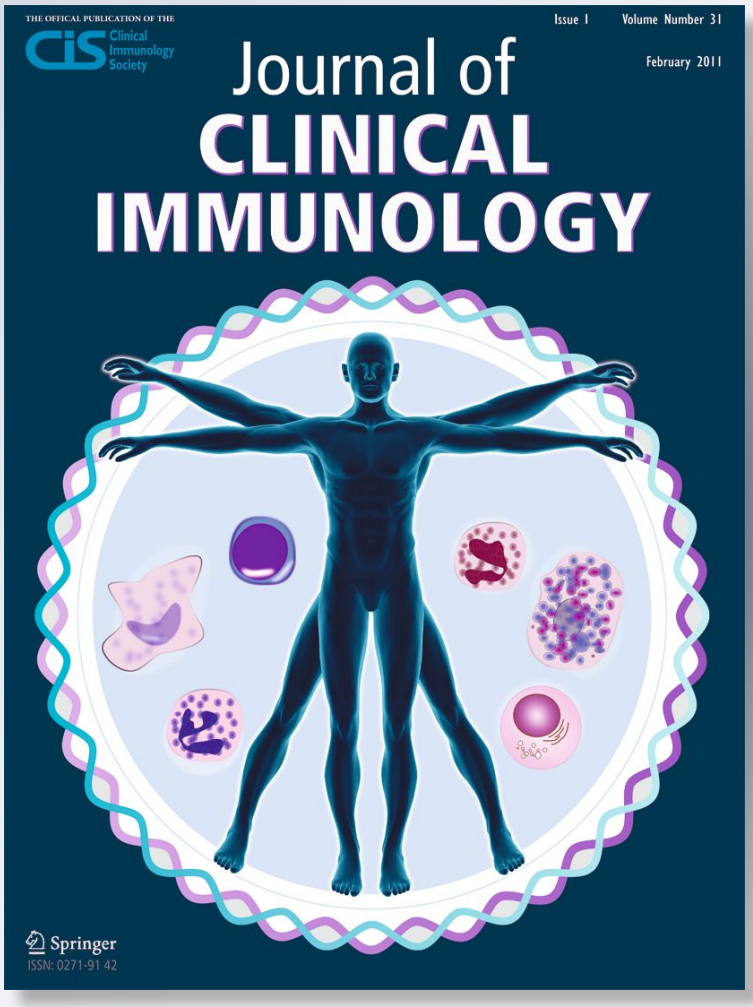
Increased Pro-inflammatory Cytokine Production After Lipopolysaccharide Stimulation in Patients with X-linked Agammaglobulinemia

María Edith González-Serrano, Iris Estrada-García, Dolores Mogica-Martínez, Alejandro González-Garay, Gabriela López-Herrera, et al.

Journal of Clinical Immunology

ISSN 0271-9142
Volume 32
Number 5

J Clin Immunol (2012) 32:967-974
DOI 10.1007/s10875-012-9706-z



Springer

J Clin Immunol (2012) 32:967–974
DOI 10.1007/s10875-012-9706-z

Increased Pro-inflammatory Cytokine Production After Lipopolysaccharide Stimulation in Patients with X-linked Agammaglobulinemia

María Edith González-Serrano · Iris Estrada-García · Dolores Mogica-Martínez · Alejandro González-Garay · Gabriela López-Herrera · Laura Berrón-Ruiz · Sara Elva Espinosa-Padilla · Marco Antonio Yamazaki-Nakashimada · Alexander Vargas-Hernández · Leopoldo Santos-Argumedo · Sergio Antonio Estrada-Parra · Francisco J. Espinosa-Rosales

Received: 25 January 2012 / Accepted: 11 May 2012 / Published online: 5 June 2012

Abstract
Purpose To evaluate the lipopolysaccharide (LPS)-induced pro-inflammatory cytokine response by peripheral blood mononuclear cells (PBMCs) from XLA patients.
Methods Thirteen patients with XLA were included in the study. LPS-induced TNF-α, IL-1β, IL-6, and IL-10 production was determined in PBMCs from patients and matched healthy controls by ELISA. Cytokine production was correlated with the severity of mutation, affected domain and clinical characteristics.
Results In response to LPS, PBMCs from XLA patients produced significantly higher amounts of pro-inflammatory

M. E. González-Serrano · G. López-Herrera · L. Berrón-Ruiz · S. E. Espinosa-Padilla · F. J. Espinosa-Rosales
Immunodeficiency Research Unit,
National Institute of Pediatrics (INP),
Av. Imán # 1, Col. Insurgentes Cuicuilco, Deleg. Coyoacán,
Mexico City ZP 04530, Mexico

M. A. Yamazaki-Nakashimada
Department of Immunology, INP,
Av. Imán # 1, Col. Insurgentes Cuicuilco, Deleg. Coyoacán,
Mexico City ZP 04530, Mexico

A. González-Garay
Department of Research Methodology, INP,
Av. Imán # 1, Col. Insurgentes Cuicuilco, Deleg. Coyoacán,
Mexico City ZP 04530, Mexico

D. Mogica-Martínez
Department of Allergy, Medical Center "La Raza",
Mexican Social Security Institute,
Calzada Vallejo y Av Jacarandas s/n, Col. La Raza,
Deleg Azcapotzalco ZP 02990, Mexico

G. López-Herrera · L. Berrón-Ruiz · A. Vargas-Hernández · L. Santos-Argumedo
Department of Molecular Biomedicine,
Center for Research and Advanced Studies (CINVESTAV-IPN),
Av. Instituto Politécnico Nacional No 2508, Col. San Pedro Zacatenco, Deleg. Gustavo A. Madero,
México City, ZP 07360, Mexico

M. E. González-Serrano · I. Estrada-García · S. A. Estrada-Parra
National School of Biological Sciences,
National Polytechnic Institute,
Plan de Ayala y Prolongación de Carpio s/n, Col Santo Tomás,
Deleg. Miguel Hidalgo,
México City ZP 11340, Mexico

F. J. Espinosa-Rosales (✉)
Unidad de Investigación en Inmunodeficiencias,
Instituto Nacional de Pediatría,
Av. Del Imán # 1, Col. Insurgentes Cuicuilco,
Delegación Coyoacán,
CP 04530 Mexico City, Mexico
e-mail: espinosa_francisco@yahoo.com.mx

cytokines and IL-10 compared to controls, and this production was influenced neither by the severity of the mutation nor the affected domain. PBMCs from patients with a history of more hospital admissions before their diagnosis produced higher levels of TNF-α. PBMCs from patients with lower serum IgA levels showed a higher production of TNF-α and IL-1β. Less severe (punctual) mutations in the Btk gene were associated with higher serum IgG levels at diagnosis.
Conclusions Our results demonstrate a predominantly inflammatory response in XLA patients after LPS stimulation and suggest a deregulation of TLR signaling in the absence of Btk. This response may be influenced by environmental factors.

Keywords X-linked agammaglobulinemia · Bruton's tyrosine kinase · LPS signaling · inflammatory response · TIRAP

Introduction

X-linked agammaglobulinemia (XLA) is a primary immunodeficiency disease resulting from mutations in the cytoplasmic Bruton's tyrosine kinase (Btk) [1]. At least 1112 different mutations have been identified, according to the BTK base [2]. In humans, affected males are highly susceptible to recurrent bacterial infections within the first year of life, which occurs following a decline in maternal immunoglobulin (Ig) levels [3]. XLA patients have an increased incidence of inflammatory diseases, such as rheumatoid arthritis and Type I diabetes mellitus, suggesting a dominant Th1 response [4, 5].

Toll-like receptors (TLRs) are members of the germline-encoded pattern-recognition receptors (PRRs), which recognize different pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharides (LPS), bacterial DNA, or single-stranded viral RNA (ssRNA) [6]. TLR4 was identified as the receptor that responds to bacterial lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria [6].

A number of recent studies have demonstrated that Btk plays an important or possibly essential role in signaling through several TLRs; for example, Btk undergoes autophosphorylation after stimulation with LPS in the human monocyte cell line THP1, in mouse bone marrow-derived macrophages and in murine splenic B lymphocytes [7–10].

Impaired LPS-induced production of TNF-α and IL-1β by peripheral blood mononuclear cells (PBMCs) and dendritic cells from XLA patients has been previously reported [11–13]. However, there is some discrepancy among these studies, [14–17] Further work has demonstrated that Btk is a component of the TLR signaling pathway. In particular, Btk interacts with the intracellular Toll/IL-1 receptor (TIR) domains of TLRs 4, 6, 8, and 9, and associates with myeloid differentiation primary response gene (88) (MyD88), toll-interleukin 1 receptor domain-containing adaptor protein (TIRAP, also known as the MyD88 adaptor-like protein or Mal), and Interleukin-1 receptor-associated kinase 1 (IRAK1), which are all key components of the TLR signaling complex [8].

Knowing that Btk has a role in TLR signaling, and that cytokine production in response to TLR4 binding may be influenced by genetic abnormalities in this gene, as well as environmental and clinical determinants, such as infections and age at diagnosis, we designed this study to evaluate pro-inflammatory cytokine production after LPS stimulation in PBMCs obtained from XLA patients, and compared it with that of PBMCs obtained from healthy controls.

Methods

Thirteen patients with an XLA diagnosis were included. Information was obtained from medical records and interviews with patients and/or parents and included the following information: age at first infection, age at diagnosis, number of hospital admissions before diagnosis, serum concentrations of IgG, IgM and IgA at diagnosis, and percentage of CD19+ cells from peripheral blood lymphocytes.

Mutation analysis to confirm XLA in 11 patients were previously reported, [18] and two mutations were confirmed recently in our laboratory. Active infection was excluded at the time of recruitment. All patients were receiving appropriate intravenous immunoglobulin (IVIG) replacement therapy. Blood samples were collected before IVIG infusions. Control subjects were healthy age-matched male donors. All studies have been approved by the institutional research and ethics committee and have been performed in accordance with the 1964 Declaration of Helsinki and his later amendments and written consent was obtained from parents and/or patients before blood was drawn.

Mononuclear Cell Separation

Peripheral blood mononuclear cells (PBMCs) were isolated from patient peripheral blood using a gradient centrifugation with BD Vacutainer® Cell preparation tubes (CPT™).

Btk Expression Analysis in Monocytes by Flow Cytometry

Intracellular Btk expression was analyzed by flow cytometry. PBMCs (1×10^6) were placed in polystyrene tubes, labeled as Btk or isotype control and incubated for 20 min on ice in RPMI+0.2 % FCS with PerCP-conjugated monoclonal anti-human CD14 BD®. After staining for CD14, cells were washed with RPMI+0.2 % FCS. Lyse/Fix Buffer BD Phosphoflow™ (300 μL) was added to each tube, and then each

tube was incubated in the dark for 10 min at 37°C; cells were then washed with wash buffer (PBS+0.5 % albumin+0.2 % sodium azide). Perm buffer II BD Phosphoflow™ (100 μL) was added to each tube, and then each tube was incubated in the dark for 30 min on ice; cells were then washed twice with wash buffer. FcBlock (BD Pharmigen™, 30 μL, 1:500 dilution) was added to each tube and incubated in the dark for 30 min on ice; cells were then washed twice with wash buffer. PE-conjugated monoclonal anti-human Btk BD Phosphoflow™ or PE-conjugated IgG_{2a} isotype control was added to the appropriate tubes, which were incubated in the dark for 30 min on ice; cells were washed twice with wash buffer. After centrifugation, the cell pellet was re-suspended in 300 μL of PBS+1 % paraformaldehyde (PFA) and maintained at 4°C until acquisition on a FACSCalibur BD Biosciences™. Analysis was performed with FlowJo v8.7 (Tree Star ™). Monocytes were gated according to forward scatter/side scatter, followed by the identification of the CD14+ cell population. CD14+ Btk-positive cells were then overlaid with the isotype control, and mean fluorescence intensity was then determined, Fig. 1 shows representative histograms of histograms of Btk expession in monocytes from a healthy control and XLA patients.

B Cell Depletion from Healthy Donor PBMCs

B cells were depleted from healthy donor PBMCs using the anti-CD19 monoclonal antibody coupled to magnetic beads (Miltenyi Biotec™) prior to placing cells in culture. Cells were incubated with the antibody-conjugated beads for 15 min on ice and were then washed with MACS buffer. The beads were magnetically removed with an MS column (Miltenyi Biotec™) and the MACS Vario Separator (Miltenyi Biotec™), and the remaining cells were stimulated as described later. To assess the elimination of B cells from the samples of healthy donors, the depleted population was stained with FITC-conjugated monoclonal anti-human CD19 antibody (BD®). The percentage of CD19+ remaining cells was determined.

TLR4 Stimulation of PBMCs from XLA Patients

PBMCs from XLA patients and healthy controls were plated in RPMI+5 % FCS at 1×10^6 cells/mL and then treated with 100 ng/mL LPS from *E. coli* 0111:B4 (San Diego, Invivogen™) or medium for 24 h at 37°C and 5 % CO_2. After stimulation supernatants were harvested and stored at −20°C.

ELISA

Concentrations of TNF-α, IL-10, IL-6, and IL-1β were determined by ELISA using R&D™ kits. The procedures were performing according to the manufacturer's instructions. Absorbance was recorded at 450 nm on a spectrophotometric ELISA plate reader (Labsystems Multiskan), using the Ascent software program. Results are expressed as the median concentration of duplicate cultures.

Classification of Mutation

Mutations were classified as severe or less severe according to a scale previously proposed by Broides et al. [19] Severe mutations include amino acid substitutions at sites that are conserved in other members of the Btk family of tyrosine kinases (Itk and Tec), frame shift mutations, splice-site alterations that occur at the invariant 2 base pairs at the beginning and end of an intron, premature stop codons and in-frame deletions. Less severe mutations include amino acid substitutions at nonconserved sites and splice-site defects at base pairs that are conserved but not invariant.

Clinical Information

At the time of blood collection, the following clinical information was obtained from charts or direct interview with parents or guardians: age at first infection, age at diagnosis, immunoglobulin (IgG, IgM, IgA) serum concentrations at

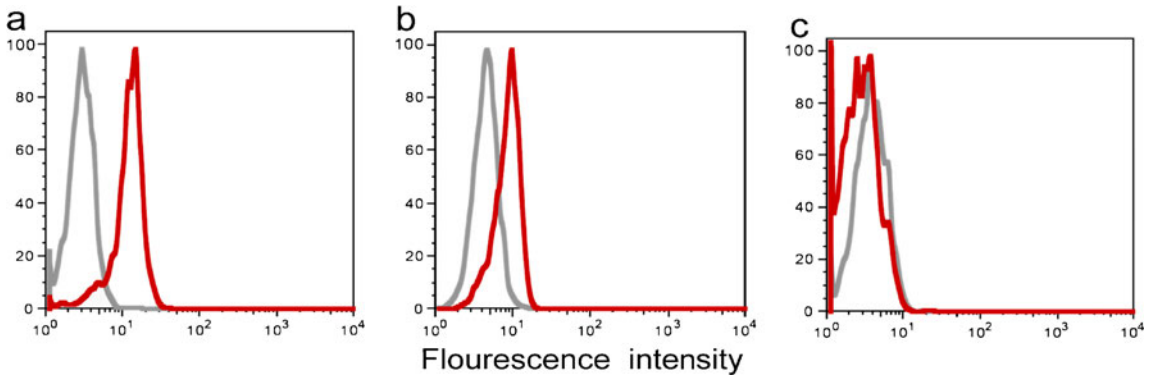


Fig. 1 Btk expression determined by flow cytometry. Image shows representative histograms of Btk expession in monocytes from a healthy control (**a**) and XLA patients: with residual (**b**) or without any (**c**) Btk expression. Lines show isotype control (gray) and Btk (red)

diagnosis, hospital admissions (due to infections) before diagnosis, and percentage of B cells in peripheral blood.

Statistical Analysis

We used SPSS 17.0 software (SPSS Inc., Chicago, Illinois) and STATA 10 (Dallas, Texas) for statistical evaluation. Qualitative data were expressed as percentages, and quantitative data were expressed as medians and ranges. We used the Mann–Whitney *U* test for comparison of median cytokine concentrations; the Spearman's rank coefficient was used for correlation analysis.

Results

Clinical Description

Table I summarizes clinical data, MFI of Btk expression in monocytes, the type of mutation and the affected domain. At the time of the study, the median age of the XLA patients in this study was 16 years (range: 9–26). The median age at diagnosis was 70 months (range 18–152). The first infections appeared at a median age of 7 months (range 3–43). Patients had a median of two hospital admissions for infections before diagnosis (range 1–6). The median level of detectable immunoglobulin at diagnosis was as follows: IgG, 83.61 mg/dL (range 6.2–282); IgM, 17.3 mg/dL (range 4.17–60.4); IgA, 9.29 mg/dL (range 0.3–25). Median percentage of CD19 + cells in XLA patients was 0.11 (range: 0–1.21). Median of MFI of Btk in monocytes was 7.04 (range 1.71–10.91). Notably, the median percentage of CD19 + cells in PBMCs of controls after the depletion procedure was 0.32 (range 0.13–1.06), which was not significantly different between XLA patients and healthy controls.

Btk Deficiency Results in Increased Production of Pro-inflammatory Cytokines and IL-10 After TLR4 Stimulation

Figure 2 shows that the PBMCs from XLA patients exhibit increased production of TNF-α, IL-1β, IL-6 and IL-10 in response to LPS stimulation as compared with PBMCs from controls. This resulted in 5-, 9- and 14-fold higher production of TNF-α and IL-1β, Il-10 and IL-6, respectively, from XLA PBMCs as compared with those obtained from healthy donors. Of note, in the absence of stimulation, there were no

Table I Clinical, laboratorial and demographic data for XLA patients

Patient	Current Age[a]	Age at first infection[b]	Age at diagnosis[b]	Hospital admissions before diagnosis	IgG[c]	IgM[c]	IgA[c]	CD19[d]	Btk[e]	Mutations	Type of mutation	Affected domain
1	16	43	70	3	109	31	0.3	0.3	7.97	cC1026→T [f]	Less severe	SH2
2	14	5	60	3	33	10	6.67	0.11	7.04	cG2085→A [f]	Less severe	SH1
3	15	5	87	1	18.5	27	25	0.2	7.29	cG1003-G1137 [g]	Severe	SH2
4	14	24	85	1	146	18.4	32.1	0	4.48	cT1895→C [f] cG2078→T [f] cG1513-G1794 [g]	Severe	SH1
5	11	18	36	1	33.3	4.17	6.67	1	4.58	518 bp insertion between exon 17 and exon 18	Severe	SH1
6	19	10	98	5	282	17	24	0.04	4.11	cA1977→G [f]	Less severe	SH1
7	16	6	92	2	199	56	0.9	0.1	10.91	cG1796→C [f]	Less severe	SH1
8	21	3	60	6	36.5	60.4	9.7	0	4.21	cT497→C [f]	Less severe	PH
9	9	6	18	1	97	25	7	1.21	8.47	cG1003-G1137 [g] 579 bp insertion between exon 10 and exon 11	Severe	SH2
10	18	7	55	1	20.3	6	7.8	0	7.42	81 pb insertion between exon 14 and exon 15	Severe	SH2
11	26	30	152	5	31	16.8	5	0.76	6.3	cG123-G141 [g]	Severe	PH
12	18	24	88	5	6.2	14.2	0.5	0.14	1.71	cA1910-A1916 [g]	Severe	SH1
13	11	4	64	4	133	16.8	21	0	10.72	cG2085→A [f]	Less severe	SH1

PH: pleckstrin homology domain, SH1: Src homology 1 domain, SH2: Src homology 2 domain. [a] in years, [b] in months, [c] mg/dl (serum concentration of all imunoglobulin isotypes were two standard deviations bellow the mean values for the patient's ages), [d] percentage of lymphocytes expressing CD19, [e] mean fluorescence intensity of Btk in monocytes, [f] missense mutations, [g] deletions

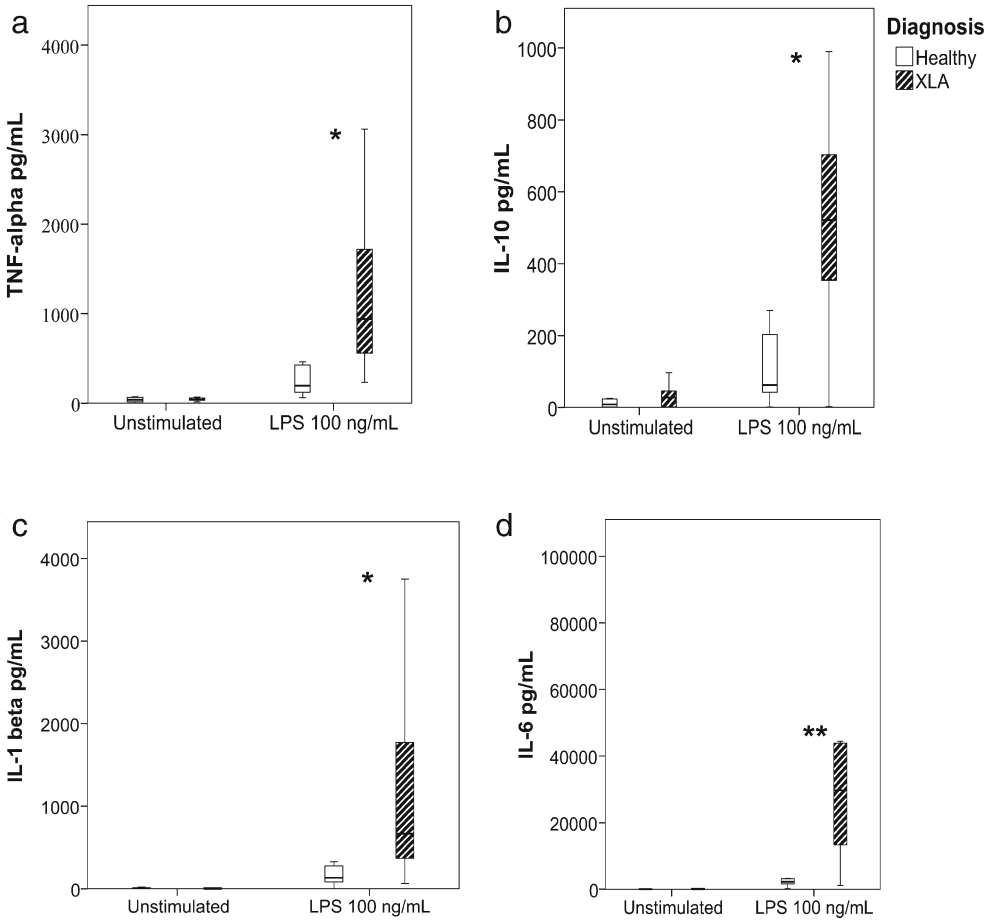


Fig. 2 TLR4-induced TNF-α, IL-10, IL-1β, and IL-6 production in XLA and healthy donors. PBMCs from XLA and healthy donors were stimulated with LPS (100 ng/mL) for 24 h, and supernatants were assayed for TNF- α (**a**), IL-10 (**b**), IL-1β (**c**), and IL-6 (**d**) production by ELISA. Significance determined by the Wilcoxon test are: *, $p<0.001$; **, $p<0.005$

differences in the production of TNF-α, IL-1β, IL-6, and IL-10 by PBMCs obtained from XLA patients and healthy controls.

Mutations and Affected Domain

Six (46 %) patients have point mutations that were considered less severe, whereas 7 (54 %) patients had splice site mutations and were considered more severe. Affected domains were SH1 (7/13, 53.84 %), SH2 (4/13, 30.76 %) and PH (2/13, 15.38 %).

Type of Mutation and Affected Domain Do Not Influence the Production of Proinflammatory Cytokines

Neither the type of mutation nor the affected domain showed a significant correlation with the production of TNF-α, IL-1β, IL-6 or IL-10 by PBMCs from XLA patients after stimulation with LPS.

Correlations Between Clinical Data, Cytokine Production and Mutation Analysis

Production of TNF-α, IL-1β, IL-6, and IL-10 by PBMCs from XLA patients in response to LPS (100 ng/mL) was analyzed to determine if there was a correlation between certain clinical manifestations (age at first infection, age at diagnosis, hospital admissions before diagnosis, serum concentration of IgG, IgA, IgM, CD19+ cells, and MFI of Btk expression in monocytes) and cytokine production. Patients whose PBMCs produced higher concentrations of TNF-α had more hospital admissions before diagnosis ($r=0.584$, $p=0.036$); these patients also had lower levels of serum IgA at diagnosis ($r=-0.605$, $p=0.028$). Similarly, patients whose PBMCs produced higher amounts of IL-1β showed lower levels of serum IgA at diagnosis ($r=-0.6$, $p=0.03$) (Fig. 3).

There was a significant correlation between type of Btk mutation and serum IgG at diagnosis, ($r=-0.577$, $p=0.038$), such that patients with mutations considered less severe had higher concentration of serum IgG at diagnosis (Fig. 4).

Discussion

A growing body of evidence suggests that Btk participates in TLR signaling; [7–13, 16, 20] however, how Btk is involved in the TLR signaling pathway is only beginning to be understood.

Studies evaluating response to TLR4 stimuli in XLA patients show some discrepancies mainly explained because

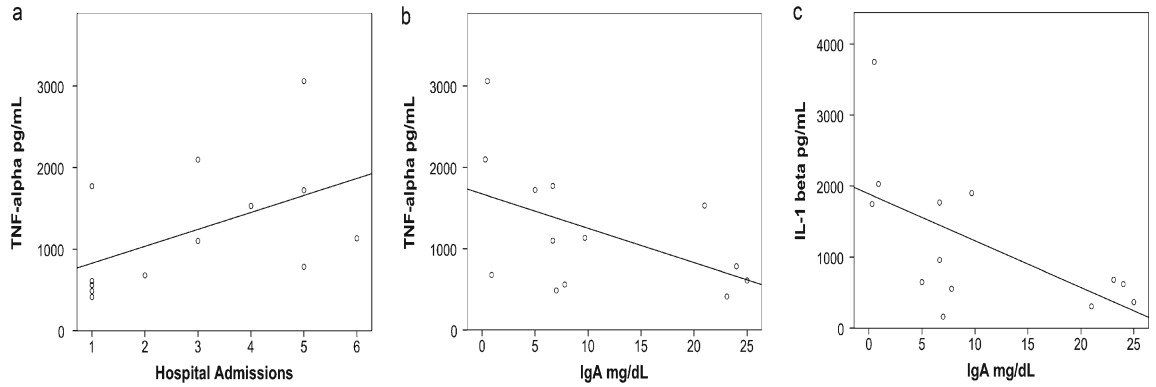


Fig. 3 Correlation between clinical manifestations of disease and cytokine production. Patients whose PBMCs produced higher amounts of TNF-α had more hospital admissions prior to diagnosis and lower serum IgA levels (r=0.584, p=0.036 and r=−0.605, p=0.028, respectively). Patients with higher production of IL-1β had lower serum IgA levels (r=−0.6, p=0.03)

of several technical and methodological differences such as the type of cells employed, which included PBMCs with [11, 12] or without a B cell depletion procedure for control cells, [14] dendritic cells derived from monocytes, [13, 15, 16] monocytes isolated from PBMCs through adherence to plastic [15]. Other variables to be considered at the moment of the sampling are the presence or absence of concurrent infections. Only one study specify this situation, [16] as well as information regarding if blood sampling was before or after immunoglobulin administration [15, 16]. Furthermore, the nature and concentration of TLR4 stimuli varies between different studies [11–16]. Our data show that in XLA patients, stimulation of PBMCs with LPS induces a significantly increased production of TNF-α, IL-1β, IL-6, and IL-10 by PBMCs, as compared with healthy controls. These data are in accordance with a number of murine studies, [9, 10, 17, 21] and with Marron et al., [15] whose paper, similar to our study, included a larger number of XLA patients (thirteen), the blood samples were collected before immunoglobulin, and the nature and concentration of LPS was similar [15]. This finding suggests that Btk is involved in TLR signaling.

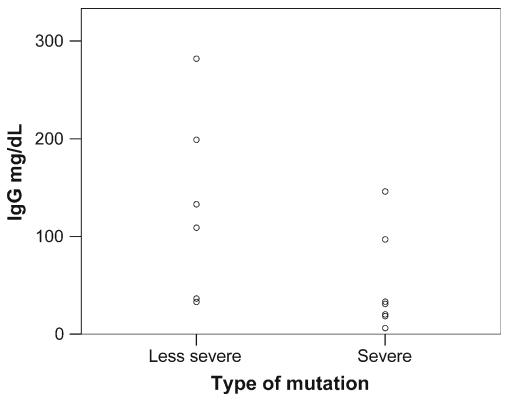


Fig. 4 Relationship between serum IgG at diagnosis and type of mutation in the Btk gene. Patients with mutations considered less severe showed higher levels of serum IgG at diagnosis, compared with those patients with mutations considered more severe (r=−0.577, p=0.038)

In order to clarify the effect of Btk absence on LPS-induced cytokine production, we compared patients with residual Btk expression with patients showing the lowest Btk expression (less than 5 MFI of Btk in monocytes), but we were unable to find significant differences between both groups, possibly because of the reduced sample size. Although interaction of Btk with adapter molecules of TLR's signaling has been demonstrated, the Btk domain implicated is unknown, and in spite of residual Btk expression in our patients, the protein is not functional (8, 22).

LPS induces tyrosine autophosphorylation of Btk and activates its kinase activity [8, 10, 11]. Jefferies et al. demonstrated that Btk interacts with the TIR domains of TLR 4, 6, 8 and 9, MyD88, TIRAP and IRAK-1 [8]. TIRAP undergoes tyrosine phosphorylation by Btk in response to TLR4 or TLR2 engagement, [20, 22] and this phosphotyrosine serves as an acceptor site for the suppressor of cytokine signaling 1 (SOCS1) protein. Binding of SOCS1 to TIRAP results in the polyubiquitination of this adaptor protein and its subsequent degradation by the 26S proteasome [22, 23]. Disturbance of this signaling cascade may potentiate TIRAP-dependent NF-κB transactivation and may be responsible for a prolonged pro-inflammatory response, which would be concordant with the excessive production of pro-inflammatory cytokines that was observed in PBMCs from XLA patients after LPS stimulation, Fig. 5. Thus, Btk seems to be acting as part of a negative regulatory mechanism limiting primary innate immune responses. Our findings contradict the results of other studies; Horwood et al. found impaired TNF-α and IL-1β production in PBMCs from XLA patients in response to LPS; [11, 12] however, neither the number of patients included nor the type of Btk mutation are mentioned. Perez de Diego et al. reported similar TNF-α production in Btk-null and control monocytes after stimulation with LPS in seven XLA patients [24]. Our patients were diagnosed later in life compared with

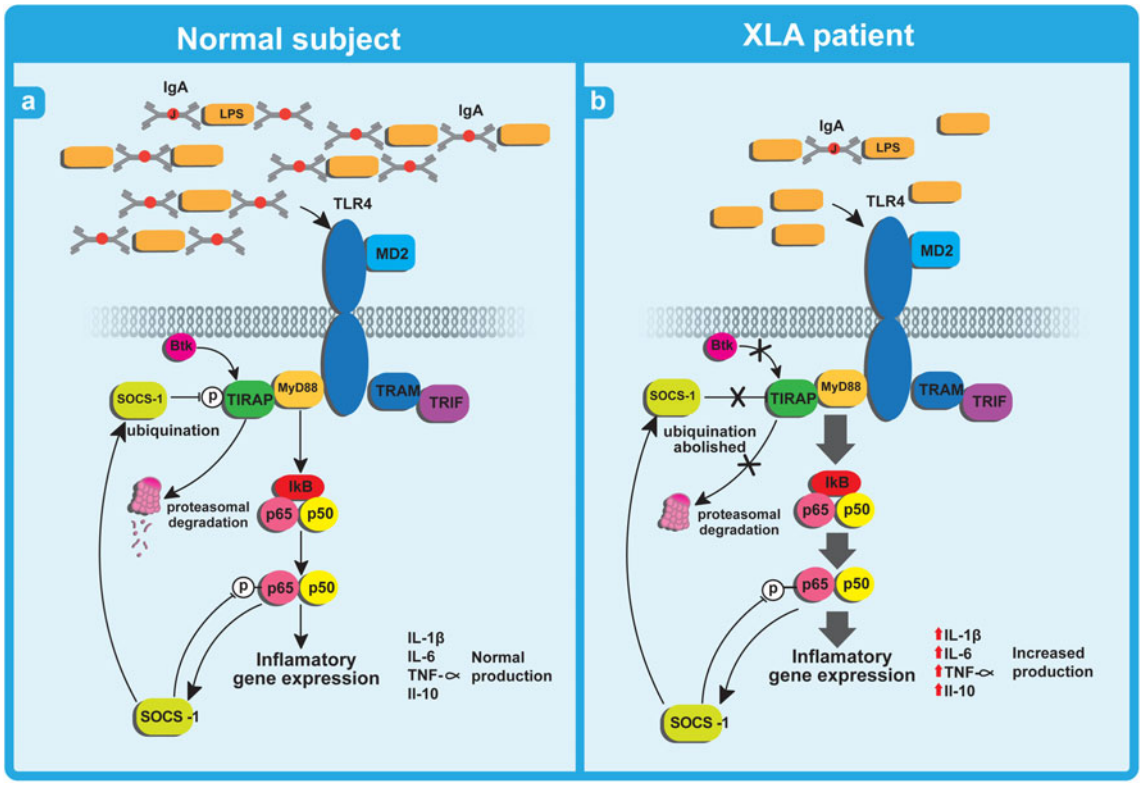


Fig. 5 TLR4 signaling. Normal Subjects (**a**) and XLA patients (**b**), in absence or deficiency of Btk, TLR4 signaling is over expressed, because of a failure of TIRAP degradation, also XLA patients have very diminished IgA, which allow more LPS available for junction to the receptor TLR4

those reported by Perez de Diego, so it is possible that the repetitive stimuli by infection in our patients could enhance the pro-inflammatory response and explain differences with patients from the study of Perez de Diego et al.

We did not find a correlation between the severity of Btk mutation, using the scale proposed by Broides et al., [19] and affected domain with clinical characteristics or cytokine production; this may be due to the small sample size used in this study. However, we have documented that patients with more severe Btk mutations have lower levels of IgG, which is similar to findings in previous reports [25].

Patients whose PBMCs produced higher concentration of TNF-α had more hospital admissions prior to diagnosis. This finding suggests that an increased number of severe infections may drive and/or potentiate a Th-1 polarized or inflammatory response in PBMCs from XLA patients. Our results resemble those reported by Paustian et al., who demonstrated that repetitive exposure to LPS induces a burst of IL-6 production by normal monocyte-derived dendritic cells [26].

Patients whose PBMCs produced more TNF-α and IL-1β after stimulation with LPS had lower serum IgA levels at diagnosis. Fernandez et al. demonstrated that intracellular dimeric IgA down regulates inflammation induced by LPS in intestinal epithelial cells by binding LPS during its intracellular trafficking and subsequently reducing NF-κB translocation. This leads to a strong attenuation of an LPS pro-inflammatory response [27]. Decreased concentration of IgA in XLA patients may enhance the interaction between TLR4 and its ligand in the gastrointestinal tract. The continuous stimulation that occurs in vivo may potentiate the inflammatory response that we observed in vitro and, in combination with a poor regulation due to an absence of or deficiency in Btk, result in a predominantly pro-inflammatory response to LPS. Clinically, this is an important finding because this hyperinflammatory response in the presence of recurrent pneumonia could play a role in the development of bronchiectasis in XLA patients.

Conclusions

We have provided evidence that LPS-induced TNF-α, IL-β, IL-6, IL-1, and IL-10 production by PBMCs is increased in XLA patients compared with matched healthy controls that is likely due to a deregulation of TLR signaling in the absence of Btk. This response may be influenced by repetitive severe infections and IgA levels in the patients. Thus, Btk seems to be acting as part of a negative regulatory mechanism limiting the primary innate immune response.

Acknowledgments The authors sincerely thank the patients and their families for their cooperation. This work was supported by the following foundations and institutions: Consejo Nacional de Ciencia y

Tecnología (CONACYT, México), grants 56836 and 153733; Instituto de Ciencia y Tecnología del Distrito Federal grant ICYTDF/326/2009; "Fundación para niñas y niños con inmunodeficiencias primarias A.C.". María Edith González Serrano and Alexander Vargas Hernández were fellows 207006 and 206710 from Conacyt respectively.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Conley ME. B cells in patients with X-linked agammaglobulinemia. J Immunol. 1985;134(5):3070–4.
2. Valiaho J, Smith CI, Vihinen M. BTKbase: the mutation database for X-linked agammaglobulinemia. Hum Mutat. 2006;27(12):1209–17. doi:10.1002/humu.20410.
3. Winkelstein JA, Marino MC, Lederman HM, Jones SM, Sullivan K, Burks AW, et al. X-linked agammaglobulinemia: report on a United States registry of 201 patients. Medicine. 2006;85(4):193–202. doi:10.1097/01.md.0000229482.27398.ad.
4. Barnett EV, Winkelstein A, Weinberger HJ. Agammaglobulinemia with polyarthritis and subcutaneous nodules. Am J Med. 1970;48 (1):40–7.
5. Sarmiento E, Mora R, Rodriguez-Mahou M, Rodriguez-Molina J, Fernandez-Cruz E, Carbone J. Autoimmune disease in primary antibody deficiencies. Allergologia et immunopathologia. 2005;33 (2):69–73.
6. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010;11(5):373–84. doi:10.1038/ni.1863.
7. Doyle SL, Jefferies CA, Feighery C, O'Neill LA. Signaling by Toll-like receptors 8 and 9 requires Bruton's tyrosine kinase. J Biol Chem. 2007;282(51):36953–60. doi:10.1074/jbc.M707682200.
8. Jefferies CA, Doyle S, Brunner C, Dunne A, Brint E, Wietek C, et al. Bruton's tyrosine kinase is a Toll/interleukin-1 receptor domain-binding protein that participates in nuclear factor kappaB activation by Toll-like receptor 4. J Biol Chem. 2003;278(28):26258–64. doi:10.1074/jbc.M301484200.
9. Lee KG, Xu S, Wong ET, Tergaonkar V, Lam KP. Bruton's tyrosine kinase separately regulates NFkappaB p65RelA activation and cytokine interleukin (IL)-10/IL-12 production in TLR9-stimulated B Cells. J Biol Chem. 2008;283(17):11189–98. doi:10.1074/jbc.M708516200.
10. Schmidt NW, Thieu VT, Mann BA, Ahyi AN, Kaplan MH. Bruton's tyrosine kinase is required for TLR-induced IL-10 production. J Immunol. 2006;177(10):7203–10.
11. Horwood NJ, Mahon T, McDaid JP, Campbell J, Mano H, Brennan FM, et al. Bruton's tyrosine kinase is required for lipopolysaccharide-induced tumor necrosis factor alpha production. J Exp Med. 2003;197(12):1603–11. doi:10.1084/jem.20021845.
12. Horwood NJ, Page TH, McDaid JP, Palmer CD, Campbell J, Mahon T, et al. Bruton's tyrosine kinase is required for TLR2 and TLR4-induced TNF, but not IL-6, production. J Immunol. 2006;176(6):3635–41.
13. Taneichi H, Kanegane H, Sira MM, Futatani T, Agematsu K, Sako M, et al. Toll-like receptor signaling is impaired in dendritic cells from patients with X-linked agammaglobulinemia. Clin Immunol. 2008;126(2):148–54.
14. Jyonouchi H, Geng L, Toruner GA, Vinekar K, Feng D, Fitzgerald-Bocarsly P. Monozygous twins with a microdeletion syndrome involving BTK, DDP1, and two other genes; evidence of intact dendritic cell development and TLR responses. Eur J Pediatr. 2008;167(3):317–21. doi:10.1007/s00431-007-0493-0.
15. Marron TU, Martinez-Gallo M, Yu JE, Cunningham-Rundles C. Toll-like receptor 4-, 7-, and 8-activated myeloid cells from patients with X-linked agammaglobulinemia produce enhanced inflammatory cytokines. J Allergy Clin Immunol. 2012;129(1):184–90. doi:10.1016/j.jaci.2011.10.009. e1-4.
16. Sochorova K, Horvath R, Rozkova D, Litzman J, Bartunkova J, Sediva A, et al. Impaired Toll-like receptor 8-mediated IL-6 and TNF-alpha production in antigen-presenting cells from patients with X-linked agammaglobulinemia. Blood. 2007;109(6):2553–6. doi:10.1182/blood-2006-07-037960.
17. Zorn CN, Keck S, Hendriks RW, Leitges M, Freudenberg MA, Huber M. Bruton's tyrosine kinase is dispensable for the Toll-like receptor-mediated activation of mast cells. Cell Signal. 2009;21 (1):79–86. doi:10.1016/j.cellsig.2008.09.010.
18. Lopez-Herrera G, Berron-Ruiz L, Mogica-Martinez D, Espinosa-Rosales F, Santos-Argumedo L. Characterization of Bruton's tyrosine kinase mutations in Mexican patients with X-linked agammaglobulinemia. Mol Immunol. 2008;45(4):1094–8. doi:10.1016/j.molimm.2007.07.022.
19. Broides A, Yang W, Conley ME. Genotype/phenotype correlations in X-linked agammaglobulinemia. Clin Immunol. 2006;118(2–3):195–200. doi:10.1016/j.clim.2005.10.007.
20. Gray P, Dunne A, Brikos C, Jefferies CA, Doyle SL, O'Neill LA. MyD88 adapter-like (Mal) is phosphorylated by Bruton's tyrosine kinase during TLR2 and TLR4 signal transduction. J Biol Chem. 2006;281(15):10489–95. doi:10.1074/jbc.M508892200.
21. Hasan M, Lopez-Herrera G, Blomberg KE, Lindvall JM, Berglof A, Smith CI, et al. Defective Toll-like receptor 9-mediated cytokine production in B cells from Bruton's tyrosine kinase-deficient mice. Immunology. 2008;123(2):239–49. doi:10.1111/j.1365-2567.2007.02693.x.
22. Mansell A, Smith R, Doyle SL, Gray P, Fenner JE, Crack PJ, et al. Suppressor of cytokine signaling 1 negatively regulates Toll-like receptor signaling by mediating Mal degradation. Nat Immunol. 2006;7(2):148–55. doi:10.1038/ni1299.
23. Kobayashi T, Takaesu G, Yoshimura A. Mal-function of TLRs by SOCS. Nat Immunol. 2006;7(2):123–4. doi:10.1038/ni0206-123.
24. Perez de Diego R, Lopez-Granados E, Pozo M, Rodriguez C, Sabina P, Ferreira A, et al. Bruton's tyrosine kinase is not essential for LPS-induced activation of human monocytes. J Allergy Clin Immunol. 2006;117(6):1462–9. doi:10.1016/j.jaci.2006.01.037.
25. Lopez-Granados E, Perez de Diego R, Ferreira Cerdan A, Fontan Casariego G, Garcia Rodriguez MC. A genotype-phenotype correlation study in a group of 54 patients with X-linked agammaglobulinemia. J Allergy Clin Immunol. 2005;116(3):690–7. doi:10.1016/j.jaci.2005.04.043.
26. Paustian C, Caspell R, Johnson T, Cohen PA, Shu S, Xu S, et al. Effect of multiple activation stimuli on the generation of Th1-polarizing dendritic cells. Hum Immunol. 2011;72(1):24–31. doi:10.1016/j.humimm.2010.10.004.
27. Fernandez MI, Pedron T, Tournebize R, Olivo-Marin JC, Sansonetti PJ, Phalipon A. Anti-inflammatory role for intracellular dimeric immunoglobulin a by neutralization of lipopolysaccharide in epithelial cells. Immunity. 2003;18(6):739–49.