells) were high, while those corresponding to Th2 responses (CCR4+) were low in human XLP patients [27]. Because, T_{FH} are a central player in the construction of long term B cell memory, which is a main defect in XLP patients, we have looked for their presence in hypogammaglobulinemic XLP. Our results indicate that in contrast to ICOS deficiency and CD40L deficiency, there are not significant differences in the proportion of CD57+, CXCR5+ T cells in the peripheral blood of XLP individuals (Coraglia A, *et al.*, 2009) (Table **1**), suggesting that functional defects as poor ICOS or IL-21 production, or inappropriate location and stability at the GC site, may underlie their lack of participation in the generation of long term memory.

Table 1.

	CXCR5, CD4/CD4 (%)	CXCR5, CD57, CD4/CD4 (%)
XLP #4 (n=6)	10.91 ± 1.43	0.55 ± 0.29
XLP #9 (n=6)	7.14 ± 0.70	0.86 ± 0.28
N (*n*=6)	11.18 ± 1..87	0.55 ± 0.22

Defects in the generation of long term B cell memory have been linked to deficient formation of GC in lymphoid tissue in *Sap-/-* mice. Lack of conventional GC formation has also been observed in human XLP [30]. Since memory B cells are a heterogeneous group, it is interesting to know if the generation of all the subsets of memory B cells is equally impaired in XLP patients. Recently, it was shown that somatically mutated IgM+, CD27+ (but not Ig-subtype switched B cells) could be found in XLP in spite of the absence of GC in secondary lymphoid organs [30]. When XLP PBMC are put in culture without exogenous stimulation [32] during 20-30 days, the proportion of IgM+, IgD+, B lymphocytes expressing CD27 increases over the initial values (Fig. **5**), suggesting that this particular group of "non switched" memory XLP B lymphocytes can be expanded *in vitro*. In fact it is interesting to note that this special type of B lymphocytes was also associated to EBV persistence in these patients [33]. As GC of gastrointestinal follicles normally contain CD27+, IgM+, IgD+, B lymphocytes [30], it is tempting to assume that their increased viability in association with EBV might be related to the observed occurrence of extranodal, gut-restricted lymphoma in these patients [33, 34]. After prolonged *in vitro* culture of XLP PBMC, most surviving cells are CD8+ T lymphocytes, and it is possible that continuous challenge with transformed EBV-infected B cells within the system provides a continuous stimulus for their differentiation and survival [33]. The remaining B lymphocytes have a "non switched" memory phenotype. *In vivo*, in addition to defective GC formation associated to poor ICOS, IL-10 and IL-21 production, selective loss of EBV transformed B lymphocytes by reaction with EBV-reactive CD8 effector cells could be part of the reaction leading to hypogammaglobulinemia and lack of memory switched B cells.

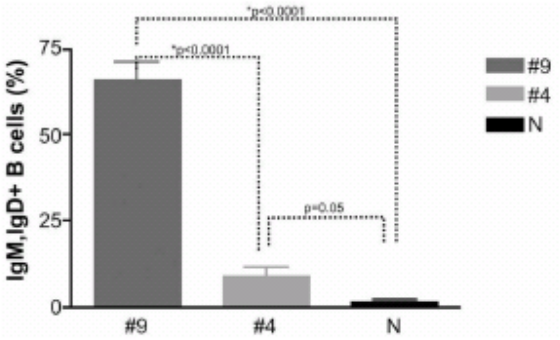

Fig. (5). Double positive IgM+, IgD+ B cells in XLP. IgM+, IgD+ cells were determined in a CD19+ B cell gate by flow cytometry. XLP patients #4 and #9 and N controls were studied: 10 monthly blood samples of XLP patients and blood samples from 10 different N donors were analyzed. X±SEM is given.

CONCLUSION

Generation of long term B cell memory is impaired in XLP patients. Multiple factors are involved in the generation of this defect, which is generally triggered by infection with EBV. While the percentage of CD4 T cells expressing the chemokine receptor CXCR5 and CD57 (T_{FH} helper cells) is not significantly reduced, it is possible that their function may be impaired in XLP. On the other hand, other types of B memory cells that do not require the GC reaction (non switched CD27+ IgM+, IgD+ memory B cells) may still persist in XLP individuals and could play a role in the eventual generation of extranodal B cell lymphoma which is one of the clinical complications of progressive XLP disease.

ACKNOWLEDGEMENTS

The authors thank Dr. N Riera and Ms M Felippo for help in cytometry studies, Dr. A Malbran and XLP patients #4 and #9 for their generous support and specimen donations. This work was done with support from CONICET, the National Agency for Science and Technology (PICT 05 38258) and Fundación Rene Baron.

REFERENCES

[1] Morra M, Howie D, Grande MS, *et al.* X-linked lymphoproliferative disease: a progressive immunodeficiency. Annu Rev Immunol 2001; 19: 657-82.

[2] Purtilo DT, Cassel CK, Yang JP, Harper R. X-linked recessive progressive combined- variable immunodeficiency (Duncan's disease). Lancet 1975; 1: 935-40.
[3] Malbran A, Belmonte L, Ruibal-Ares B, Baré P, Bracco MME. X-linked lymphoproliferative syndrome, EBV infection and impaired regulation of cell-mediated cytotoxicity. Medicina (B Aires) 2003; 63: 70-6.
[4] Nichols KE, Harkin DP, Levitz S, *et al*. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. Proc Natl Acad Sci USA 1998; 95: 13765-70.
[5] Nichols KE, Ma CS, Cannons JL, Schwartzberg PL, Tangye SG. Molecular and cellular pathogenesis of X-linked lymphoproliferative disease. Immunol Rev 2005; 203: 180-199.
[6] Latour S, Veillette A. The SAP family of adaptors in immune regulation. Semin Immunol 2004; 16: 409-19.
[7] Czar MC, Kersh EN, Mijares LA, *et al*. Altered lymphocyte responses and cytokine production in mice deficient in the X-linked lymphoproliferative disease geneSH2D1A/DHSP/SAP. Proc Natl Acad Sci USA 2001; 98: 7449-54.
[8] Crotty S, McCausland M, Aubert RD, Wherry EJ, Ahmed R. Hypogammaglobulinemia and exacerbated CD8 T-cell mediated immunopathology in SAP-deficient mice with chronic LCMV infection mimics human XLP disease. Blood 2006; 108: 3085-93.
[9] Harris DP, Haynes L, Sayles PC, *et al*. Reciprocal regulation of polarized cytokine production by effector B and T cells. Nat Immunol. 2000; 1(6): 475-82.
[10] McHeyzer-Williams MG. B cells as effectors. Curr Opin Immunol 2003; 15: 354-61.
[11] Tangye SG, Avery DT, Hodgkin PD. A division-linked mechanism for the rapid generation of Ig-secreting cells from human memory B cells. J Immunol 2003; 170: 261-9.
[12] Vinuesa CG, Tangye SG, Moser B, Mackay CR. Follicular B helper T cells in antibody responses and autoimmunity. Nat Rev Immunol 2005; 5: 853-65.
[13] Schmitt N, Morita R, Bourdery L, *et al*. Human dendritic cells induce the differentiation of IL-21-producing T follicular helper-like cells through interleukin-12. Immunity 2009; 31: 158-69.
[14] Ma CS, Suryani S, Avery DT, *et al*. Early commitment of naïve human CD4+ T cells to the follicular helper (TFH) lineage is induced by IL-12. Immunol Cell Biol 2009; 1: 1-11.
[15] King C, Tangye SG, Mackay CR. T follicular helper (TFH) cells in normal and dysregulated immune responses. Annu Rev Immunol. 2008; 26: 741-66.
[16] Mc Heyzer-Williams LJ, Pelletier N, Mark L, Fazilleau N, McHeyzer-Williams MG. Follicular helper T cells as cognate regulators of B cell immunity. Curr Opin Immunol 2009; 21: 266-73.
[17] Bossaller L, Burger J, Draegge R, *et al*. ICOS deficiency is associated with severe reduction of CXCR5+ CD4 germinal center Th cells. J Immunol 2006; 177: 4927-32.
[18] Kim CH, Lim HW, Kim JR, Rott L, Hillsamer P, Butcher EC. Unique gene expression program of human germinal center T helper cells. Blood 2004; 104: 1952-60.
[19] Kim CH, Rott LS, Clark-Lewis I, Campbell DJ, Wu L, Butcher EC. Subspecialization of CXCR5+ T cells: B helper activity is focused in a germinal center-localized subset of CXCR5+ T cells. J Exp Med 2001; 193: 1373-81.
[20] Rashid A-U, Rahn H-P, Sallusto F, Lipp M, Müller G. Follicular B helper T cell activity is confined to $CXCR5^{hi}ICOS^{hi}$ CD4 T cells and isindependent of CD57 expression. Eur J Immunol 2006; 36: 1892-903.
[21] Nurieva RI, Chung Y, Martinez GJ, *et al*. Bcl6 mediates the development of T follicular helper cells. Science 2009; 325: 1001-5.
[22] Yu D, Rao S, Tsai LM, *et al*. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. Immunity 2009; 31: 1-12.
[23] Johnston RJ, Poholek AC, DiToro D, *et al*. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science 2009; 325:1006-10.
[24] Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B interactions underlie germinal centre formation. Nature 2008; 455: 764-9.
[25] McCausland M, Yusuf I, Tran H, Ono N, Yanagi Y, Crotty S. SAP regulation of follicular helper CD4 T cell development and humoral immunity is independent of SLAM and Fyn kinase. J Immunol 2007; 178: 817-28.
[26] Fazilleau N, Mark L, McHeyzer-Williams LJ, McHeyzer-Williams MG. Follicular helper T cells: lineage and location. Immunity 2009; 30: 324-35.
[27] Malbran A, Belmonte L, Ruibal-Ares B, *et al*. Loss of circulating CD27+ memory B cells and CCR4+ T cells occurring in association with elevated EBV loads in XLP patients surviving primary EBV infection. Blood 2004; 103: 1625-31.
[28] Morra M, Barrington RA, Abadia-Molina AC, *et al*. 2005. Defective B cell responses in the absenceof SH2D1A. Proc Natl Acad Sci USA 2005; 102: 4819-23.
[29] Ma CS, Pittaluga S, Avery DT, *et al*. Selective generation of functional somatically mutated IgM+CD27+, but not Ig-isotype switched, memory B cells in X-linked lymphoproliferative disease. J Clin Investig 2006; 116: 322-33.
[30] Cannons JL, Yu LJ, Jankovic D, *et al*. SAP regulates T-cell mediated help for humoral immunity by a mechanism distinct from cytokine regulation. J Exp Med 2006; 6: 1551-65.
[31] Ruibal Ares B, Belmonte L, Baré P, *et al*. Monocyte differentiation and HIV replication after prolonged culture of peripheral mononuclear cells from HIV-infected individuals. Cell Immunol 2001; 210: 11-20.
[32] Belmonte L, Parodi C, Baston M, *et al*. Increased viability after non-stimulated peripheral blood mononuclear cell (PBMC) culture in patients with X-linked lymphoproliferative disease (XLP). Clin Immunol 2009; 133(1): 86-94.
[33] Shaffer AL, Rosenwald A, Staudt LM. Lymphoid malignancies: the dark side of B-cell differentiation. Nat Rev Immunol 2002; 2: 920-32.

Received: September 29, 2009 Revised: October 2, 2009 Accepted: October 15, 2009