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CD23b isoform expression in human schistosomiasis identifies a novel subset of activated B cells

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Abstract

Resistance to schistosomiasis is associated with increased levels of serum parasite-specific IgE. IgE exerts its functions through its cellular receptors, FcεRI and FcεRII/CD23; however, its functional significance requires further characterization in humans. We previously reported that increased levels of CD23+ B cells correlate with resistance to schistosomiasis in hyper-exposed populations and sought to define their potential function and relationship with IgE. We found that CD23+ B cells are a heterogeneous cell population with functional and phenotypic differences. Circulating CD23+ B cells are uniquely activated in schistosomiasis and express the CD23b isoform and CXCR5, the homing receptor for lymphoid follicles. High CXCR5 expression by CD23+ B cells was associated with the capacity to home to cognate ligand, CXCL13. CD23-bound IgE cross-linking increased surface expression of CXCR5 suggesting that CD23+ B cells home directly into the lymphoid follicles upon antigen capture. As human schistosomiasis is an intravascular parasitic infection associated with a high antigenic burden in the blood, circulating CD23+ B cells may play a role in capture and shuttling of antigens directly to splenic follicles, highlighting a new role for circulating B cells. This function likely plays an important role in the development of protective immunity to infection with schistosomes.

Introduction

Resistance to schistosomiasis is associated with increased levels of serum parasite-specific IgE (10). The functional significance of IgE requires further characterization in humans, but the antibody may facilitate parasite attrition or immune responses (20, 21). IgE exerts its functions through its cellular receptors, FcεRI and FcεRII/CD23, which are expressed by a variety of cells (19). We previously reported that increased levels of CD23+ B cells correlate with resistance to schistosomiasis in hyper-exposed populations (3, 34). CD23 is a 45 kD type II membrane glycoprotein and contains an α-helical coiled-coil stalk region, which facilitates oligomerization of membrane-bound receptors (22). Trimerization of CD23 greatly increases the affinity of IgE to meet, or exceed, that of FcεRI (1.45 X 10^8 M^{-1})(28). CD23+ B cells circulate in the bloodstream pre-loaded with IgE indicating a probable role for CD23-bound IgE in mediating some of the effector functions of IgE in schistosomiasis (34).

We demonstrated that CD23-bound parasite-specific IgE induces kinase activation in B cells, but the role(s) of these signaling pathways remains unclear in host resistance (21). Indeed, the immunobiology of CD23 is highly complex. B cells express both isoforms of human CD23, CD23a and CD23b, which differ only in their cytosolic domains (42). CD23a is constitutively expressed by many cell types, including B cells, while CD23b is induced by exposure to certain factors, most notably IL-4 (14, 18). The gene for CD23 is located on chromosome 19 from where the two isoforms are generated by individual promoters and alternative RNA splicing (11, 29). Functionally, the CD23 isoforms appear distinct as well. Whereas CD23b controls IgE-dependent cytotoxicity by macrophages (39), CD23a mediates endocytosis of bound ligands by B cells (25). This corresponds well to other findings that the isoforms are associated with

different signaling cascades; CD23b up-regulates cAMP and iNOS in macrophages while CD23a mediates increased intracellular calcium (9, 30).

CD23-bound IgE by B cells is thought to augment antigen presentation of captured antigens to T cells, but other roles, such as transportation of immune complexes to splenic follicles, have been demonstrated in mice (23). However, although CD23b is inducible, the function of this isoform in human B cells is unknown. We therefore sought to better define the role of CD23+ B cells in human schistosomiasis. We demonstrate that circulating CD23+ B cells are uniquely activated and express CD23b, as well as CXCR5 (1). CXCR5 levels are generally increased by activated B cells upon receiving a positive signal from T cells. CXCR5 expression licenses the activated B cells to enter germinal centers to continue on a path of differentiation (37). Here, we provide evidence that CD23 plays a role in CXCR5 regulation to promote the capture and transportation of intravascular antigens directly into lymphoid follicles to augment immunity to schistosomiasis.

Methods

Study area and population

This study was approved by the Institutional Review Board of Boston University (BU IRB), the Scientific Steering Committee of the Kenya Medical Research Institute (KEMRI), and the National Ethics Review Committee of Kenya. The study was conducted along the shores of Lake Victoria, approximately 80 km from Kisumu city, western Kenya among adults males exposed as car washers (n=45) and fisherman (n=10; Table 1). Occupationally-exposed laborers have relatively longer contacts with the lake water, raising their average rates of infection (26). Uninfected Kenyan subjects were recruited from KEMRI (n=5).

Upon informed consent, peripheral blood was drawn into heparinized tubes for experiments outlined below. Stool samples were examined for *S. mansoni* eggs and for other helminth ova by the modified Kato-Katz method (Vestergaard Frandsen) (2 slides each, 3 stool specimens obtained over several days). Subjects positive for *S. mansoni* were treated with 40 mg/kg praziquantel; those positive for other helminth ova were treated with 400 mg of albendazole as previously described (34).

Blood and tissue samples

Peripheral blood was purchased from Source Leukocytes (NY Biologics; n= 12) and was used to characterize and isolate circulating B cells from unexposed/uninfected population. Fresh, surgically discarded tonsils (n=10), peripheral lymph nodes (PLN; n= 3), and spleens (n= 2) were purchased from the Pathology Department at BU (Boston, MA) or from the National Disease Research Institute (Philadelphia, PA, USA) and processed as previously described (15). Briefly, minced lymphoid tissues were gently homogenized and passed over a 70 µM cell strainer (Falcon) to obtain a single cell suspension followed by Ficoll gradient to isolate mononuclear cells. B cells were isolated from mononuclear cells from tissues, or peripheral blood mononuclear cells (PBMC), by negative selection magnetic beads with a resulting 97-99% purity of CD19+ B cells (Miltenyi, Auburn, CA; Invitrogen, Carlsbad, CA). The CD23+ Ramos B cell line was purchased from ATCC (Manassas, VA).

Flow cytometry

B cells were evaluated in fresh, whole blood samples for surface expression of CD23 and CXCR5. 100 µl /tube of heparinized whole blood were incubated with fluorescently labeled antibodies purchased from BD Pharmingen (San Jose, CA) at 4^{o}C for 30 minutes. Red blood cells were lysed with 2ml FACS Lysing Buffer (BD Pharmingen). Assessment of surface

expression on B cells was performed with gates generated with anti- CD19 and the appropriate isotype controls for each sample. Other flow cytometry was performed using standard protocols (35).

CD23 gene expression

Total RNA was extracted from ~1 million purified B cells using a commercially available kit from QIAGEN (Valencia, CA). The extracted RNA was treated with DNase and heated at 37ºC for 30 min and then at 65ºC for an additional 30 min. DNA-free mRNA was subjected to RT-PCR with SuperScript III One -Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen) to determine CD23a and CD23b mRNA expression and β-actin mRNA as a control. Primers for CD23a and b were previously published (33). The DNA was labeled with 2 µl of EvaGreen™ dye (Biotium Inc., California, USA). PCR products were resolved by agarose gel electrophoresis to identify bands reflecting levels of expression of CD23a and CD23b mRNA expression. Relative expression of CD23a and CD23b was assessed with ImageJ (rsbweb.nih.gov/ij).

Cell culture

Tonsil and Ramos B cells were cultured overnight with 20 ng/ml of IL-4 to upregulate nascent surface CD23. The following day, B cells were subjected an IgE-binding protocol to load nascent CD23 molecules with IgE (21). B cells were rotated in TBS buffer containing 2mM $CaCl_2$ with 20 µg of NP-specific IgE (AbD Serotec, Oxford, UK). B cells loaded with antigen-specific IgE were stimulated with NP-BSA (Biosearch Technologies, Novato, CA) or anti-IgE (2 µg/ml; Sigma-Aldrich, St. Louis, MO). Isotype control was used at 2 µg/ml (eBioscience, San Diego, CA).

In some experiments, B cells were activated with one of the following: stimulatory anti-CD23 (2 µg/ml; eBioscience) to cross-link CD23, Fab anti-BCRµ (Jackson ImmnnoReseach Inc.; 2 µg/ml); anti-CD40 (R&D Systems), Pam3CSK4 (InvivoGen, San Diego, CA; 1 µg/ml), soluble CD21 (XpressBio, Thurmont, MD; 1 µg/ml), schistosome egg antigen (SEA; 5 µg/ml) or schistosome adult worm preparation (SWAP; 5 µg/ml, generous gifts from Dr. W. Evan Secor, CDC, Atlanta, GA). B cells were also treated with recombinant IL-10, IL-4, IL-2, IL-7, and IL-13 (eBioscience; 10-20 ng/ml).

Chemotaxis assay

Purified B cells were untreated or treated for 18 hours with IL-4 or anti-CD40, washed, and subjected to a chemotaxis assay. Assays for B cell chemotaxis were performed using 8 µm Costar Transwell plates (Corning). Bottom chambers contained recombinant human CXCL13 (R&D Systems) at 1000 ng/ml in cell culture media. B cells (5×10^5) were placed in the upper chamber and incubated for 4 hours at 37°C. Cells that had migrated were enumerated and presented as the percentage that migrated based on input over the media control.

Intracellular phospho-specific flow cytometry

CD23-activation was assessed by phospho-kinase activity with phospho-flow. B cells were cultured in the presence of stimuli for 10 minutes and fixed with paraformaldehyde and permeabilized with BD Phosflow Perm II Buffer (21). Cells were vortexed and incubated with fluorescently labeled anti-phospho-SYK (pY352)/Zap70(Y319) (BD Pharmingen) at room temperature for 30 min in the dark, washed, and evaluated by flow cytometry.

Statistical analyses

Statistical analyses were performed using GraphPad Prism (GraphPad Software). One-way analysis of analysis of variance with Dunn's post-test and the Mann-Whitney *U* test were

used for multiple- or single-group comparisons, respectively. Possible correlations were examined using Spearman's rank correlation test. Group sample sizes differ among the tests because some patient samples were unavailable.

Results

Schistosome antigens do not alter surface expression of CD23

CD23 expression is high on B cells during the development of immunity to schistosomiasis (3, 34). We sought to better define host-parasite interactions that might lead to changes in levels of CD23+ B cells. In general, subjects with schistosomiasis have a larger range of CD23+ B cell percentages compared to North American populations suggesting that schistosome infection may lower CD23 expression levels in some individuals (Fig. 1A). However, crude schistosome antigens, SEA and SWAP, did not directly alter expression of surface CD23 on splenic (Fig. 1B) or peripheral lymph node B cells from unexposed/uninfected donors (not shown). Further, schistosome antigens did not affect surface levels of CD23 on B cells from subjects hyper-exposed to schistosomes (Fig 1D &E).

CD23 upregulation on B cells is induced by cytokines, including IL-4 (Fig. 1B and C) IL-13 (Fig. 1C), IL-2 (not shown) as well as by CD40 stimulation and BCRμ cross-linking (Fig. 1B and C). IL-4 also induces an increase in CD23 on B cells from subjects with schistosomiasis (Fig 1D and E). In contrast, IL-10, which is elevated in schistosomiasis (8), reduces basal levels of CD23 (Fig. 1B). IL-7, a B cell growth factor, has a null effect on CD23 surface levels (Fig. 1C).

Schistosomiasis, caused by intravascular parasites, greatly raises the systemic antigenic burden and Toll-like receptor (TLR) expression by B cells (38). Fig. 1C demonstrates that TLR2

ligands also increase CD23 expression. Thus, CD23 levels are likely affected by many factors relevant to schistosomiasis.

CD23b is elevated relative to CD23a on B cells of adults exposed to S. mansoni

CD23 exists in two isoforms detectable only intracellularly by mRNA expression levels. To determine which isoform of CD23 was dominant in schistosomiasis, purified B cells were subjected to RT-PCR for expression of CD23a and CD23b. Figure 2 demonstrates that CD23a is the predominant isoform expressed in uninfected/unexposed subjects (Fig. 2A; upper and lower panels). However, CD23b is higher in individuals hyper-exposed to schistosomes (Fig. 2A; upper and lower panels), although a modest increase in CD23b levels was noted in Kenyan individuals who indicated no current infection with schistosomes (Fig. 2A; lower panel).

Whereas CD23a is constitutively expressed by B cells (apparently regardless of surface levels), CD23b is inducible, most notably by IL-4 (Fig. 2B). To determine whether schistosome antigens affected CD23 isoform expression, we incubated B cells with SEA or SWAP and found no affect on CD23b (Fig. 2C) or CD23a (not shown) mRNA levels (Fig. 2). In contrast, CD40 and BCRμ stimulation were strong inducers of CD23b expression by B cells, in addition to IL-4 (Fig. 2C).

CD23+ B cells express CXCR5 in schistosomiasis

B cells that are stimulated through either the BCR or CD40 are thought to be retained in the lymphoid tissue and to not circulate in the blood (1). However, it was recently shown that CD23+ B cells play a role in transportation of immune complexes from the blood to the follicular regions of the spleen (23). CXCR5 has a role in directing B cells to the lymphoid follicles, germinal centers (GC), and Peyer's patches (1). We therefore sought to evaluate expression levels of CXCR5 in schistosomiasis. CXCR5 expression was evident on B cells from individuals

with schistosomiasis (Fig.3A). Further, CXCR5 expression by B cells was correlated with expression of CD23 both by the proportion of cells that express CXCR5 and CD23 (Fig. 3B) and by the level of CD23 and CXCR5 (mean fluorescence intensity; MFI; Fig. 3C). Thus, high levels of CD23, which arise during the development of resistance, are also associated with increased expression of CXCR5 (34).

CD40 stimulation induces CD23^{high}CXCR5^{high} B cells and mobilization to CXCL13

As CD23b+CXCR5+ B cells appeared to be a unique subset of activated, circulating B cells, we sought to determine the potential stimuli necessary to generate these cells *in vivo*. IL-4 is a strong inducer of surface CD23 (Fig. 1B and C) and CD23b expression (Fig. 2B and C) and a hallmark of helminthiasis is indicators of increased IL-4 production, such as IgE and eosinophilia (16, 32). However, IL-4 reduces CXCR5 expression (Fig. 3D; middle panel and E). In addition, whereas BCRμ cross-linking induced CD23 (Fig. 1C), CXCR5 levels were reduced (see Fig. 4C) consistent with B cells requiring a signal from T cells to enter the GC. In contrast, CD40 stimulation induced both CD23b (Fig. 2C) and CXCR5 on B cells (Fig. 3E).

Although CXCR5 is expressed by most B cells, there appears to be a threshold level required for the ability to respond to chemokine (1). To test if experimentally generated CXCR5+ B cell populations had functional differences, we tested their migratory potential in chemotaxis assays to the cognate chemokine CXCL13. CD40-stimulated B cells migrated towards CXCL13, whereas IL-4 treated B cells demonstrated reduced chemotaxis compared to untreated B cells (Fig. 3F). Inflamed tonsils contain several populations of CXCR5+ and CD23+ B cells indicating the physiological significance of the experimentally-generated B cells in the lymphoid tissues (Fig. 3G). These results suggest that CD23+ B cells represent several distinct populations of cells with differing CD23 isoform expression, homing potential, and function.

CD23 cross-linking increases CXCR5 expression

CD23-bound IgE is thought to play a role in antigen capture for presentation to T cells (17). This process is thought to be mediated by CD23a through an ITIM-like motif that allows internalization of the captured antigen (27). The CD23b internal nucleotide sequence differs from CD23a and there is no known role for CD23b in human B cells. As mentioned above, recent results suggest an important function for CD23+ B cells in immune complex transport to the follicles in mice (23). In schistosomiasis, antigen capture and transport to the follicles via CXCR5 is likely a highly relevant process in the context of the chronic, intravascular infection. Thus, we speculated that there would be an additional role for CD23b-bound IgE cross-linking in directing B cells towards the follicles.

As CD23 correlated with CXCR5 in schistosomiasis, we tested whether CD23 cross-linking affected surface CXCR5. Tonsil or Ramos B cells were treated with IL-4 to upregulate nascent CD23 and were experimentally bound by NP-specific IgE. We found that increasing levels of monomeric IgE exposure reduced CXCR5 surface expression (Fig. 4A). However, cross-linking NP-specific cell bound IgE with NP-BSA or anti-IgE increased CXCR5 levels suggesting that antigen capture may drive B cells directly into the follicles and demonstrating the importance of antigen in mediating the effect (Fig. 4B).

The effect of schistosome antigens on CXCR5

We recently reported that schistosome antigens reduced B cell activation levels (21). Interestingly, schistosome egg antigens, but not adult worm antigens, reduced surface levels of CXCR5 on B cells (Fig. 4C). These results suggest that the homing receptor, CXCR5, is a target of immuno-evasive tactics highlighting the potential importance of the receptor in generating immunity to schistosomiasis.

IL-10 was a strong stimulator of CXCR5 and was able to overcome the effects of SEA (Fig. 4C). However, CD23 levels are reduced in response to IL-10 (Fig. 1B), perhaps supporting a role for other activation mechanisms, such as through CD40, in generating $CD23^{high}CXCR5^{high}$ B cells. TLR2 ligand also increased both CXCR5 and CD23 expression illustrating another possible stimulator of the activated CD23+ B cells in schistosomiasis (Fig. 4C)(15). For comparison, CXCR4 expression, which homes activated B cells to an area of the lymphoid tissue involved in memory B cell and plasma cell differentiation, was reduced upon CD23-cross-linking (Fig. 4D).

CD23 ligation with CD21 activates B cells similar to CD23-cross-linking

We reported that CD23-cross-linking is an important mediator in B cell signaling, particularly of SYK activation (21). In addition to the role of CD23-bound IgE, B cells have been shown to transport immune complexes in a CD21-dependent manner to follicular dendritic cells (FDC) in the GC (12). In contrast to mouse CD23, human CD23 possesses a C-terminal tail that binds CD21 (2). CD21 is the complement 2 receptor and can also exist in a soluble form (sCD21) which binds to complement-coated pathogen molecules. Because CD23b appears not to have an endocytosis signal, we hypothesized that this isoform may have a role in responding to CD21 ligation. CD23 is known to interact with CD21 on two sites of CD21. For these studies, we used a polypeptide of rhuCD21 spanning amino acids 30-280 (out of 1092) and 1-2 of the short consensus repeats (SCR), which contains the necessary binding for CD23, but does not cross-link CD23 molecules. B cells were treated with IL-4 to induce CD23b expression followed by stimulation with soluble rhuCD21. Interestingly, CD23 ligation with sCD21 induced CXCR5 expression (Fig. 5A) as well as a strong phospho-SYK response (Fig. 5B) in B cells, similar to the effect mediated by CD23 cross-linking (21).

284 **Discussion**

285 CD23 expression by B cells is associated with the development of resistance to
286 schistosomiasis (3, 34). We previously demonstrated that CD23-bound IgE augments B cell
287 responses to schistosome antigens, thereby identifying a possible function of IgE in resistance
288 (21). Here we show that CD23-bound IgE may be important in influencing B cell homing
289 mechanisms. Because soluble- or complexed-antigens must be transported to the lymphoid
290 follicles by specialized cells, specific subpopulations of macrophages and marginal zone B cells
291 are required for antigen transportation to- and within- the lymph node structures (7, 12, 40). In
292 mice, CD23+ B cells were also shown to bind IgE-immune complexes in blood. Capture of the
293 IgE-, but not IgG2a-, immune complexes induced rapid homing of the B cells directly into the
294 follicular areas of the spleen (23). Antigens complexed by IgE have been shown to have potent
295 immunostimulatory effects through CD23+ B cells, similar to an adjuvant (17). Direct
296 trafficking of antigen into the follicles by CD23+ B cells resulted in augmentation of T cell
297 organization in the T-B cell borders of the T cell zone and an overall enhanced immune response
298 to the CD23-transported antigen (23). These observations are likely clinically relevant in the
299 context of schistosomiasis. Here, we present evidence that circulating CD23+ B cells in humans
300 also transport immune complexes directly into the lymphoid follicles, which may play a role in
301 the Th2-mediated immunity associated with resistance to schistosomiasis (24, 32).

302 We found that circulating B cells in schistosomiasis expressed a predominance of CD23b
303 and that surface CD23 levels were correlated with expression of CXCR5. CXCR5 expression
304 licenses activated B cells to enter germinal centers in response to cognate chemokine, CXCL13,
305 produced by follicular dendritic cells in the GCs (1). Therefore, CXCR5 expression is generally
306 regulated by T cells through CD40-CD40L interactions in the B cell areas of the lymphoid

tissues (5) . Experimentally-generated CD23b+CXCR5^{high} B cells readily responded to CXCL13 in chemotaxis assays suggesting that populations of CD23+ B cells in schistosomiasis have a propensity to traffic into lymphoid follicles. Cross-linking of CD23 also enhanced CXCR5 expression demonstrating that CD23-bound IgE mediated capture of antigen may itself increase follicular homing mechanisms in the absence of T cell help or regulation. Why CD23+ *B cells* would play an important role in the IgE-mediated transport of antigen is not clear, but their specific function(s) may include concentrating antigen in certain regions of the tissue or initiating steps in CD23-mediated antigen presentation to T cells upon arrival to the GC.

Whereas the CD23a- associated endocytosis signal is thought to be important in the antigen presentation by CD23+ B cells, the role of CD23b remains undefined in human B cells (33). Thus, the inducible CD23b isoform likely has other roles during an immune response. It is possible that the lack of an endocytosis signal allows for the efficient transport of antigen without ensuing internalization by the B cell. On the other hand, both CD23a- and -b were shown to transport antigens within gastrointestinal epithelial human cells, a process which requires internalization of the IgE-antigen complex (33, 41). Sessile cells, like mucosal epithelium, may utilize different mechanisms of immune complex shuttling than B cells and this requires further characterization. CD23b likely also has a role in complement-mediated transportation of immune complexes by B cells through the CD21-binding C-terminal tail, which increases CXCR5 surface expression as well.

B cell migration towards the germinal center normally initiates a pathway of differentiation to memory B cells or plasma cells (4). However, B cells activated by CD23-bound IgE will likely not class switch and differentiate because the antigen captured is non-cognate for the B cell receptor. This supposition is illustrated by our observation that CD23-

cross-linking reduced CXCR4 surface level, which induces plasmablasts to home to the outer edges of the GC where they differentiate into plasma cells and memory B cells (36). Nevertheless, two photon laser-scanning microscopy demonstrated that naïve follicular mantle B cells continually visit the GC (13). The authors speculated that occasional antigen-specific B cells would recognize cognate antigen and join in the pre-existing germinal center. Our results, and those of Hjelm *et al*, suggest that some of the follicular mantle B cells, all of which are CD23+ in mice, may have transported antigen into the GC (23). This hypothesis may explain the lack of clonal relationship between B cell populations found in the dark zone containing germ-line encoded V regions with those expressed by B cells in the GC as well as the lack of clonal relationship amongst each other in human tonsils (31). And, once B cells have released their cargo, they may no longer bound by the chemokines in the FDC region, which is consistent with our observation that IgE monomers reduce CXCR5 surface levels, and the cells subsequently reenter the circulation.

We have demonstrated that CD23-mediated signaling dominants over that mediated by BCRμ illustrating the potential importance of this pathway (21). Because CD23 is expressed by BCRμ+ cells, it would stand to reason that antigen capture in the bloodstream by the low affinity BCR would also occur in intravascular schistosomiasis (21). Our results demonstrate that BCR-cross-linking reduces CXCR5 surface levels. Thus, the dominance of CD23-IgE activation may be significant where the transportation of specific antigens (those that bind antigen-specific IgE) is critical for the development of immunity. Interestingly, our previous report indicates that schistosome antigens inherently suppress human B cell function (21). Here we show that exposure to schistosome egg antigens reduces CXCR5 levels on B cells through undefined mechanisms. Resistant individuals likely have the ability to prevail over the immuno-evasive

tactics by maintaining a high level of both surface CXCR5 and CD23 (3, 34). Our data indicate there may be multiple mechanisms to generate the uniquely activated CD23b+CXCR5^{high} B cells in schistosomiasis. For example, CD40 stimulation upregulates both CD23b and CXCR5 and can overcome the effects of SEA on CXCR5 expression. In addition, TLR ligands may also promote an increase in CXCR5^{high} CD23^{high} B cells as schistosomiasis is associated with elevated microbial and endogenous TLR ligands and TLR2+ B cells (38). Nevertheless, we also observed a modest increase in CD23b expression by B cells in schistosome-uninfected Kenyans (n=5) suggesting that CD23b expression may rise through multiple mechanisms. Overall, as we begin to better understand the role of IgE in protective immunity in human helminthiasis, we can develop improved vaccines and adjuvants for controlling disease (6). Further characterization of the functional significance of CD23 expression by B cells may shed light on human immunological mechanisms critical for understanding multiple diseases.

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Figure legends

Figure 1: Schistosomes do not alter CD23 surface levels. A. Circulating CD23+ B cell percentages were compared between subjects with schistosomiasis (n=55) and North Americans (NA; n=12). CD23+ B cells levels are higher in NA; $P<0.0001$. B. Purified splenic B cells were cultured in the presence of stimuli indicated on figure for 48 hours or left untreated (No Tx). rIL-4 (20 ng/ml) and anti-CD40 (1µg/ml) increase CD23 whereas crude schistosome antigens (SEA, 5µg/ml; soluble egg antigen and SWAP, 5 µg/ml; soluble adult worm preparation) have no effect. rIL-10 (10 ng/ml) reduces basal levels of CD23. C. Isolated peripheral lymph node cells were cultured in the presence of stimuli indicated on figure. rIL-4 (20 ng/ml), rIL-13 (10 ng/ml), anti-BCRµ (2 µg/ml), and Pam3CSK4 (TLR2 ligand; 1 µg/ml) increase CD23 levels, whereas IL-7 (20 ng/ml) has a null effect. D & E. Purified circulating B cells from subjects with schistosomiasis were cultured in the presence of IL-4 and schistosome antigens (SEA and SWAP). IL-4 increased both the percentage (D) and mean fluorescence intensity (MFI; E) of CD23 but there was no effect from schistosome antigens (n= 13-19; $P<0.0001$).

Figure 2. CD23b expression predominates in schistosomiasis. A. Purified B cells were subjected to RT-PCR to measure the expression of CD23b compared to CD23a. CD23a is the predominant isoform in B cells from unexposed/uninfected North American blood samples (n=4). CD23b is the predominant isoform in Kenyans populations both hyper-exposed (n=5; upper and lower panels) and uninfected (n=8; lower panel). B. CD23a is constituitively expressed by B cells. IL-4 induces expression of CD23b. Shown is data from Ramos B cells.

C. Quantified expression levels of CD23b in response to stimuli indicated on figure. No Tx: no treatment. Note: anti-CD40 does not induce CD23b expression in Ramos B cells. Shown is the effect on tonsil B cells (n=4).

Figure 3. CXCR5 expression and function by CD23+ B cells. A. CXCR5 expression on CD19+ B cells in fresh blood of individual with schistosomiasis. B. The percentage of CD23+ B cells correlates with the percentage of CXCR5+ B cells in schistosomiasis; n=33, r=0.39, *P*=0.02. C. The level (mean fluorescence intensity; MFI) of CD23 correlates with the level of CXCR5 on B cells in schistosomiasis; n=26, r=0.45, *P*= 0.02. D. IL-4 and anti-CD40 stimulate different populations of CD23+CXCR5+ B cells. Tonsil B cells were treated for 18 hours with 10 ng/ml of IL-4 or 1 µg/ml of stimulatory anti-CD40. Representative of 6 experiments with 6 tonsils. E. IL-4 reduces CXCR5 levels, whereas anti-CD40 increases expression. Gray fill: untreated cells; gray line: IL-4 (10 ng/ml); black line: anti-CD40 (1 µg/ml). Representative of 6 experiments with 6 tonsils. F. IL-4 reduces the chemotactic response to CXCL13 whereas CD40 stimulatory increases mobilization of B cells; n=4 tonsils, *P*=0.03. F. *Ex vivo* levels of CD23 and CXCR5 expression on CD19+ B cells from a tonsil.

Figure 4. CD23-bound IgE cross-linking increases CXCR5 but reduces CXCR4 expression. A. Increasing levels of exogenous IgE reduce surface levels of CXCR5 on IL-4-treated tonsil B cells. B cells were incubated for 18 hours and evaluated by flow cyometry. Gray fill: untreated B cells. Representative of 3 experiments with 3 tonsils. B. CD23-bound IgE cross-linking increases surface levels of CXCR5. NP-specific IgE was cross-linked by NP-BSA (thick black line) or anti-IgE (thin black line). Gray fill: untreated B cells. NP-BSA in the absence of IgE or isotype control did not affect CXCR5 levels (not shown). Representative of 4

experiments with 4 tonsils. C. The effect of B cell stimuli on CXCR5 levels. Tonsil B cells were treated with the stimuli indicated on figure for 18 hours and CXCR5 levels were assessed by flow cytometry. Pam3CSK4 (TLR2 ligand), anti-CD40, and IL-10 increased levels of CXCR5 whereas SEA, anti-BCR, and IL-4 reduced levels. SWAP had no effect on CXCR5 surface levels; n=4 tonsils; *P<0.05 compared to untreated (No Tx) B cells. D. CD23-cross-linking reduces CXCR4 expression on Ramos B cells. 1µg/ml anti-CD23 (gray line); 5 µg/ml (thick black line) compared to untreated cells (gray fill). Representative of 6 separate experiments. Similar results were obtained with tonsil B cells.

Figure 5: sCD21 activates B cells. A. Tonsil B cells were treated for 18 hours with IL-4 and washed and re-plated with 2 µg/ml of sCD21 for an additional 18 hours. sCD21 induced the expression of CXCR4 as well as CD40. B. sCD21 (thick black line) and anti-CD23 (thin black line; 2 µg/ml) induces phosphorylation of SYK in Ramos B cells compared to untreated B cells (gray fill).

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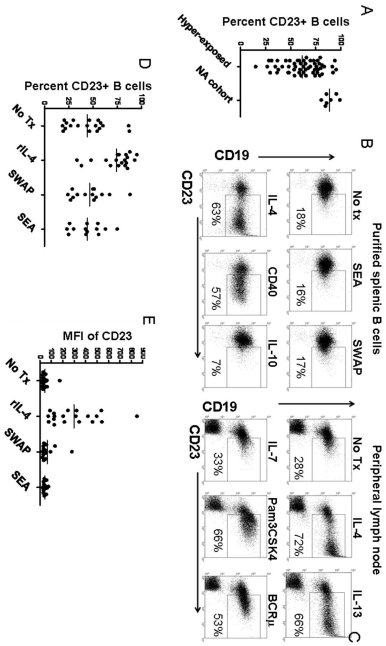

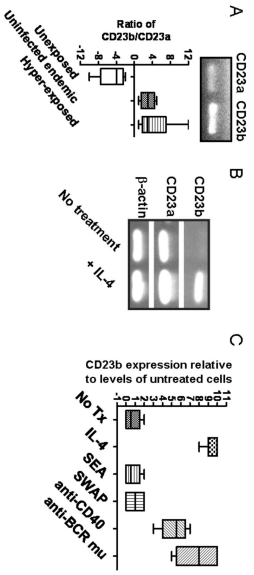

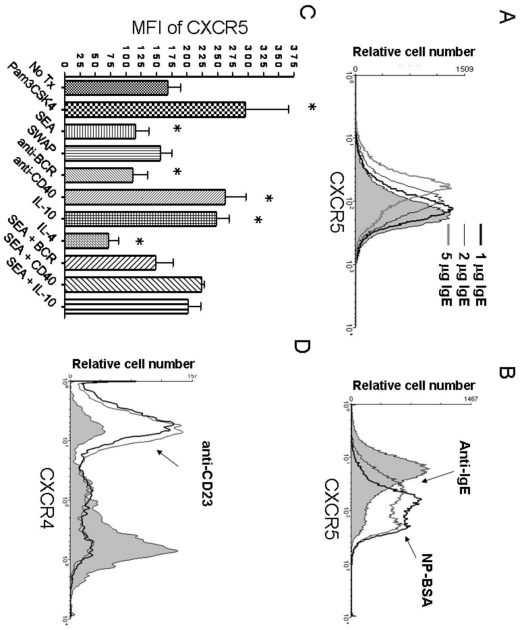

Table 1

	N	Age	Eggs per gram feces (EPG)
Car washers	45	25 +/- 4.4	9.5 +/- 16.9 (range 0-92)
Fishermen	10	39.4 +/- 12.4	479.3 +/- 792.0 (range 4-2880)