HHS Public Access

Author manuscript

Nat Immunol. Author manuscript; available in PMC 2013 November 01.

Published in final edited form as:

Nat Immunol. 2013 May ; 14(5): 514–522. doi:10.1038/ni.2569.

Trypanosoma cruzi trans-sialidase initiates an ROR-γt–AHR-independent program leading to IL-17 production by activated B cells

Daniela A Bermejo[1], **Shaun W Jackson**[2,4], **Melisa Gorosito-Serran**[1], **Eva V Acosta-Rodriguez**[1], **Maria C Amezcua-Vesely**[1], **Blythe D Sather**[2,4], **Akhilesh K. Singh**[2,4], **Socheath Khim**[2,4], **Juan Mucci**[5], **Denny Liggitt**[6], **Oscar Campetella**[5], **Mohamed Oukka**[2,3,4], **Adriana Gruppi**[1,7], and **David J Rawlings**[2,3,4,7]

[1]Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET). Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. Córdoba. Argentina

[2]Department of Pediatrics, University of Washington School of Medicine, Seattle, WA 98195

[3]Department of Immunology, University of Washington School of Medicine, Seattle, WA 98195

[4]Seattle Children's Research Institute, Seattle, WA 98101

[5]Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín, B1650WGA San Martín, Argentina

[6]Department of Comparative Medicine, University of Washington School of Medicine, Seattle, WA 98195

Abstract

We identified B cells as a major source for rapid, innate-like interleukin 17 (IL-17) production *in vivo* in response to *Trypanosoma cruzi* infection. IL-17$^+$ B cells exhibited a plasmablast phenotype, outnumbered T_H17 cells and were required for optimal response to this pathogen. Using both murine and human primary B cells, we demonstrate that exposure to parasite-derived

Correspondence should be addressed to D.J.R. (drawling@u.washington.edu) **or A. G.** (agruppi@fcq.unc.edu.ar), **David J. Rawlings, M.D.**, Center for Immunity and Immunotherapies, Seattle Children's Research Institute, 1900 Ninth Avenue, Seattle, WA 98101, Tel. (206) 987-7450, Fax (206) 987-7310, **Adriana Gruppi, PhD.**, Inmunología. Departamento de Bioquímica Clínica. Facultad de Ciencias Químicas. Universidad Nacional de Córdoba. Haya de la Torre y Medina Allende. Ciudad Universitaria. Córdoba (5000). Argentina., Tel.: +54 351-4344973., Fax: +54 351-4333048.

[7]A.G. and D.J.R. contributed equally to this work.

AUTHOR CONTRIBUTIONS

DAB performed most of the experiments and analyzed the results; SWJ performed experiments, including cell transfer studies, immunohistochemistry and additional analyses; MGS helped with trypomastigote cultures and studies of *T. cruzi* infected μMT mice; MCAV performed Q-PCR studies and analysis; BDS established and helped to perform human tonsillar assays; AKS performed Q-PCR studies and IL-17 related assays; SK assisted with mouse colony management and cell transfer experiments; DL performed and interpreted tissue histopathology studies; JM and OC provided trans-sialidase, anti-trans-sialidase Abs and provided technical and intellectual contributions to perform sialylation and desialylation assays. EVAR and MO significantly contributed to study design and analysis, AG and DJR conceived, designed and supervised the study. SWJ, EVAR, AG and DJR wrote the manuscript. All authors reviewed the manuscript before submission.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

trans-sialidase *in vitro* was sufficient to trigger modification of the cell surface mucin, CD45, leading to Btk-dependent signaling and IL-17A or IL-17F production via an ROR-γt and AHR-independent transcriptional program. Our combined data suggest that generation of IL-17$^+$ B cells may be an unappreciated feature of innate immune responses required for pathogen control or IL-17-mediated autoimmunity.

Interleukin 17 (IL-17) is a proinflammatory cytokine that contributes to host protection against a range of infectious pathogens by inducing neutrophil recruitment and secretion of inflammatory mediators[1]. The IL-17 cytokine family comprises six related proteins: IL-17A (also called IL-17), IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25) and IL-17F. The best-studied members, IL-17A and IL-17F, share the highest homology and are coordinately secreted by multiple subsets of immune cells as homodimers or IL-17A–IL-17F heterodimers[2].

The description of new sources and mechanisms responsible for IL-17 production may have critical relevance in the understanding of IL-17-mediated immune responses during infection and autoimmunity. In addition to its impact in bacterial and fungal infections, emerging data implicate IL-17 in the control of selected parasitic pathogens[3–5]. Consistent with this theme, recent work has suggested an important role for IL-17 in resolution of infection with the protozoan parasites, *Trypanosoma cruzi* (*T. cruzi*) and *Trypanosoma congolense*[6–8]. Indeed, prior work from our group indicates that IL-17 exerts its protective function following exposure of mice to *T. cruzi*, in part, by recruiting suppressive IL-10–producing neutrophils that modulate interferon-γ (IFN-γ) production and thereby limit tissue damage[9]. In the absence of this IL-17 dependent response, infected animals exhibit markedly increased liver pathology and overwhelming wasting disease.

During the course of *T. cruzi* infection, we observed that IL-17 was produced by multiple cell populations including: NKT cells and γδ, CD4$^+$ (T_H17) and CD8$^+$ (T_C17) T cells[9]. Each of these hematopoietic-derived cell subsets has previously been identified as an IL-17 producing population[1,10]. Interestingly, we also observed a predominant cell population, present during peak parasitemia, lacking relevant lineage markers for each of these lineages. In this study, we have identified this new cellular source of IL-17 and determined the signals required to promote IL-17 production by such cells in response to *T. cruzi* infection. Our combined data provide the first demonstration that B lineage cells secrete IL-17 in response to challenge with an infectious pathogen. B cell-intrinsic IL-17A production was triggered via a novel signaling cascade in response to a *T. cruzi*-derived extracellular enzyme and was required for efficient parasite control.

RESULTS

T. cruzi infection triggers generation of IL-17$^+$ B cells

To identify the cell populations responsible for IL-17 production during *T. cruzi* infection, we characterized the phenotype of IL-17A–producing cells in mice infected with 10,000 trypomastigotes of *T. cruzi* (Y strain)[11]. Surprisingly, most IL-17A-producing cells in the spleen at day 10 post-infection lacked CD3 expression. Instead, these cells consistently

expressed the prototypical B lineage cell surface protein, CD19, as well as lower amounts of the B cell antigen, B220 (Fig. 1a). Although $CD4^+$ IL-17A-producing (T_H17) cells were generated during *T. cruzi* infection, IL-$17A^+$ $B220^+$ cells significantly outnumbered T_H17 cells at days 10 and 19 post infection (Fig. 1b) and no significant increase in $CD8^+$ IL-17-producing cells occurred at either time-point. Analyzing additional B cell markers, we determined that a proportion of $CD19^+$ IL-$17A^+$ cells expressed the plasmablast or plasma cell marker, CD138, but lacked the germinal center markers, GL7 and PNA (Fig. 1c and data not shown). These observations suggested that plasma cell-committed B cells, but not germinal center B cells, are able to produce IL-17. In agreement, immunofluorescence analysis of the spleen (Fig. 1d) identified an IgM^{hi} IL-17^+ cell population outside the (less strongly staining IgM^{lo}) splenic follicle and proximal to the central arteriole (T cell zone), a finding consistent with the abundant extrafollicular plasmablast response previously characterized during *T. cruzi* infection[12].

To verify these results, we quantified IL-17A mRNA in total splenocytes and in sorted $CD19^+$ $B220^+$ B cells versus $CD19^-$ $B220^-$ non-B cells derived from infected mice. Abundant IL-17A mRNA was present upon stimulation of $CD19^+$ $B220^+$ B cells (Fig. 1e). In contrast, transcripts were undetectable in B cells isolated from non-infected mice (not shown). $B220^-$ non-B cells from infected mice also exhibited abundant IL-17A mRNA expression, suggesting that a subset of non-B cells produced somewhat higher amounts of IL-17 transcripts on a per cell basis compared with B cells. Next, we directly measured IL-17A in culture supernatants from purified, splenic-derived, $CD19^+B220^+$ cells from infected versus uninfected control animals. In the absence of any additional stimulus, B cells from infected animals spontaneously secreted IL-17A and cytokine production was further enhanced using PMA plus ionomycin stimulation (Fig. 1f). No IL-17A production was detected in either stimulated or resting B cells cultures established using uninfected animals.

To confirm the B cell origin of the $CD19^+$ $B220^+$ IL-17^+ cells induced during *T. cruzi* infection, we studied this population in μMT mice, animals that lack mature B cells. $B220^+$ IL-17^+ cells were undetectable in *T. cruzi* infected μMT mice (Fig. 1g,h). Development of this population was restored, however, in recipients of adoptively transferred mature B cells (Fig. 1g,h). IL-17^+ cells induced in B cell-reconstituted, infected μMT mice showed a surface phenotype indistinguishable from that observed in infected wild-type mice ($B220^{dim}CD19^+CD138^+GL7^-PNA^-$ IL-17^+ cells; Fig. 1g).

Taken together, these results provide the first demonstration that B cells are able to secrete the proinflammatory cytokine IL-17A in response to challenge with an infectious pathogen. Further, these data indicate that, during *T. cruzi* infection, IL-17 producing B cells are a major source of this cytokine, outnumbering T_H17 cells.

B cell IL-17A production promotes *T. cruzi* parasite control

To test the physiologic relevance of IL-17 production by B cells during *T. cruzi* infection, we adoptively transferred wild-type or IL-17A-deficient B cells into μMT recipients and infected these animals with *T. cruzi*. While equivalent B cell reconstitution was achieved in all recipient animals (Supplementary Fig. 1), control μMT mice, and μMT recipients of IL-17A-deficient B cells, exhibited increased parasitemia compared with recipients of wild-

type B cells (Fig. 2a) and, ultimately, this lead to accelerated mortality (Fig. 2b). Lack of B cell IL-17A production was associated with elevated IFN-γ and tumor necrosis factor (TNF) concentrations (Fig. 2c,d). These differences correlated with increased tissue damage as manifest by transaminitis (Fig. 2e). Thus, B cells are the major producers of IL-17A during acute *T. cruzi* infection and B cell-intrinsic IL-17A production is critical for the protective response to this pathogen.

B cells utilize alternative IL-17 triggering signals

We next determined the signals leading to B cell IL-17 production during *T. cruzi* infection. The cytokines, IL-23 and IL-6, strongly promote IL-17 production by γδ and $CD4^+$ T cells[1,10]. Surprisingly, infected IL-23R-deficient or IL-6-deficient mice showed no difference in the number of $B220^+$ $IL\text{-}17^+$ cells in comparison to infected wild-type mice, implying that these signals were not required for B cell IL-17A production (Supplementary Fig. 2a). Further, B-T cell cooperation through the CD40 pathway[13] was also not required as similar numbers of IL-17-producing $B220^+$ cells were detected in infected CD40-deficient versus control mice (Supplementary Fig. 2a).

Microbial products that trigger C-type lectin- and Toll-like-receptors (TLR) promote IL-17 production by innate cells[10]. Moreover, TLR ligands are critically involved in B cell activation and differentiation[14,15] and mediate the immune recognition of *T. cruzi*[16]. Thus, we analyzed whether direct triggering of TLRs or the B cell antigen receptor (BCR) induced IL-17 production by purified B cells *in vitro*. No significant IL-17 production was detected in cultures supernatants of normal splenic B cells stimulated with various doses of Zymosan (a dectin-1 and TLR2 ligand), lipopolysaccharide (LPS, TLR4 ligand), CpG (TLR9 ligand), anti-IgM or CpG plus anti-IgM (Supplementary Fig. 2b), indicating that TLR, BCR or dual cross-linking were insufficient to induce potent IL-17 production. Consistent with these data, we observed no defect in $B220^+$ $IL\text{-}17^+$ generation in infected μMT mice that were recipients of adoptively transferred mature B cells lacking a key adaptor protein required for TLR signaling, MyD88 (data not shown).

T. cruzi triggers $IL\text{-}17^+$ B cell generation *in vitro*

As *T. cruzi* is a complex microorganism that initiates a cascade of direct and indirect lineage-specific activation events *in vivo* [16], we determined whether *in vitro* parasite exposure was sufficient to initiate IL-17 production by purified B cells. Indeed, live *T. cruzi* trypomastigotes rapidly induced parasite dose-dependent IL-17A production by follicular mature (FM) B cells (Fig. 3a and data not shown). Similarly, antigen from total homogenates of *T. cruzi* trypomastigotes induced detectable, albeit less, IL-17A secretion by total splenic B cells (Fig. 3a). Of note, the purity (>90–95%) and phenotype of flow cytometry-sorted B cells was confirmed in this and all subsequent experiments.

Interestingly, using highly purified splenic B cell subsets, mature (marginal zone or FM), but not immature (transitional) B cells, secreted both IL-17A and IL-17F following exposure to live parasites (Fig. 3b,c). Lack of cytokine expression by transitional B cells was not due to reduced cell viability (data not shown). Importantly, neither total spleen cells from RAG2-deficient mice nor wild-type non-B or purified T cells produced IL-17A after *T. cruzi*

stimulation *in vitro* (Supplementary Fig. 2c–d), indicating that a contaminating non-B or -T cell population or residual T cells could not account for IL-17 production in response to parasite encounter.

Additionally, we evaluated the participation of key B cell surface proteins in triggering IL-17 production *in vitro*. Consistent with our *in vivo* data, cytokine production was not altered by loss of the co-stimulatory receptor, CD40, or the TLR signaling adaptor, MyD88. We also observed no impact on IL-17 production by restricting BCR specificity to recognition of a non-parasite associated antigen (hen-egg lysozyme; Supplementary Fig. 2e). Thus, direct exposure of B cells to *T. cruzi* trypomastigotes was sufficient to initiate B cell IL-17 production.

T. cruzi trans-sialidase drives IL-17$^+$ B cell formation

Polyclonal B cell activation is a hallmark of acute *T. cruzi* infection[17,18], implying that a range of *T. cruzi* surface antigens might be capable of triggering an IL17 program. We noted that IL-17A–producing B cells were most abundant at peak parasitemia, a time point coinciding with a strong polyclonal B cell activation. Therefore, we investigated whether candidate polyclonal activators expressed on the surface of *T. cruzi* might be sufficient to trigger B cell IL-17 production. We focused our efforts on *T. cruzi* trans-sialidase, an abundant, parasite cell surface, GPI-anchored enzyme required for parasite invasion[19] and previously shown to exhibit potent T cell-independent B cell mitogenic activity[20]. Strikingly, recombinant, enzymatically active, trans-sialidase efficiently triggered, dose-dependent, IL-17A production by murine B cells (Fig. 3d).

T. cruzi trans-sialidase is comprised of an N-terminal catalytic domain and a long C-terminal domain containing of multiple 12-amino-acid (DSSAHSTPSTPA) tandem repeats, also known as the Shed-Acute-Phase-Antigen (SAPA)[21]. Trans-sialidase activity is critical for *T. cruzi* pathogenesis[22,23] and a natural point-mutation (Tyr342His), that abrogates enzymatic activity while retaining N-terminal lectin-like activity, prevents many immunological alterations induced by trans-sialidase *in vivo* [23]. In contrast, strong antigenicity[21] and B cell mitogenic activity[20] is present in the SAPA motifs of the C-terminal moiety. We therefore analyzed IL-17A secretion following B cell stimulation with recombinant, Tyr342His mutant trans-sialidase. While mutant trans-sialidase was still able to trigger some IL-17A production, cytokine production was significantly lower than with active trans-sialidase (Fig. 3d); indicating that enzymatic activity is required for a robust response and that either the SAPA repeats or residual N-terminal lectin activity may partially mediate cytokine production. To further assess the role for SAPA repeats, we utilized a neutralizing mAb directed against the catalytic site of trans-sialidase[22,24], a reagent that blocks both enzymatic function and residual lectin activity. Anti-trans-sialidase, but not control, mAb treatment abrogated trans-sialidase- or trypomastigote-induced IL-17 production (Fig. 3e), implicating trans-sialidase as the sole *T. cruzi* molecule required in this process.

Glycoconjugate remodeling promotes B cell IL-17 secretion

Trans-sialidase exhibits a unique enzymatic activity, first described in trypanosomatids, that promotes transfer of α(2,3)-linked sialic acid from host glycoconjugates to acceptor proteins

on the parasite surface or to recipient host molecules[25]. In the absence of suitable acceptors, trans-sialidase also displays neuraminidase activity leading to hydrolysis of α(2,3)-linked sialyl residues[26]. We evaluated the role for these alternative enzymatic activities in IL-17 secretion by B cells in our experimental system. We first tested whether IL-17A production was induced after hydrolysis of sialyl residues using neuraminidases derived from candidate bacteria species including *Vibrio cholerae* (specific for α(2,3)-linked sialyl residues) and *Clostridium perfringens* (able to hydrolyze α(2,3); α(2,6) and α(2,8)-linked sialyl residues). No IL-17A was detected upon B cell stimulation with several doses of bacterial neuraminidases, suggesting that desialylation of B cell-surface molecules was not sufficient to trigger of IL-17 production (Fig. 3f and data not shown).

Because trans-sialidase is able to sialylate lymphocyte membrane proteins *in vivo*[26] and protein specific sialylation is associated with alterations in immune function[27,28], we tested whether trans-sialidase-catalyzed modification of host B cell surface molecules might promote IL-17 production. For this, B cells were cultured with BSA under serum-free conditions to avoid presence of soluble donor or acceptors of sialyl residues. Production of IL-17 in the presence of trans-sialidase or parasites was detected only when the sialyl donor, α(2,3)-sialyl-lactose, but not the control sugar, α(2,6)-sialyl-lactose, was present, linking sialylation of B cells with IL-17A production (Fig. 3g).

To better mimic physiological conditions, where serum glycoproteins act not only as donors but also as suitable residue acceptors, we carried out experiments where lactose was added as a sialyl residue acceptor. Under these conditions, the production of IL-17 was markedly enhanced (Fig. 3g). Together, these data supported the notion that the simple acquisition of sialyl residues on the B cell triggers modest IL-17 secretion whereas the more complete surface glycoconjugate remodeling that occurs in the presence of sialyl donor or acceptor molecules promotes a vigorous IL-17 response program.

CD45 is required for generation of IL-17$^+$ B cells

Trans-sialidase has been reported to catalyze sialylation of host acceptor molecules including the protein-tyrosine phosphatase, CD45, on the surface of T lymphocytes[25]. While never directly tested, these events have been postulated to promote immune abnormalities induced by this enzyme. As an initial screen for the potential role for CD45 in the B cell IL-17 program, we utilized a CD45-specific inhibitor (N-(9,10-Dioxo-9,10-dihydro-phenanthren-2-yl)-2,2-dimethyl-propionamide). We observed a significant reduction in IL-17A and IL-17F production when sorted B cells were co-incubated with *T. cruzi* trypomastigotes and increasing doses of inhibitor (Fig. 4a, Supplementary Fig. 3a and data not shown).

Next we assessed the genetic requirement for CD45 in the *in vitro* IL-17 effector response using B cells isolated from CD45-deficient mice. In contrast to wild-type B cells, CD45-deficient B cells failed to produce IL-17A or -17F in response to parasite exposure (Fig. 4a and Supplementary Fig. 3a). Further, in contrast to wild-type counterparts, B cells from CD45-deficient mice adoptively transferred into recipient μMT mice, were unable to restore the B220$^+$ IL-17$^+$ population generated following *T. cruzi* infection (Fig. 4b–c).

Galectins are sugar-binding proteins that participate in multiple immune responses[29] and galectin-1 and -3 binding to CD45 can modulate T lymphocyte survival[30]. These lectins are produced by B cells in response to *T. cruzi* infection[31,32] and might recognize sialylation changes induced by trans-sialidase. Parasite induced IL-17, however, was unimpaired by B cell-intrinsic loss of Galectin-1 or-3 ruling out a role for these lectins in these events (Fig. 4d).

IL-17$^+$ B cell production requires Src and Btk signals

We next addressed the potential intracellular signaling pathways leading to B cell IL-17 production. Consistent with the key role for CD45 in regulating Src family kinase activity, Src inhibition markedly attenuated IL-17 production in trypomastigote-stimulated B cells (Fig. 4e and Supplementary Fig. 3b). As previous work implicated the non-receptor tyrosine kinase, Btk, in trans-sialidase–mediated B cell mitogenic activity[33], we analyzed whether Btk or Tec kinases and other downstream Btk effectors, including PKCβ and NF-κB[34, 35] were required. Addition of specific, covalent Btk-Tec inhibitors (AVL-292 or CNX-652) strongly reduced IL-17A, and abrogated IL-17F, production (Fig. 4f and Supplementary Fig. 3c). In agreement, little or no IL-17 was detected following activation of B cells derived from Btk-deficient mice and IL-17 cytokine production was abrogated in parasite-stimulated Btk-Tec doubly deficient B cells (Fig. 4f and Supplementary Fig. 3c). Of note, deletion of Tec kinases or addition of various inhibitors had no appreciable impact on B cell survival in these assays (data not shown). Consistent with these findings, CD19$^+$ IL-17$^+$ B cells were markedly decreased in Btk-deficient mice following *in vivo* T. cruzi infection (Fig. 4g)

Interestingly, IL-17 production by B cells cultured with *T. cruzi* was not affected by the deficiency of a key Btk effector, PKCβ (Fig. 4h). PKCβ signaling is essential for BCR-driven NF-kB activation. Consistent with the lack of requirement for PKCβ, pharmacologic inhibition of NF-kB also had no impact on IL-17 production (Supplementary Fig. 4a). Together, these results demonstrate that *T. cruzi* triggers a CD45–Src-family–Btk-Tec dependent signaling cascade that initiates B cell-specific IL-17 production.

B cells do not utilize the 'canonical' IL-17 program

The transcription factor, RORγt, is essential for differentiation of naïve T cells into T_H17 cells and subsequent expression of IL-17[2,36]. Studies, to date, have also demonstrated a requirement RORγt in all IL-17 producing lineages including 'innate' IL-17 cells[10]. Based on these data, we evaluated of the requirement for RORγt in B cell IL-17 production. Surprisingly, when stimulated using either *T. cruzi* trypomastigotes or trans-sialidase, RORγt-deficient and wild-type B cells produced similar concentrations of IL-17A and IL-17F (Fig. 5a). Consistent with the requirement for trans-sialidase in these events, IL-17 production was blocked in both populations by addition of the anti-trans-sialidase mAb.

The related nuclear receptor, RORα, can synergize with RORγt to promote T_H17 lineage development. We stimulated purified wild-type B cells with trypomastigotes in the presence, or absence, of the RORα- and RORγt-specific inhibitor, SR1001, and observed no effect on B cell IL-17 production (Fig. 5b). The ligand-dependent transcription factor, aryl hydrocarbon receptor (AHR), also promotes T_H17 lineage development and production of

IL17 and the T_H17 cytokine, IL-22[37]. Purified, trypomastigote-stimulated, AHR-deficient and wild-type B cells generated similar amounts of IL-17A (Fig. 5b). In concert with these *in vitro* data, *T. cruzi* infection lead to equivalent numbers of $CD19^+IL\text{-}17^+$ B cells in RORγt-deficient, AHR-deficient and wild-type mice (Fig. 5c).

As an alternative means to address the role for RORγt, we performed immunofluorescence studies using spleens obtained from non-infected vs. *T. cruzi* infected RORγt reporter mice. While all T cells expressed the RFP reporter[38] due to transient expression of RORγt during thymocyte development[39], we failed to identify IgM^+RFP^+ B cells following infection (Fig. 5d). Consistent with these findings and the intact $CD19^+IL17^+$ response in *T. cruzi* infected IL-23R-deficient and IL-6-deficient mice (Supplementary Fig. 2a), IL-17 production in B cells stimulated *in vitro* was unaffected by B intrinsic loss of IL-6 or combined absence of both the IL-21- and IL-23 receptors (Supplementary Fig. 4b).

Together, these results demonstrate that exposure to *T. cruzi* trans-sialidase initiates a RORγt-, RORα-and AHR-independent, B cell transcriptional program; a program that bypasses the canonical signals utilized by T_H17 and innate IL-17 cells.

T. cruzi promotes human B cell IL-17 production

To determine whether this signaling cascade was operational in primary human B cells in response to *T. cruzi*, we carried out *in vitro* studies using purified tonsillar $CD19^+$ B cells. Cells were cultured in media alone, or in association with recombinant human IL-10 or the B cell cytokine, BAFF, to facilitate cell survival, and concurrently stimulated with *T. cruzi* trypomastigotes. Human IL-17 production was consistently triggered in response to parasite exposure (Fig. 6). In contrast to murine B cells, IL-17F was slightly more abundant than IL-17A. IL-17F was detectable in cultures supernatants stimulated with parasites alone, and potentiated by addition of IL-10 or BAFF. IL-17A was identified only in parasite-stimulated, IL-10 or BAFF-supplemented, cultures. Importantly, consistent with our findings using murine B cells, IL-17 production was abrogated by addition of an anti-trans-sialidase mAb. Further, addition of CD45- or Btk-specific inhibitors significantly reduced IL-17 production. Cell viability was improved in cultures containing IL-10 or BAFF and the addition of inhibitors had no appreciable impact on survival (data not shown). Thus, *T. cruzi* trans-sialidase drives IL-17 production in human B cells via an analogous CD45–Btk-dependent signaling program.

DISCUSSION

Our combined findings identify B cells as a major source for rapid, innate-like IL-17 production in response to *T. cruzi* infection. $IL\text{-}17^+$ B cells were present at the time of peak parasitemia and outnumbered T_H17 cells throughout the course of infection. Notably, acute *T. cruzi* infection is associated with polyclonal B cell activation and, congruent with this response, $IL\text{-}17^+$ B cells exhibited a plasmablast phenotype ($CD19^+B220^{dim}GL7^-CD138^+$). Thus, our data suggest that, in addition to antibody secretion, IL-17 production may comprise an additional key effector function for plasma cells in this infection. Consistent with this idea, B cell-intrinsic, IL-17A production was required for efficient control of parasitemia and regulation of parasite inflammatory responses. Strikingly, B cell IL-17

production in response to *T. cruzi* trypomastigotes occurred via a novel pathway triggered by parasite-derived trans-sialidase. Our data support a model whereby this enzyme directly modifies the B cell surface protein, CD45 (or indirectly impacts CD45 activity via modification of another B cell surface protein), thereby triggering Src- and Btk-Tec kinase-family dependent intracellular signaling. These signals, ultimately, drive abundant IL-17 production via a ROR-γt–AHR-independent transcriptional program. Importantly, this signaling program operated in both murine and human primary B cells.

Genetic deletion or pharmacological blockade of CD45, respectively, abrogated B cell IL-17 production. Thus, specific alterations in CD45 structure or localization unique to trans-sialidase-modified B cells appear to be directly or indirectly required to initiate IL-17 production. The receptor-like protein tyrosine phosphatase, CD45, is expressed on all nucleated hematopoietic cells and comprises one of the most abundant cell surface glycoproteins. CD45 plays an essential role in B and T cell antigen receptor signal transduction and also modulates integrin and cytokine receptors signals by controlling the relative threshold of sensitivity to external stimuli[40]. Notably, the functional activity of CD45 is precisely regulated via the structural features of its extracellular domain. Distinct lymphoid populations express specific CD45 isoforms due to alternative splicing of exons within the extracellular domain. Developing and activated lymphocytes also differ with respect to extent of extracellular domain glycosylation as well as site-specific O- and N-glycan sialylation, and such micro-heterogenous changes impact cell survival and activation[41]. Interestingly, in contrast to B cells, exposure of primary T cells to *T. cruzi* trypomastigotes failed to trigger IL-17 production despite previous evidence for trans-sialidase-mediated modification of CD45 on T cells. Similarly, trypomastigote-stimulated myeloid cells and neutrophils also did not generate IL-17. Thus, trans-sialylation of CD45 (or of CD45 interacting molecules) on B cells likely functions in a novel manner to initiate signaling. Both the enzymatic and residual N-terminal lectin-activity of trans-sialidase, but not the repetitive C-terminal SAPA motifs, were required for maximal B cell IL-17 production *in vivo*. These findings suggest that trans-sialidase triggers a unique manner of CD45 compartmentalization or oligomerization that initiates the IL-17 transcriptional program. This program operated independently of key candidate B cell surface receptors including CD40 and MyD88-dependent innate receptors. CD45 oligomerization may help to explain why these events did not require antigen-specific BCR recognition. However, whether this program requires the scaffold function of an intact BCR, integrins or other B cell surface receptor(s) remains to be determined. Interestingly, unlike mature B cells, purified transitional B cells failed to respond to *T. cruzi* trypomastigotes (or trans-sialidase), implying that a B cell stage-specific CD45 isoform or a mature transcriptional development program[42] is essential for these events.

CD45 modulates signal transduction thresholds as either a positive or negative regulator of Src family kinases, with its dominant activity dependent upon its precise localization. Using inhibitors and genetic models we demonstrated a requirement for both Src- and Btk-Tec kinases in this pathway B cells. Based upon our previous work[43], we hypothesize that trans-sialylation of CD45 initiates Src kinase-dependent trans-phosphorylation of the activation loop tyrosine within Btk-Tec kinases. Activated Btk is capable of directly trans-

phosphorylating a range of signaling effectors including PLC-γ2, adaptor proteins and transcription factors[34,44]. Surprisingly, IL-17 production did not require the Btk-dependent, essential BCR downstream effectors PKCβ and NF-κB. We are currently utilizing high sensitivity mass-spectroscopy to identify proximal signaling intermediates modified in response to trans-sialidase exposure in primary B cells.

The orphan nuclear receptor and transcription factors, RORγt and RORα, play an essential role in the IL-17 program in T_H17, T_C17 and innate IL-17 producing cells including γδ T cells and *i*NKT, NK, and LTi-like cells[2,10,36]. In naïve T cells, initial and sustained expression of RORγt is coordinately controlled via signaling inputs from key environmental cytokines (IL-1, IL-6, TGF-β, IL-21 and IL-23) that mediate STAT3 activation and the interplay of additional transcription factors. Additional effectors, including AHR, also function to sustain IL-17 production in RORγt-expressing cells. Thus, one of the most surprising findings in this study was that generation of IL-17$^+$ B cells occurred independently of RORγt, RORα and AHR expression or activity; and, similarly, that the B cell IL-17 program was unimpeded by lack of IL-6, IL-21 or IL-23R. Notably, while neutrophils are also capable of producing IL-17[45], it remains unknown whether these cells require RORγt, raising the possibility that other innate cells might utilize RORγt-independent programs for IL-17 production. Our findings set the stage for assays designed to identify proximal effectors and nuclear factors required for B cell IL-17 transcription; work that may provide new insight into the biology of this cytokine family.

In summary, our findings demonstrate that B cells can be driven to rapidly secrete IL-17A and IL-17F in response to challenge with an infectious pathogen and that IL-17$^+$ B cells comprise a major source for this cytokine during *T. cruzi* infection. Our data leave several important open questions. Most importantly, these observations suggest that generation of IL-17$^+$ B cells may be an unappreciated feature of the innate immune response required for pathogen control in other disease settings. In this context, several bacterial species, including *Streptococcus pneumoniae*, *Campylobacter jejuni* and *Pasteurella multocida*, also express trans-sialidases[46–49]. Further, both B lineage cells and IL-17 are strongly implicated in a range of autoimmune disorders. Indeed, low-level, chronic *T. cruzi* infection leads to life-threatening chronic inflammatory sequelae that comprise a major disease burden in parasite endemic regions[50]. Thus, it will be important to determine the role(s) for IL-17$^+$ B cells in this clinically important setting as well as in candidate autoimmune disorders.

ONLINE METHODS

Mice

Control C57BL6, BALBc, μMT, IgHEL (Hen egg lysozyme-specific immunoglobulin transgenic), *Il23r*$^{-/-}$, *Il21r*$^{-/-}$ *Il23r*$^{-/-}$, *Il6*$^{-/-}$, *Myd88*$^{-/-}$, *Cd40*$^{-/-}$, *Rag2*$^{-/-}$, *Btk*$^{-/-}$, *Btk*$^{-/-}$ *Tec*$^{-/-}$, *Pkcb*$^{-/-}$, *Ahr*$^{-/-}$, RORγt-GFP homo- and heterozygotes, and RORγt-cre×ROSA-RFP mice were maintained in the specific pathogen–free barrier facilities at Seattle Children's Research Institute. Animal studies were carried out with the approval of Seattle Children's Research Institute Institutional Animal Care and Use Committee. Spleens from control C57BL6, *Cd45*$^{-/-}$ and *Il17a*$^{-/-}$ animals were kindly donated by A. Weiss (UCSF) and D. Artis (U Penn), respectively.

For experiments performed in Argentina, C57BL6 mice were originally obtained from School of Veterinary, La Plata National University (La Plata, Argentina) and were housed in School of Chemical Sciences animal facility where all experiments were performed in compliance with the Institutional Review Board and Ethical Committee of the School of Chemical Sciences, National University of Cordoba. B6.Cg-Lgals3^{tm1Poi} Lgals1^{tm1Rob}J (*Gal-1*$^{-/-}$*Gal-3*$^{-/-}$) mice (Jackson Laboratory) were bred at the UNSAM animal facilities in accordance with the Institutional Review Board and Ethical Committees.

Infections with *T. cruzi*

T. cruzi parasites (Y-Br strain, kindly provided by K. Norris, U. Pittsburgh) were cultured in NIH 3T3 fibroblasts and harvested as previously described[51]. 6–8 week-old mice were infected by intraperitoneal injection of 1×10^4 Tp diluted in Glucose 2%-PBS solution[51]. Tp dose was selected to maximize level of parasitemia without impacting survival[11]. Uninfected normal littermates were injected with PBS and processed in parallel. Level of whole blood parasitemia was measured by counting viable trypomastigotes in a Neubauer counting chamber[18, 31].

Adoptive B cell transfer experiments

Purified splenic B cells (>95% purity) from C57BL6, *Il17a*$^{-/-}$, *Cd45*$^{-/-}$, *Il23r*$^{-/-}$, *Il6*$^{-/-}$, *Myd88*$^{-/-}$ or *Cd40*$^{-/-}$ mice were obtained by CD43$^+$ cell depletion (Miltenyi Biotec) or sorting by flow cytometry (as below) and transferred into μMT recipients ($3.5–5\times10^7$ B cells per recipient). 5 days post-transfer, recipient mice were infected with 1×10^4 trypomastigotes via intraperitoneal injection.

Flow cytometry and cell sorting

Single cell suspensions were obtained by dissociating spleens with frosted glass slides. Erythrocytes were lysed with ammonium chloride potassium phosphate (ACK) buffer. Viable leukocytes numbers was determined by trypan blue exclusion using a Neubauer counting chamber. Single cell suspensions were incubated with fluorescently labeled antibodies for 20 min at 4 °C. The following antibodies were used for flow cytometry: anti-B220-PECy7 (RA3-6B2); anti-CD19-Alexa Fluor 700 or PECy7 (1D3); anti-GL-7-Alexa Fluor 647 (GL7); anti-CD4-PeCy5 (RM-5), anti-CD8-PerCPCy5.5 (53–6.7), anti-CD24-APC (M169)); anti-CD21 (7G6) and anti-CD138-Biotin (281-2) followed by Streptavidin-APC-Cy7; and anti-IL-17-PE (eBio17B7) from (eBioscience). Anti-PNA-FITC was purchased from Vector (USA). Data were collected on a LSR II or FACs-CANTO flow cytometer (BD Biosciences) and analyzed using FlowJo software (TreeStar).

For intracellular cytokine staining, cells were stimulated with 50 ng/ml PMA, 500 ng/ml ionomycin and GolgiStop (BD Biosciences) for 5 h. After surface-staining, cells were fixed and permeabilized with CytofixCytoperm (BD Biosciences) according manufacturer's instructions.

For cell sorting, B cells were enriched using CD43 MicroBeads (Miltenyi Biotec) prior to surface staining and sorting using a FACSAria sorter with Diva software (BD Biosciences). Sort gates were defined based on B220, CD19, CD21, CD23 and CD4 expression: FM B

cells ($CD21^{int}CD24^{int}$ $CD23^{+}$), MZ B cells ($CD21^{hi}CD24^{hi}CD23^{-}$) and T1 B cells ($CD21^{lo}CD24^{hi}CD23^{+}$). Sort purities were >97%.

Tonsillar human B cells

Tonsils (discarded surgical specimens) were obtained in accordance with the guidelines of the Institutional Review Board of Seattle Children's Hospital and Seattle Children's Reseach Insitute. Mononuclear cells were isolated by mechanical disruption of tonsil tissue followed by Ficoll-Hypaque gradient centrifugation. Highly pure B cells (>98% purity) were obtained by depletion of magnetically labeled non-B cells (B Cell Isolation Kit II; Miltenyi Biotec).

B cell cultures

Sorted splenic B cells ($CD19^{+}$ $B220^{+}$) or B cell sub-populations (FM, MZ and T1) from uninfected or *T. cruzi* infected (day 10 post infection) mice were cultured *in vitro* with varying doses of trypomastigotes or with media, $F(ab')_2$ anti-IgM (10 μgml; Jackson ImmunoResearch Laboratories), TLR agonists (LPS 10 μg/ml (Sigma); CpG 2 μg/ml;; Zymozan 50 μg/ml (Invivogen)) or *T. cruzi* trans-sialidase (active or Tyr342His inactive forms; 5 μg/ml[52]; or *T. cruzi* antigen. In separate experiments, B cells were cultured with trypomastigotes in the presence of: anti-trans-sialidase-neutralizing mAb[22] or a IgG2a isotype control; CD45 Inhibitor: N-(9,10-Dioxo-9,10-dihydro-phenanthren-2-yl)-2,2-dimethyl-propionamide (Calbiotech); Src inhibitor: PP2, 4-Amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-d]pyrimidine (EMB Millipore); Btk inhibitors AVL-292 and CNX-652 (kindly provided by Avila Therapeutics); NFκB inhibitor BAY 11-7082; RORγt and RORα inhibitor SR1001[53] kindly provided by T Burris (Scripps) or DMSO as control.

For human B cell stimulation, B cells were incubated in 96-well plates (10^6 cells per well in 200 μl RPMI 1640 supplemented with 10% FBS) for 72 h with 10 ng/ml soluble BAFF or IL-10 in the presence of trypomastigotes with different concentrations of AVL-292 (50 nM), DMSO (control), anti-trans-sialidase-neutralizing mAb or CD45 Inhibitor (0.25 nM). After 72 h, supernatants were analyzed for IL-17 production by ELISA.

Sialylation and desialylation assays

Sorted $B220^{+}$ splenocytes from uninfected mice were cultured with different doses of bacterial *Vibrio cholerae* (SIGMA N6514) and *Clostridium perfringens* neuraminidases (SIGMA N2876) for 72 h. In separate experiments, B cells were cultured with 30 mM of α-(2,3)sialyllactose (α-2,3-SL) and α-(2,6)sialyllactose (α-2,6-SL) in medium containing FBS or 2% BSA with or without acceptor lactose for 72 h. IL-17A production in the supernatants was determined by ELISA.

Cytokine quantification

Cytokine concentrations in supernatants from *in vitro* cultures and in sera were determined by ELISA using paired antibodies for human or murine IL-17A or IL-17F (eBiosciences) and murine IFN-γ and TNF (eBiosciences) according to manufacturer's instructions.

Transaminase quantification

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity was measured using commercial kits according to manufacturer's instructions.

Reverse transcription of mRNA and quantification by real time PCR

Sorted splenic B cells ($CD19^+$ $B220^+$) and non-B cells ($CD19^-$ $B220^-$) from uninfected and *T. cruzi* infected (10 d post infection) mice were stimulated for 5 h with 50 ng/ml PMA, 500 ng/ml ionomycin. Cells were incubated with TRIzol™ (Life Technologies) and RNA was extracted according to the manufacturer's instructions and stored at −70 °C. RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Invitrogen, USA) at 42 °C for 60 min. One microgram of RNA was used to generate initial cDNA species. Real-time PCR reactions for mouse IL-17A and HPRT were performed using the following primers pairs and probes IL-17A (Mm00439618_m1) and HPRT (Mm00446968_m1) (Applied Biosystems). HPRT was used as standard to normalize cDNA loading[54].

Immunofluorescence

Spleens from uninfected and *T. cruzi* infected (10 d post infection) wild-type and RORγt-Cre-ROSA RFP mice were fixed in 4% paraformaldehyde and embedded in OCT prior to sectioning. 8 μm sections were rehydrated in staining buffer (PBS, 1% goat serum, 1% BSA, and 0.1% Tween-20), pre-incubated with anti-mouse CD16CD32 (Fc Block™, BD Biosciences), then stained with antibodies including: anti-IgM-FITC (Southern Biotech), anti-IL-17A-PE (eBioscience) anti-CD3-Alexa647 (Invitrogen) and anti-B220-FITC (BD Biosciences). Images were acquired using a DM6000B microscope, DFL300 FX camera, and Application Suite Advanced Fluorescence software (all from Leica).

Statistical analysis

Statistical significance of comparisons of mean values was assessed by a two-tailed Student's *t*-test or nonparametric Mann-Whitney *U*-test and, in the case of multiple experimental group comparisons, by one-way ANOVA with Bonferroni correction using GraphPad software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We thank N. Scharping and F. Fiocca-Vernengo for technical assistance and K. Sommer and M. Schwartz and other members of the Rawlings laboratory for valuable suggestions and discussions. We also thank K. Norris (U Pittsburgh) for providing Y strain *T. cruzi*; A. Weiss (UCSF) and G. Sonnenberg and D. Artis (U Penn) for $Cd45^{-/-}$ and $Il17a^{-/-}$ mice, respectively; and T. Burris (Scripps) for the SR1001 inhibitor. We are grateful to P. Abadie and MP Crespo for cell sorting and F. Navarro for animal care in Argentina. This work was supported by NIH grants HD037091, HL075453, AI084457 and AI071163 (to DJR) and AI 075589 (to OC); a Rheumatology T32 Postdoctoral award (5T32AR007108), Child Health Research Career Development Award (NICHD K12HD043376) and Rheumatology Research Foundation Scientist Development Award (to SWJ); and CONICET, ANPCyT (FONCYT) and SECYTUNC (to AG). DAB, MCAV and MGS thank CONICET for fellowship support.

REFERENCES

1. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol. 2009; 27:485–517. [PubMed: 19132915]
2. Gaffen SL. Recent advances in the IL-17 cytokine family. Curr Opin Immunol. 2011; 23:613–619. [PubMed: 21852080]
3. Kelly MN, et al. Interleukin-17interleukin-17 receptor-mediated signaling is important for generation of an optimal polymorphonuclear response against Toxoplasma gondii infection. Infect Immun. 2005; 73:617–621. [PubMed: 15618203]
4. Pitta MG, et al. IL-17 and IL-22 are associated with protection against human kala azar caused by Leishmania donovani. J Clin Invest. 2009; 119:2379–2387. [PubMed: 19620772]
5. Rudner XL, Happel KI, Young EA, Shellito JE. Interleukin-23 (IL-23)-IL-17 cytokine axis in murine Pneumocystis carinii infection. Infect Immun. 2007; 75:3055–3061. [PubMed: 17403873]
6. da Matta Guedes PM, et al. IL-17 produced during Trypanosoma cruzi infection plays a central role in regulating parasite-induced myocarditis. PLoS Negl Trop Dis. 2010; 4:e604. [PubMed: 20169058]
7. Miyazaki Y, et al. IL-17 is necessary for host protection against acute-phase Trypanosoma cruzi infection. J Immunol. 2010; 185:1150–1157. [PubMed: 20562260]
8. Mou Z, Jia P, Kuriakose S, Khadem F, Uzonna JE. Interleukin-17-mediated control of parasitemia in experimental Trypanosoma congolense infection in mice. Infect Immun. 2010; 78:5271–5279. [PubMed: 20855512]
9. Tosello Boari J, et al. IL-17RA signaling reduces inflammation and mortality during Trypanosoma cruzi infection by recruiting suppressive IL-10-producing neutrophils. PLoS Pathog. 2012; 8:e1002658. [PubMed: 22577359]
10. Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. Nat Rev Immunol. 2010; 10:479–489. [PubMed: 20559326]
11. Bryan MA, Guyach SE, Norris KA. Specific humoral immunity versus polyclonal B cell activation in Trypanosoma cruzi infection of susceptible and resistant mice. PLoS Negl Trop Dis. 2010; 4:e733. [PubMed: 20625554]
12. Bermejo DA, et al. Trypanosoma cruzi infection induces a massive extrafollicular and follicular splenic B-cell response which is a high source of non-parasite-specific antibodies. Immunology. 2011; 132:123–133. [PubMed: 20875075]
13. Noelle RJ, Ledbetter JA, Aruffo A. CD40 and its ligand, an essential ligand-receptor pair for thymus-dependent B-cell activation. Immunol Today. 1992; 13:431–433. [PubMed: 1282319]
14. Meyer-Bahlburg A, Rawlings DJ. Differential impact of Toll-like receptor signaling on distinct B cell subpopulations. Front Biosci. 2012; 17:1499–1516.
15. Rawlings DJ, Schwartz MA, Jackson SW, Meyer-Bahlburg A. Integration of B cell responses through Toll-like receptors and antigen receptors. Nat Rev Immunol. 2012; 12:282–294. [PubMed: 22421786]
16. Tarleton RL. Immune system recognition of Trypanosoma cruzi. Curr Opin Immunol. 2007; 19:430–434. [PubMed: 17651955]
17. Minoprio P, Bandeira A, Pereira P, Mota Santos T, Coutinho A. Preferential expansion of Ly-1 B and CD4- CD8- T cells in the polyclonal lymphocyte responses to murine T. cruzi infection. Int Immunol. 1989; 1:176–184. [PubMed: 2518657]
18. Bermejo DA, et al. BAFF mediates splenic B cell response and antibody production in experimental Chagas disease. PLoS Negl Trop Dis. 2010; 4:e679. [PubMed: 20454564]
19. Schenkman S, Eichinger D, Pereira ME, Nussenzweig V. Structural and functional properties of Trypanosoma trans-sialidase. Annu Rev Microbiol. 1994; 48:499–523. [PubMed: 7826016]
20. Gao W, Wortis HH, Pereira MA. The Trypanosoma cruzi trans-sialidase is a T cell-independent B cell mitogen and an inducer of non-specific Ig secretion. Int Immunol. 2002; 14:299–308. [PubMed: 11867566]

21. Alvarez P, Leguizamon MS, Buscaglia CA, Pitcovsky TA, Campetella O. Multiple overlapping epitopes in the repetitive unit of the shed acute-phase antigen from Trypanosoma cruzi enhance its immunogenic properties. Infect Immun. 2001; 69:7946–7949. [PubMed: 11705983]
22. Risso MG, Pitcovsky TA, Caccuri RL, Campetella O, Leguizamon MS. Immune system pathogenesis is prevented by the neutralization of the systemic trans-sialidase from Trypanosoma cruzi during severe infections. Parasitology. 2007; 134:503–510. [PubMed: 17166319]
23. Leguizamon MS, Mocetti E, Garcia Rivello H, Argibay P, Campetella O. Trans-sialidase from Trypanosoma cruzi induces apoptosis in cells from the immune system in vivo. J Infect Dis. 1999; 180:1398–1402. [PubMed: 10479182]
24. Buschiazzo A, et al. Trypanosoma cruzi trans-sialidase in complex with a neutralizing antibody: structurefunction studies towards the rational design of inhibitors. PLoS Pathog. 2012; 8:e1002474. [PubMed: 22241998]
25. Muia RP, et al. Identification of glycoproteins targeted by Trypanosoma cruzi trans-sialidase, a virulence factor that disturbs lymphocyte glycosylation. Glycobiology. 2010; 20:833–842. [PubMed: 20354005]
26. Mucci J, Risso MG, Leguizamon MS, Frasch AC, Campetella O. The trans-sialidase from Trypanosoma cruzi triggers apoptosis by target cell sialylation. Cell Microbiol. 2006; 8:1086–1095. [PubMed: 16819962]
27. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. Science. 2006; 313:670–673. [PubMed: 16888140]
28. Kuijf ML, et al. TLR4-mediated sensing of Campylobacter jejuni by dendritic cells is determined by sialylation. J Immunol. 2010; 185:748–755. [PubMed: 20525894]
29. Rabinovich GA, Toscano MA. Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. Nat Rev Immunol. 2009; 9:338–352. [PubMed: 19365409]
30. Earl LA, Baum LG. CD45 glycosylation controls T-cell life and death. Immunol Cell Biol. 2008; 86:608–615. [PubMed: 18607388]
31. Zuniga E, Rabinovich GA, Iglesias MM, Gruppi A. Regulated expression of galectin-1 during B-cell activation and implications for T-cell apoptosis. J Leukoc Biol. 2001; 70:73–79. [PubMed: 11435488]
32. Acosta-Rodriguez EV, et al. Galectin-3 mediates IL-4-induced survival and differentiation of B cells: functional cross-talk and implications during Trypanosoma cruzi infection. J Immunol. 2004; 172:493–502. [PubMed: 14688359]
33. Gao W, Pereira MA. Trypanosoma cruzi trans-sialidase potentiates T cell activation through antigen-presenting cells: role of IL-6 and Bruton's tyrosine kinase. Eur J Immunol. 2001; 31:1503–1512. [PubMed: 11465107]
34. Rawlings DJ, Sommer K, Moreno-Garcia ME. The CARMA1 signalosome links the signalling machinery of adaptive and innate immunity in lymphocytes. Nat Rev Immunol. 2006; 6:799–812. [PubMed: 17063183]
35. Moreno-Garcia ME, Sommer KM, Bandaranayake AD, Rawlings DJ. Proximal signals controlling B-cell antigen receptor (BCR) mediated NF-kappaB activation. Adv Exp Med Biol. 2006; 584:89–106. [PubMed: 16802601]
36. Dong C. Genetic controls of Th17 cell differentiation and plasticity. Exp Mol Med. 2011; 43:1–6. [PubMed: 21270506]
37. Esser C, Rannug A, Stockinger B. The aryl hydrocarbon receptor in immunity. Trends Immunol. 2009; 30:447–454. [PubMed: 19699679]
38. Eberl G, Littman DR. Thymic origin of intestinal alphabeta T cells revealed by fate mapping of RORgammat+ cells. Science. 2004; 305:248–251. [PubMed: 15247480]
39. He YW, Deftos ML, Ojala EW, Bevan MJ. RORgamma t, a novel isoform of an orphan receptor, negatively regulates Fas ligand expression and IL-2 production in T cells. Immunity. 1998; 9:797–806. [PubMed: 9881970]
40. Hermiston ML, Xu Z, Weiss A. CD45: a critical regulator of signaling thresholds in immune cells. Annu Rev Immunol. 2003; 21:107–137. [PubMed: 12414720]

41. Hernandez JD, Klein J, Van Dyken SJ, Marth JD, Baum LG. T-cell activation results in microheterogeneous changes in glycosylation of CD45. Int Immunol. 2007; 19:847–856. [PubMed: 17606981]
42. Andrews SF, Rawlings DJ. Transitional B cells exhibit a B cell receptor-specific nuclear defect in gene transcription. J Immunol. 2009; 182:2868–2878. [PubMed: 19234182]
43. Rawlings DJ, et al. Activation of BTK by a phosphorylation mechanism initiated by SRC family kinases. Science. 1996; 271:822–825. [PubMed: 8629002]
44. Rawlings DJ. Bruton's tyrosine kinase controls a sustained calcium signal essential for B lineage development and function. Clin Immunol. 1999; 91:243–253. [PubMed: 10370369]
45. Li L, et al. IL-17 produced by neutrophils regulates IFN-gamma-mediated neutrophil migration in mouse kidney ischemia-reperfusion injury. J Clin Invest. 2010; 120:331–342. [PubMed: 20038794]
46. Yu H, et al. A multifunctional Pasteurella multocida sialyltransferase: a powerful tool for the synthesis of sialoside libraries. J Am Chem Soc. 2005; 127:17618–17619. [PubMed: 16351087]
47. Cheng J, et al. Multifunctionality of Campylobacter jejuni sialyltransferase CstII: characterization of GD3GT3 oligosaccharide synthase, GD3 oligosaccharide sialidase, and trans-sialidase activities. Glycobiology. 2008; 18:686–697. [PubMed: 18509108]
48. Gut H, King SJ, Walsh MA. Structural and functional studies of Streptococcus pneumoniae neuraminidase B: An intramolecular trans-sialidase. FEBS Lett. 2008; 582:3348–3352. [PubMed: 18775704]
49. Cheng J, et al. Trans-sialidase activity of Photobacterium damsela alpha2,6-sialyltransferase and its application in the synthesis of sialosides. Glycobiology. 2010; 20:260–268. [PubMed: 19880425]
50. Gutierrez FR, Guedes PM, Gazzinelli RT, Silva JS. The role of parasite persistence in pathogenesis of Chagas heart disease. Parasite Immunol. 2009; 31:673–685. [PubMed: 19825107]
51. Norris KA, Schrimpf JE, Szabo MJ. Identification of the gene family encoding the 160-kilodalton Trypanosoma cruzi complement regulatory protein. Infect Immun. 1997; 65:349–357. [PubMed: 9009282]
52. Campetella OE, Uttaro AD, Parodi AJ, Frasch AC. A recombinant Trypanosoma cruzi trans-sialidase lacking the amino acid repeats retains the enzymatic activity. Mol Biochem Parasitol. 1994; 64:337–340. [PubMed: 7935611]
53. Solt LA, et al. Suppression of TH17 differentiation and autoimmunity by a synthetic ROR ligand. Nature. 2011; 472:491–494. [PubMed: 21499262]
54. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25:402–408. [PubMed: 11846609]

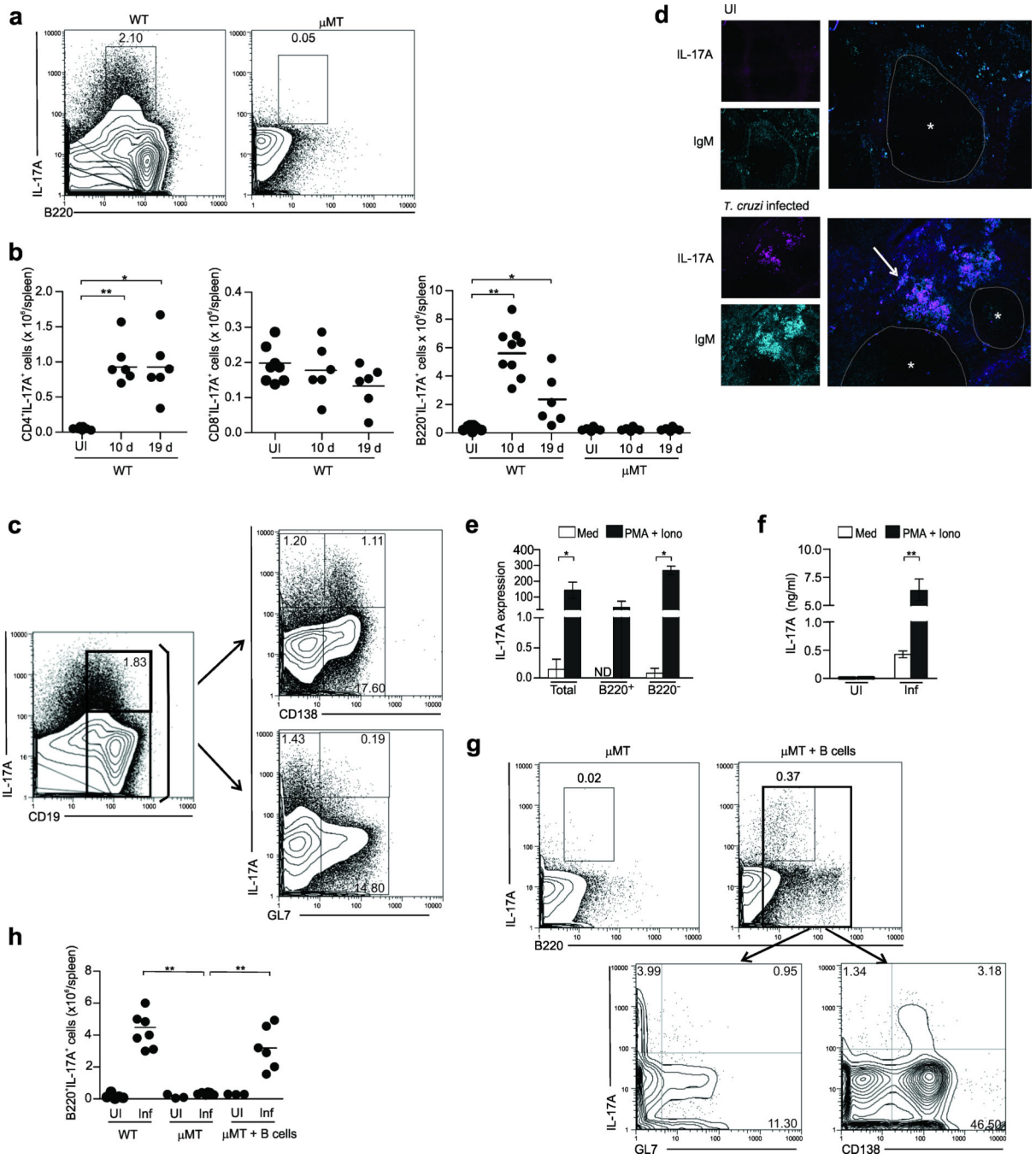

Figure 1. B cells from *T. cruzi* infected mice produce IL-17

(**a**) Representative flow cytometry plots showing IL-17A expression in B220$^+$ cells in the spleen of wild-type (WT) and μMT mice infected with *T. cruzi* at 10 days (d) post-infection. (**b**) Number of IL-17A-expressing splenic CD4$^+$, CD8$^+$ and B220$^+$ cells in uninfected (UI), or 10 and 19 d *T. cruzi*-infected mice (WT or μMT). Each symbol denotes a single animal and the line represents the mean value in each group (6–9 animals per group). Data are representative of 4 independent experiments. (**c**) IL-17A expression (at 10 d) in total splenocytes from *T. cruzi*-infected WT mice (left) and in gated CD19$^+$ cells expressing the

plasma cell marker, CD138, or the GC B cell marker, GL7 (right). Data are representative of 3 experiments. (**d**) Immunofluorescence staining of splenic sections from UI and *T. cruzi* infected (10 d) mice showing IL-17A and IgM expression (magenta and cyan, respectively, left; and merged images, right). Arrow indicates IL-17^{+}IgM^{+} cells. Dashed lines surround less strongly staining (e.g. IgM^{lo}) B cell follicles (*). Data are representative of 3 experiments. (**e**) IL-17A mRNA expression in total, sorted B220^{+} and B220^{-} splenocytes from infected mice cultured in media alone or with PMA and ionomycin (PMA+Iono). HPRT was used for normalization (10 d, 3 replicates per condition). (**f**) IL-17A production by B220^{+} splenocytes from UI or infected (Inf) mice cultured with medium or PMA+Iono for 72 h (10 d, 3 replicates per condition). Data (e–f) are representative of 2 experiments. (**g**) IL-17A^{+} B220^{+} cells in *T. cruzi*-infected μMT or B-cell reconstituted μMT (μMT + B cells) mice (10 d). Lower panel shows IL-17A expression predominantly within the B220^{+}CD138^{+} gate. Data are representative of 3 experiments. (**h**) Number of IL-17^{+} B220^{+} cells within the spleen of UI or Inf WT, μMT or B cell reconstituted μMT mice (10 d; 6–7 mice per condition). Data are representative of 3 experiments. All error bars denote s.e.m. *, $P \leq 0.05$ **, $P \leq 0.005$ (calculated by Mann-Whitney *U*-test).

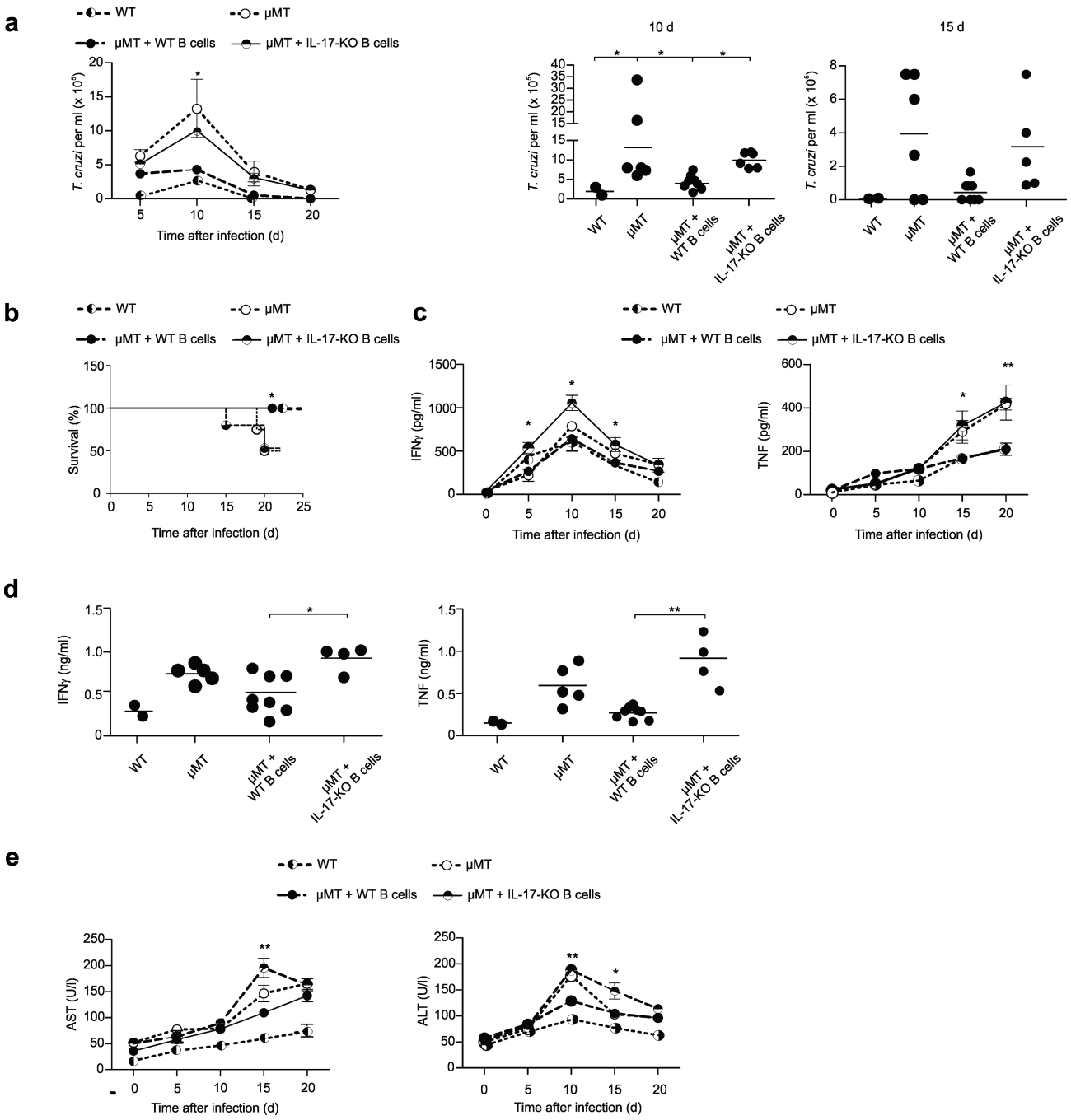

Figure 2. B cell IL-17A production is required for *T. cruzi* infection control

Control WT, µMT, or µMT mice reconstituted with WT or *Il17a*$^{-/-}$ B cells, were infected with *T. cruzi* and evaluated for: **(a)** Parasitemia (left panel: kinetic; right panels: day 10 and 15); **(b)** Survival based on Kaplan-Meier survival curve; **(c)** Serum IFNγ and TNF concentrations; and, **(e)** plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity. Surviving animals were sacrificed at 20 d and **(d)** IFN-γ and TNF production by unstimulated splenocytes was quantified (72 hour *in vitro* culture). Each symbol denotes a single animal and the line represents the mean value in each group.

All error bars denote s.e.m. *, $P \leq 0.05$ **, $P \leq 0.005$ (calculated by Mann-Whitney *U*-test). Results shown are from 2 independent experiments with 3–4 animals per group in each experiment.

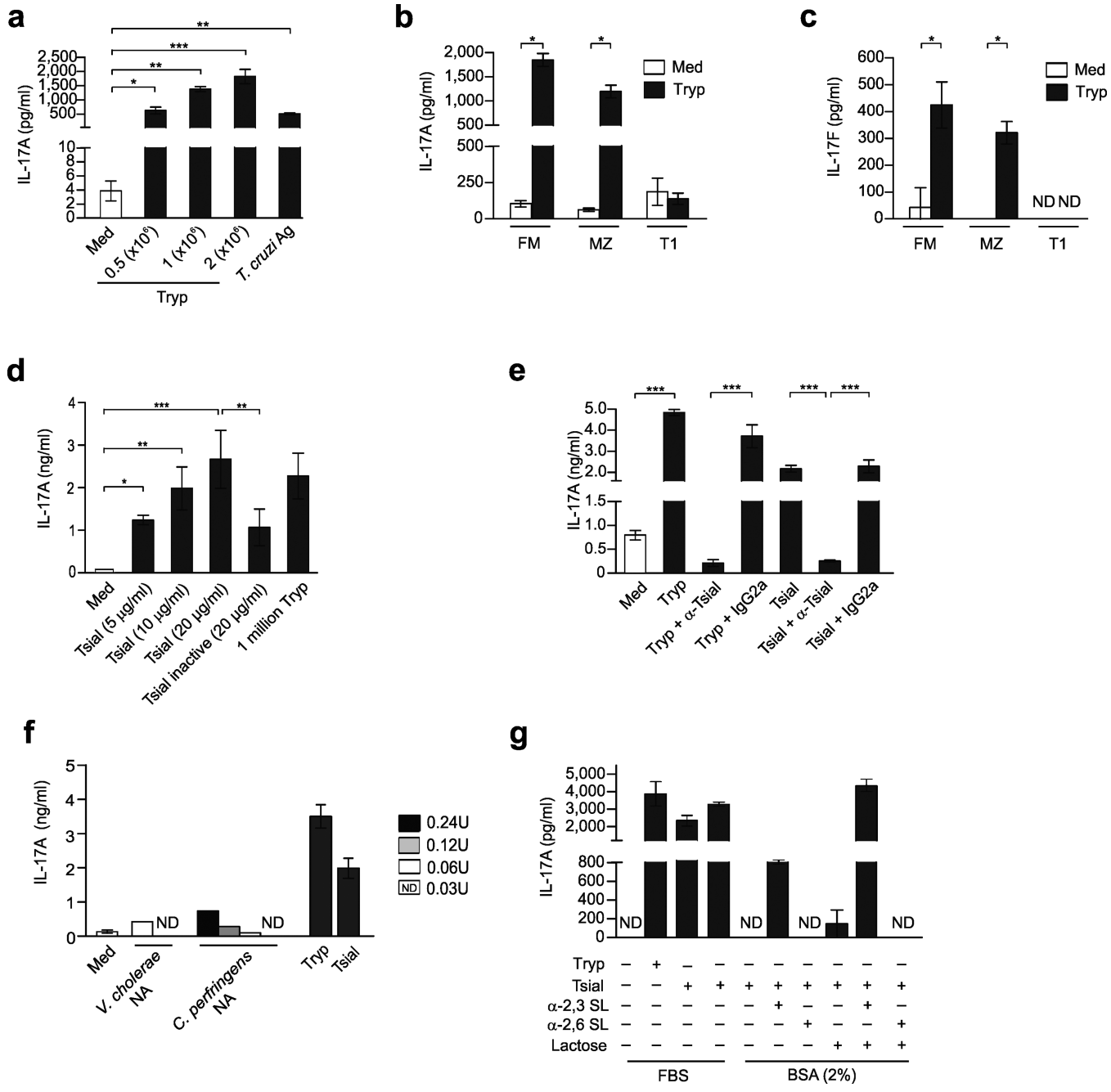

Figure 3. Exposure to *T. cruzi* trypomastigotes or purified trans-sialidase is sufficient to trigger B cell IL-17 production

(**a,d–g**) IL-17A secretion by B220^{+}CD19^{+} cells or (**b–c**) IL-17A and IL-17F production by follicular (FM), marginal zone (MZ) and transitional 1 (T1) B cell subsets purified from spleens of UI WT mice and stimulated for 72 h with: **(a)** various doses of live *T. cruzi* trypomastigotes (Tryp) or *T. cruzi* antigen (Ag); or (**b–g**) 1 × 10^{6} Tryp or different doses of active vs. inactive trans-sialidase (Tsial) with or without a Tsial-neutralizing mAb (α-Tsial) or IgG2a isotype control. **(f)** B220^{+}CD19^{+} cells from UI WT mice were cultured with Tryp, Tsial or different doses of bacterial *V. cholerae* and *C. perfringens* neuramidases (NA). **(g)**

$B220^+CD19^+$ cells from WT mice were cultured with Tryp or Tsial for 72 h in medium containing FBS or 2% BSA with alpha(2,3)-sialyllactose (α-2,3-SL) and alpha(2,6)-sialyllactose (α-2,6-SL) or in identical media lacking acceptor lactose. All error bars denote s.e.m. *, $P \leq 0.05$ **, $P \leq 0.005$ (calculated by Mann-Whitney *U*-test). Results are representative of 3 independent experiments with 2 replicates per condition. ND: None detectable.

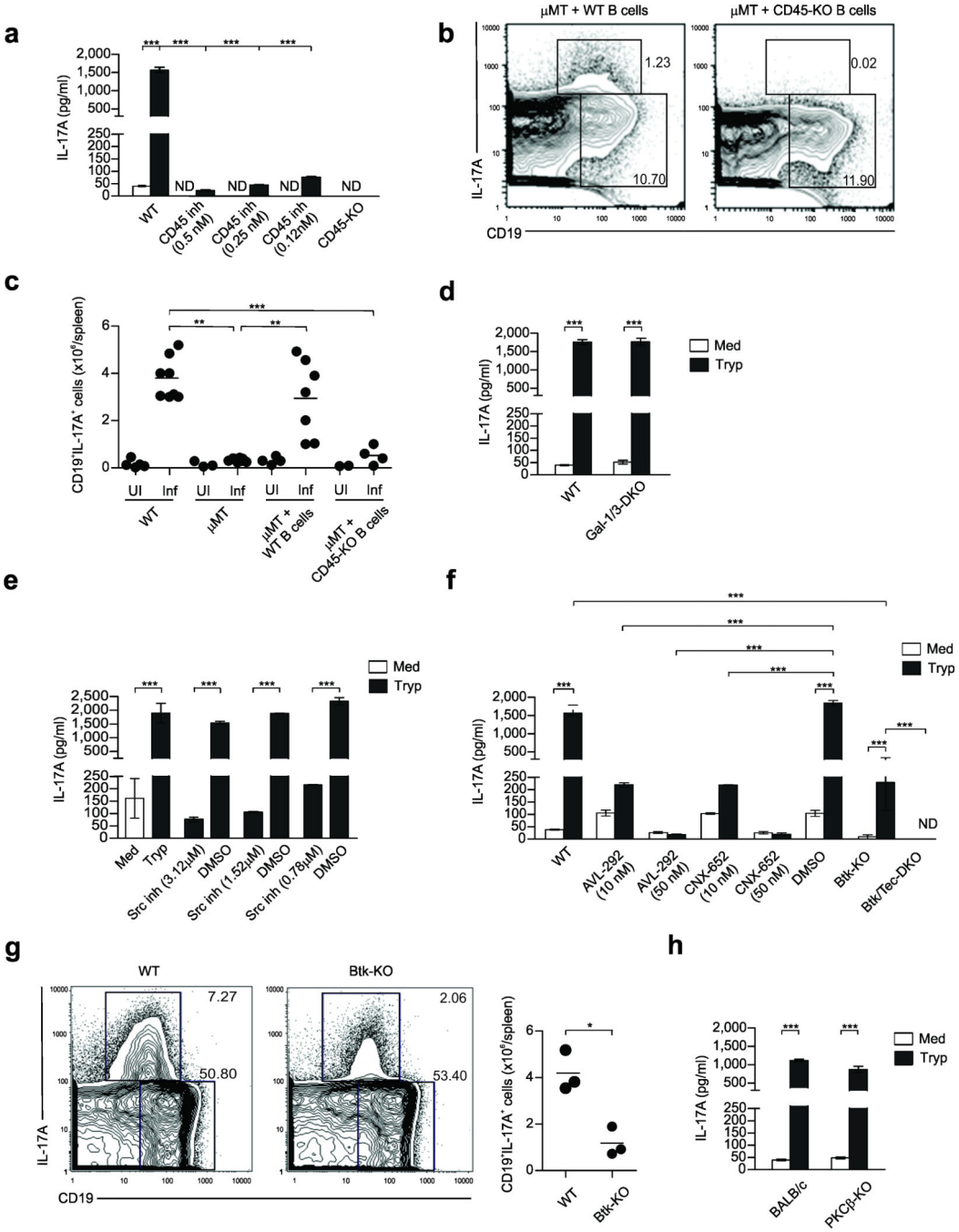

Figure 4. CD45 and Btk are required for B cell IL-17 production in response to *T. cruzi* trypomastigotes or purified trans-sialidase

(**a**) IL-17A production in follicular B cells from WT mice in response to *T. cruzi* Tryp and treated with or without various doses of CD45 inhibitor. Similar analysis was performed using B cells isolated from *Cd45*$^{-/-}$ mice. (**b**) IL-17A expression in CD19^{+} splenic cells from 10 d infected µMT mice that were recipients of adoptively transferred WT (left) or *Cd45*$^{-/-}$ B cells (right). (**c**) Number of CD19^{+}IL-17A^{+} cells in spleen of UI vs. 10 d infected animals including: WT or µMT mice, or µMT recipients of WT vs. *Cd45*$^{-/-}$ B cells. Each

symbol denotes a mouse and the line represents the median value in each group. (**d**) *In vitro* IL-17A secretion by $B220^{+}CD19^{+}$ cells derived from WT vs. *Gal-1*$^{-/-}$*Gal-3*$^{-/-}$ mice in response to Tryp-stimulation. (**e**) IL-17A production by WT FM B cells stimulated with Tryp and treated with different concentration of Src inhibitor (PP2) vs. DMSO control. (f) IL-17A production by WT FM B cells stimulated with Tryp and treated with increasing doses of Btk-specific inhibitors (AVL-292 or CNX-652) vs. DMSO control. IL-17A production by Tryp stimulated FO B cells derived from *Btk*$^{-/-}$ and *Btk*$^{-/-}$*Tec*$^{-/-}$ mice is also shown. (**g**) Representative FACS plots (left panels) and total number of splenic $CD19^{+}IL\text{-}17A^{+}$ cells (right panel) in *T. cruzi* infected (10 d) WT or *Btk*$^{-/-}$ mice. (h) IL17A production in Tryp stimulated purified B cells derived from BALB/c (control) vs. *Pkcβ*$^{-/-}$ mice. All *in vitro* analyses were performed at 72 h and results are representative of three (a–c) or two (d–g) independent experiments for each strain or condition. Data are shown as means ± s.e.m. *, $P \leq 0.05$ **, $P \leq 0.005$ (calculated by one-way ANOVA with Bonferroni correction (a–f, h) and Mann-Whitney *U*-test (g)).

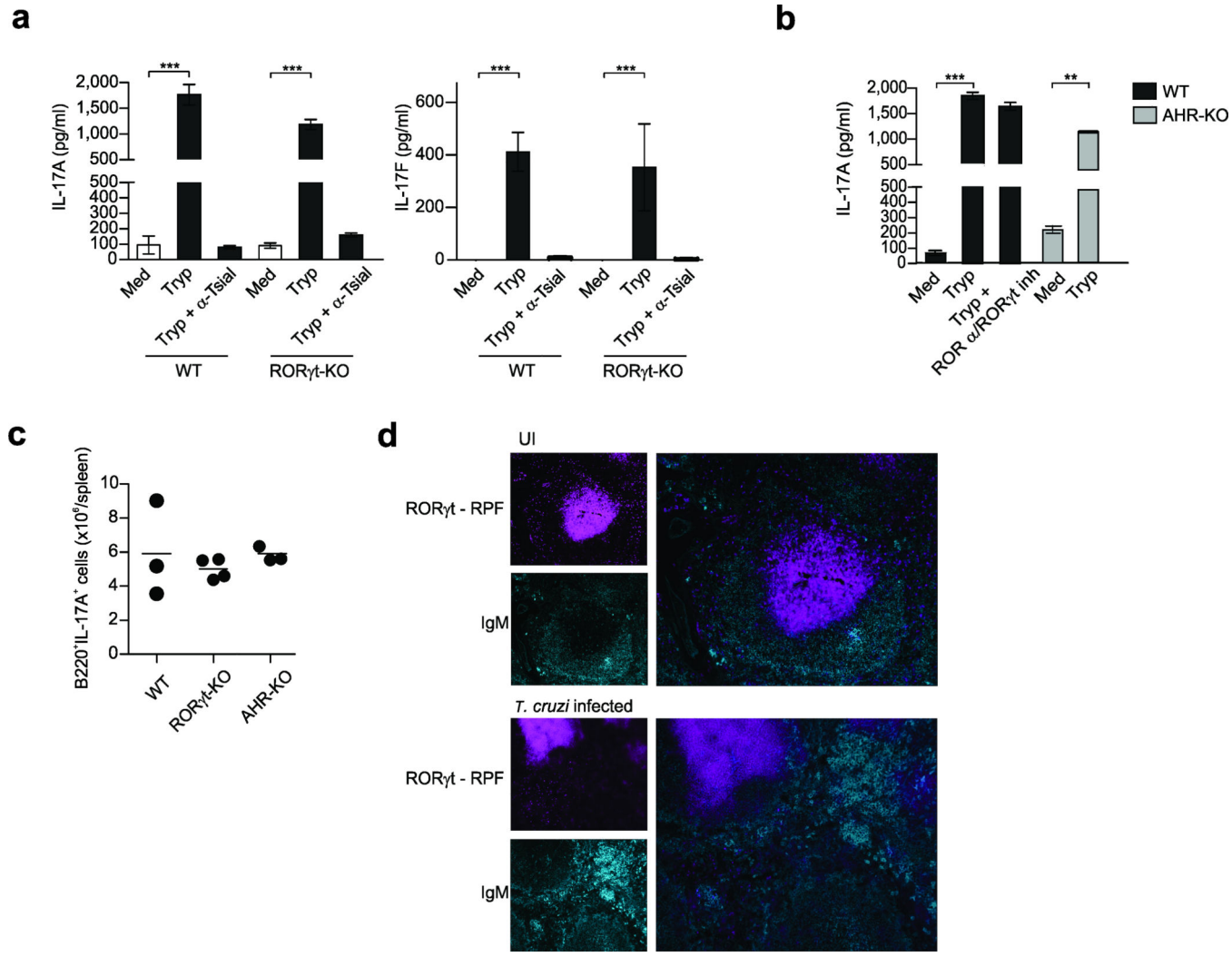

Figure 5. RORγt and AHR are not required for B cell IL-17 production in response to *T. cruzi*

(**a**) IL-17 secretion by WT and *Rorγt*$^{-/-}$ splenic B cells cultured *in vitro* for 72 hr with *T. cruzi* Tryp, with or without Tsial-neutralizing mAb (α-Tsial) (4 replicates per condition). (**b**) IL-17A production in non-stimulated and Tryp-stimulated, WT B cells (in presence or absence of the RORγt and RORα inhibitor SR1001, 25μM) and *Ahr*$^{-/-}$ B cells. Data are shown as means ± s.e.m. *, P ≤ 0.05 **, P ≤ 0.005 (calculated by one-way ANOVA with Bonferroni correction). (**c**) Number of splenic B220^{+}IL-17A^{+} cells in *T. cruzi* infected (10 d) WT, *Rorγt*$^{-/-}$ and *Ahr*$^{-/-}$ mice. Each symbol denotes a mouse and the line represents the median value in each group. (**d**) *Rorγt* locus activation cannot be detected *in vivo* in B cells following *T. cruzi* infection. RORγt-cre × ROSA-RFP reporter mice were infected with *T. cruzi* and spleens collected at 10 d post-infection. ROSA-RFP (magenta) and IgM (cyan) expression in spleen sections from UI (upper panels) vs. 10 d-infected (lower panels) animals. Results are representative of three (a), two (b, c), or one (d) experiment for each strain or condition.

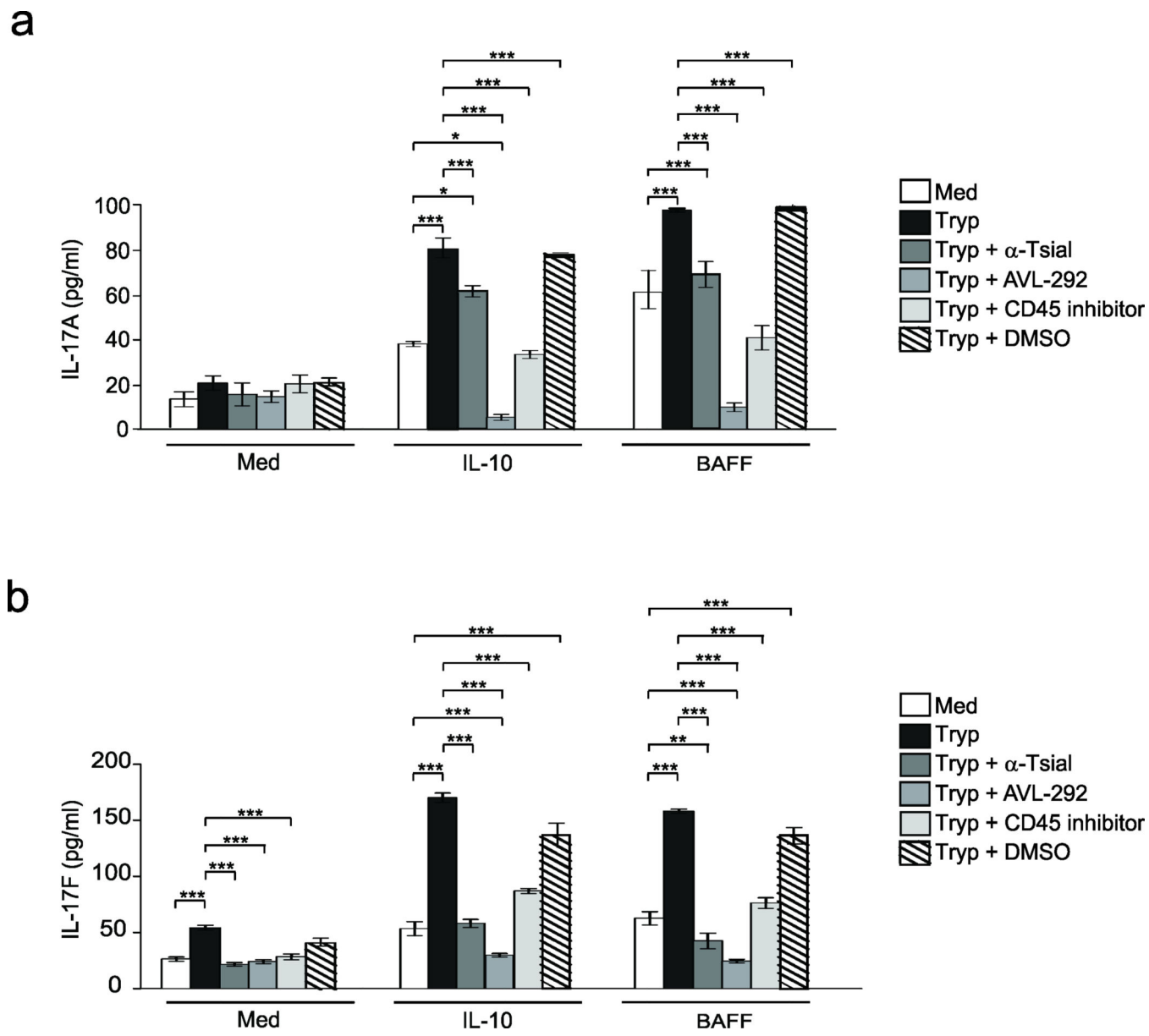

Figure 6. Primary human B cells stimulated with *T. cruzi* produce IL-17

Purified human $CD19^+$ tonsillar B cells were cultured for 72 hr in presence of recombinant IL-10 or BAFF and incubated with *T. cruzi* Tryp with or without α-Tsial mAb. B cells were also treated with Btk (AVL-292) or CD45 inhibitors or DMSO control. Production of (**a**) IL-17A or (**b**) IL-17F determined by ELISA. Data (3 replicates per condition) are shown as means ± s.e.m. *, $P \leq 0.05$ **, $P \leq 0.005$ (calculated by one-way ANOVA with Bonferroni correction). Results are representative of 3 independent experiments.