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CD23-Bound IgE Augments and Dominates Recall Responses through Human Naive B Cells

Qyana K. Griffith,* YanMei Liang,* Daniel O. Onguru,† Pauline N. Mwinzi,† and Lisa M. Ganley-Leal*

Human peripheral blood BCRμ^+ B cells express high levels of CD23 and circulate preloaded with IgE. The Ag specificity of CD23-bound IgE presumably differs from the BCR and likely reflects the Ag-specific mix of free serum IgE. CD23-bound IgE is thought to enhance B cell Ag presentation to T cells raising the question of how a B cell might respond when presented with a broad mix of Ags and CD23-bound IgE specificities. We recently reported that an increase in CD23$^+$ B cells is associated with the development of resistance to schistosomiasis, highlighting the potential importance of CD23-bound IgE in mediating immunity. We sought to determine the relationship between BCR and CD23-bound IgE-mediated B cell activation in the context of schistosomiasis. We found that crude schistosome Ags downregulate basal B cell activation levels in individuals hyperexposed to infectious worms. Schistosome-specific IgE from resistant, occupationally exposed Kenyans recovered responses of B cells to schistosome Ag. Furthermore, cross-linking of CD23 overrode intracellular signals mediated via the BCR, illustrating its critical and dominating role in B cell activation. These results suggest that CD23-bound IgE augments and dominates recall responses through naive B cells. ***The Journal of Immunology*****, 2011, 186: 000–000.**

High levels of serum parasite-specific IgE are associated with resistance to human schistosomiasis, but the functional significance is undefined (1–4). Indeed, the bona fide roles of IgE in the human immune system remain elusive. We sought to evaluate the role that IgE receptor-bearing cells might have in IgE-mediated immunity and recently reported that an increase in circulating CD23$^+$ B cells was associated with the development of resistance to schistosomiasis in occupationally exposed Kenyans (5). CD23 is the low-affinity IgE receptor and has broad cellular distribution in humans (6). Up to 80% of circulating B cells express CD23 at relatively high surface levels, illustrating a potentially important role in immunity (5). In fact, describing CD23 as having low affinity for IgE is misleading because oligomers of CD23 can bind IgE with equal affinity as the high-affinity IgE receptor (FcεRI) (7). Thus, CD23$^+$ B cells circulate preloaded with CD23-bound IgE (5). The Ag specificity of CD23-bound IgE presumably differs from the BCR and likely reflects the Ag specific mix of free serum IgE. CD23 is predominantly expressed by BCRμ^+ B cells, raising the question of how CD23-bound IgE might affect the ability of the B cell to respond to a broad mix of Ags.

In both humans and mice, CD23-bound IgE is thought to enhance B cell Ag presentation to T cells when CD23-bound IgE is cross-linked by Ag and endocytosed (8, 9). The significance of this pathway is demonstrated by the ability of CD23-activated BCRμ^+ B cells to present Ag to naive T cells (10). Thus, murine CD23$^+$ B cells bound by OVA-specific IgE can enhance the expansion of OVA-specific T cells and IgG (but not IgE) by B cells other than those that express CD23-bound IgE (10). The activation of Ag-specific T cells allows for subsequent enhancement of cognate B and T cells to cross-talk and expand. This immunostimulatory effect of CD23-bound IgE is similar to a strong adjuvant and is Ag-specific in mice (11).

Whereas Ag-specific IgG is enhanced by CD23-mediated B cell responses, CD23 appears to be primarily a negative regulator of IgE in mice (12). This is highlighted by the observation that variants of CD23 expressed by New Zealand Black and 129/SvJ strains of mice fail to bind IgE, resulting in an excessive IgE response (13, 14). However, the influence of CD23-bound IgE on human B cell-mediated immunity is poorly described. Experimental evidence suggests that cross-linking CD23-bound IgE on B cells increases the recycling of HLA-DR complexes to the surface of the cell, suggesting a role in Ag presentation (15); however, there are conflicting reports as to whether surface CD23 is a negative or positive regulator of IgE in humans (9, 16). We therefore sought to better define the effect of CD23-bound IgE on human B cell activation and present a potential role for CD23$^+$ B cells in the development of resistance to schistosomiasis.

Materials and Methods

Human subjects

This study was approved by the Institutional Review Board of Boston University, the Scientific Steering Committee of the Kenya Medical Research Institute, and the National Ethics Review Committee of Kenya. The study participants with schistosomiasis were men employed as car washers and thus occupationally exposed to schistosome transmission as they washed cars while standing in a lake, as described elsewhere (5). Stool samples were examined for *Schistosoma mansoni* eggs and for other helminth ova by the modified Kato-Katz method (Vestergaard-Frandsen) (two

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Abbreviations used in this article: P-, phospho-; SWAP, soluble adult worm crude Ag preparation.

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slides each, three 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 albendazole as previously described (5).

Ag-specific IgE

Human sera from schistosomiasis or unexposed/uninfected subjects was screened for IgE reactivity to schistosome soluble adult worm crude Ag preparation (SWAP; gift from Dr. Evan Secor, Centers for Disease Control and Prevention) or dust mite Ag Derp2 (Indoor Biotechnologies) by ELISA with reagents from SouthernBiotech. ELISA plates were coated with 5 μg/ml SWAP or 1 μg/ml dust mite Ag in PBS, and serum was evaluated for levels of Ag-specific IgE at a 1:10 dilution in 1% BSA/PBS (17). Sera was used as pooled dust mite-specific IgE^{high} derived from uninfected/unexposed donors (n = 4 donors), SWAP-specific IgE^{high} (n = 6 donors), or polyclonal IgE derived from uninfected/unexposed donors (n = 4 donors), which was not reactive for dust mite or SWAP, but had similar levels of total IgE as measured by ELISA (3500 ± 520 ng/ml; Sigma-Aldrich/SouthernBiotech). Myeloma IgE (unknown specificity) was purchased from Diatec.

CD23-mediated B cell activation

Peripheral blood was purchased from Source Leukocytes (New York Biologics; n = 10) and was used to isolate circulating B cells from unexposed/uninfected population. Upon informed consent, peripheral blood was drawn from Kenyan occupationally exposed car washers (n = 22). Fresh, surgically discarded tonsils and spleens were purchased from the Pathology Department at Boston University Medical Center (Boston, MA) or from National Disease Research Interchange (Philadelphia, PA) and processed as previously described (n = 14 tonsils; n = 3 spleens) (18). Briefly, minced tonsils and spleens were gently homogenized and passed over a 70-μm cell strainer (BD Falcon) to obtain a single-cell suspension followed by Ficoll gradient to isolate mononuclear cells. B cells were isolated by negative selection magnetic bead isolation with 97–99% purity (Miltenyi Biotec, Invitrogen). The $CD23^+$ Ramos B cell line was purchased from American Type Culture Collection.

Peripheral blood B cells are CD23-bound IgE-positive (Fig. 1*A*) and were activated with anti-IgE (2 μg/ml; Sigma-Aldrich) or anti-CD23 (2 μg/ml; eBioscience) Abs to cross-link CD23 (Fig. 1*B*). Isotype control was used at 2 μg/ml (eBioscience). Tonsil B cell expression of CD23 is variable (Fig. 1*C*, *right*) and, generally, levels of CD23-bound IgE are lower than those in peripheral blood (Fig. 1*D*, *middle*). Furthermore, tonsils contain few CD23-negative IgE-positive B cells, such as memory $BCR\varepsilon^+$ B cells (Fig. 1*C*, *right*). Thus, in some experiments, B cells were cultured overnight with 20 ng/ml IL-4 to upregulate nascent surface CD23 (Fig. 1*D*, *left*). The following day, B cells were subjected to the IgE-binding assay to load nascent CD23 molecules with IgE. B cells were rotated in TBS buffer containing 2 mM $CaCl_2$ plus 20 μl per 500,000 B cells of heat-inactivated serum for 2 h to induce dust mite-specific IgE^{high}, SWAP-specific IgE^{high}, or polyclonal IgE binding to CD23 (Fig. 1*D*, *right*) (19). Twenty micrograms of myeloma IgE (Diatec) was bound to B cells as a control in some experiments. No other Ab isotype bound to $CD23^+$ B cells under these conditions, and CD23-bound IgE is stable in culture for up to 3 d (not shown). B cells loaded with Ag-specific IgE were stimulated with 2 μg per million cells of dust mite Ag (Derp2; Indoor Biotechnologies), 5 μg per million cells of SWAP, or 1 μg/ml anti-IgE (Fig. 1*B*). CD23-mediated activation was compared with Fab anti-BCRμ (Jackson ImmnnoReseach Laboratories; 2 μg/ml) cross-linking, as CD23 is expressed primarily by $BCR\mu^+$ B cells and both receptors are used in Ag capture. Stimulatory anti-CD40 (R&D Systems; 1 μg/ml), anti-CD3 and anti-CD28 (eBioscience; 1 μg/ml each), *Escherichia coli* LPS (1 μg/ml; Sigma-Aldrich); Pam2CSK4 (eBioscience; 1 μg/ml), schistosome egg Ag (5 μg/ml; gift from Evan Secor, Centers for Disease Control), and recombinant IL-10 and IL-4 (eBioscience; 20 ng/ml) were used as a controls in some experiments. Abs to CD86, CD80, HLA-DR, and CD74 were used to assess B cell activation after 18 h in culture by flow cytometry and were purchased from BD Pharmingen. Secreted IgM was measured in cell-free supernatants by ELISA with reagents from Sigma-Aldrich.

Intracellular phospho-specific flow cytometry and Western blot

CD23 activation was assessed by phospho-kinase activity with phosphoflow (20) or Western blot at 5 min to 2 h poststimulation. For phosphoflow, cells were fixed with paraformaldehyde and permeabilized with BD Phosflow Perm II buffer following stimulation. Cells were then incubated with fluorescently labeled Abs at room temperature for 30 min in the dark and washed. The following phospho-specific PE-conjugated mAbs were purchased from BD Pharmingen (San Diego, CA): phospho-ERK1/2 (pT202, pY204), phospho-p38 (pT180, pY182), phospho-SYK (pY352)/Zap70(Y319), phospho-BTK (pY551), phospho-BLNK (pY84), and phospho-ATK (S473). For Western blot analysis, anti-Lyn (pT507; Cell Signaling Technology) and phospho-BTK (pY551; BD Pharmingen) were used in standard protocol. Ab to β-actin and total BTK were used as a protein loading control (BD Pharmingen). Group means were subjected to analyses with ANOVA (GraphPad Software).

Results

Intracellular signaling in CD23- or BCRμ-stimulated B cells

$CD23^+$ B cells circulate preloaded with IgE (Fig. 1*A*), indicating that $BCR\mu^+$ B cells have multiple Ig surface receptors, likely of many differing specificities. Previous studies have demonstrated that Ag captured via CD23-bound IgE is processed and presented on surface MHC class II within hours (15), suggesting that CD23 is highly efficient in inducing Ag presentation functions. These

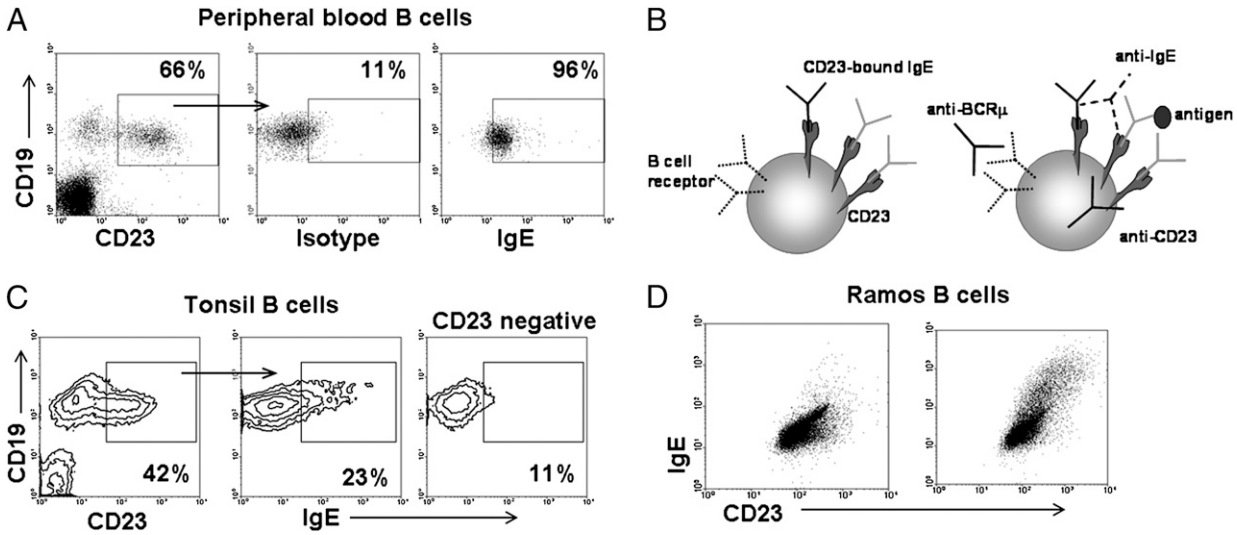

FIGURE 1. CD23-bound IgE on B cells. *A*, Circulating B cells express CD23 and are preloaded with IgE. Shown are B cells evaluated in fresh whole blood from a Kenyan car washer hyperexposed to schistosomes. *B*, Schematic depicting the complexity of surface-bound receptors on $BCR\mu^+$ B cells (*left*) and activation protocols used in this study (*right*). *C*, The level of CD23 (*left*) and CD23-bound IgE on freshly isolated tonsil $CD23^+$ B cells (*middle*) or $CD23^-$ B cells (*right*). *D*, Ramos B cells before (*left*) and after IgE binding assay (*right*). Shown are B cells bound by SWAP IgE^{high}.

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observations raise the question of how a B cell would "decide" which receptor (CD23 or BCR) or Ag, is the priority in generating a response. To answer these questions, we first compared early B cell responses to CD23 or BCR cross-linking by assessing kinase phosphorylation pathways (20). CD23-mediated B cell signaling pathways in response to CD23 activation are poorly defined. The human CD23a isoform has a similar amino acid sequence as the canonical ITIM; however, it is not clear whether this sequence dictates an endocytosis signal or an immunosuppressive ITIM (21). We thus assessed phospho (P)-kinases within the well-described BCR Ag capture signaling pathways for comparison with CD23 (20).

Signaling through CD23 was similar in tonsil, peripheral blood, and Ramos B cells except for basal levels of P-ERK1/2, which we previously reported were higher in circulating B cells (22). Anti-IgE, anti-CD23, and Ag-specific cross-linking of cell-bound IgE (Fig. 1*B*, 1*D*) induced the same cascade of events in B cells. Similar to BCR-mediated stimulation, cross-linking of CD23 induced phosphorylation of Lyn (not shown), SYK (Fig. 2*A*), and ERK1/2 (Fig. 2*C*). In general, the kinetics of kinase activity were similar between the BCR and CD23 (Fig. 2*F*). However, in contrast to BCR cross-linking, CD23 activation did not lead to phosphorylation of BLNK (Fig. 2*B*), p38, or Akt (not shown). Furthermore, activation through CD23 led to a reduction in the basal levels of P-BTK (Fig. 2*D–F*). Thus, CD23-mediated signaling induces Lyn, Syk, and ERK1/2 phosphorylation, but results in a null effect on BLNK and p38, likely through inhibition of P-BTK (Fig. 2*G*) (23). These results were used as a tool to probe how B cells respond to physiological stimulation through CD23.

Parasite-specific IgE recovers B cell responses to schistosomes

High levels of parasite-specific IgE are associated with resistance to schistosomiasis (1). However, while susceptible individuals have low levels of parasite-specific IgE, their levels of polyclonal IgE are not necessarily different from those of resistant individuals (L. Ganley-Leal and P.N. Mwinzi, unpublished observations). This indicates that there is a deficiency in the development of parasite-specific IgE in susceptibility to schistosomiasis. However, it is not clear why some individuals develop parasite-specific IgE, whereas others do not.

We tested the effect of crude schistosome Ags on B cell activation and found that, in general, B cells from unexposed/uninfected donors were unresponsive to crude schistosome Ags (up to 50 μg/ml) as measured multiple parameters, including surface expression of CD69, cytokines (not shown), and IgM production (Fig. 3*A*). As this approach would model responses in a primary infection, these results suggested that schistosome Ags may contain components that are inherently immunosuppressive for human B cells. To determine a possible mechanism, kinase activity in B cells was assessed, and Fig. 3*B* and 3*E* demonstrate that schistosome Ags have a modest, but significant, effect on reducing the basal levels of P-SYK. This effect was observed in circu-

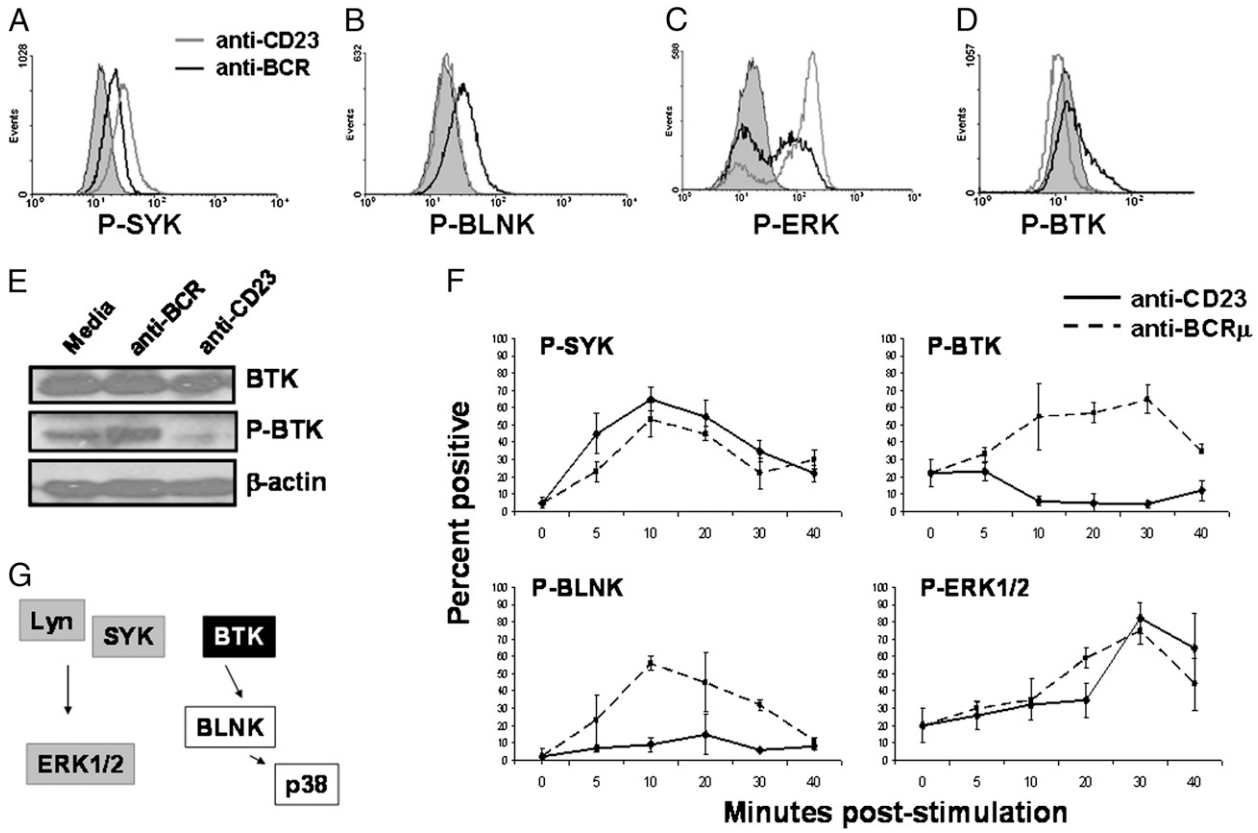

FIGURE 2. CD23 cross-linking induces activation of Syk and ERK1/2. *A*, Cross-linking CD23 (anti-CD23; gray line) results in the phosphorylation of SYK similar to anti-BCRμ (black line) compared with untreated B cells (gray fill) in Ramos B cells 5 min poststimulation. Isotype control has a null affect on B cell activation (not shown). *B*, Anti-CD23 does not activate BLNK (10 min poststimulation), but has a strong effect on ERK1/2 (*C*; 30 min poststimulation). *D*, Anti-CD23 reduces basal levels of P-BTK as confirmed by Western blot (*E*); 10 min poststimulation. *F*, Comparison of the kinetics of kinase phosphorylation following CD23 and BCR cross-linking for SYK (*upper left*), BTK (*upper right*), BLNK (*lower left*), and ERK (*lower right*). Ramos B cells. Time points later than 40 min did not reveal delays in CD23-mediated kinase activation for BLNK or BTK (up to 2 h). *G*, Cross-linking of CD23 has a positive effect (gray fill) on Lyn, Syk, and ERK1/2 phosphorylation, a negative effect (black fill) on BTK phosphorylation, and null effect (open) on BLNK and p38.

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lating B cells from susceptible, occupationally exposed Kenyans, circulating B cells from unexposed/uninfected subjects, and tonsil B cells from unexposed/uninfected donors and may explain the null effect of schistosome Ags on other measures of B cell activation (Fig. 3*A*).

Because CD23 cross-linking induces robust P-SYK, we tested how CD23-bound SWAP-specific IgE^{high} would affect this response (Fig. 3*C*). CD23-bound SWAP-specific IgE^{high} recovered SYK phosphorylation of schistosome-treated B cells from chronically exposed subjects (Fig. 3*D*, 3*E*). CD23-bound SWAP-specific IgE^{high} also recovered responses to schistosome Ags by tonsil B cells (Fig. 3*E*). Thus, a potential role for IgE in human schistosomiasis immunity is to augment recall responses by naive B cells. In the absence of sufficient parasite-specific IgE, B cells would be prone to the downregulatory effect of schistosomes, and host immunity may fail to develop to protective levels.

CD23-mediated activation dominates that mediated by BCR

CD23-bound IgE is likely of a higher affinity and avidity for Ags than low-affinity, broad reactive BCRμ. Thus, it is possible that upon secondary pathogen invasion, both CD23 and BCR might be cross-linked simultaneously on a B cell (Fig. 4*A*). We tested how B cells would respond to dual signals by globally cross-linking BCR (anti-BCRμ) and CD23 (anti-CD23) receptors across a population of naive B cells. Interestingly, CD23-mediated signaling reduced levels of P-BTK, even in the presence of a strong BCR signal (Fig. 4*B*, 4*C*). Importantly, this was apparent under more physiological conditions. BCR-mediated P-BTK was reduced when CD23-bound dust mite IgE^{high} (Fig. 4*A*) or SWAP IgE^{high} (not shown) was cross-linked with cognate Ag, which results in a presumably much lower strength signal than anti-BCRμ (or anti-CD23; Fig. 4*D*). These results are consistent with the interpretation that responses via CD23-bound IgE dominate over those mediated by the BCR.

Effects of CD23 on specific Ag presentation mechanisms

CD23-bound IgE-mediated Ag presentation has been shown to be a potent stimulator of T cells, although the mechanism remains unclear (10). We tested the effects of CD23-mediated B cell activation on surface expression of costimulatory molecules, CD86 and CD80, as well as HLA-DR but found no effect (not shown). The HLA-DR–associated invariant chain, CD74, is a MHC class II chaperone and plays a role in Ag presentation (24). CD74 and HLA-DR are associated at the cell surface. Upon cell activation, proteolytic cleavage of internalized CD74 results in disengagement of HLA-DR and allows binding of cognate antigenic peptides for Ag presentation (25). A change in detectable surface CD74 may therefore reflect increased Ag presentation capacity by MHC class II. Thus, we tested the effect of CD23-mediated B cell activation on CD74 surface levels (Fig. 4*E*). Whereas anti-BCRμ

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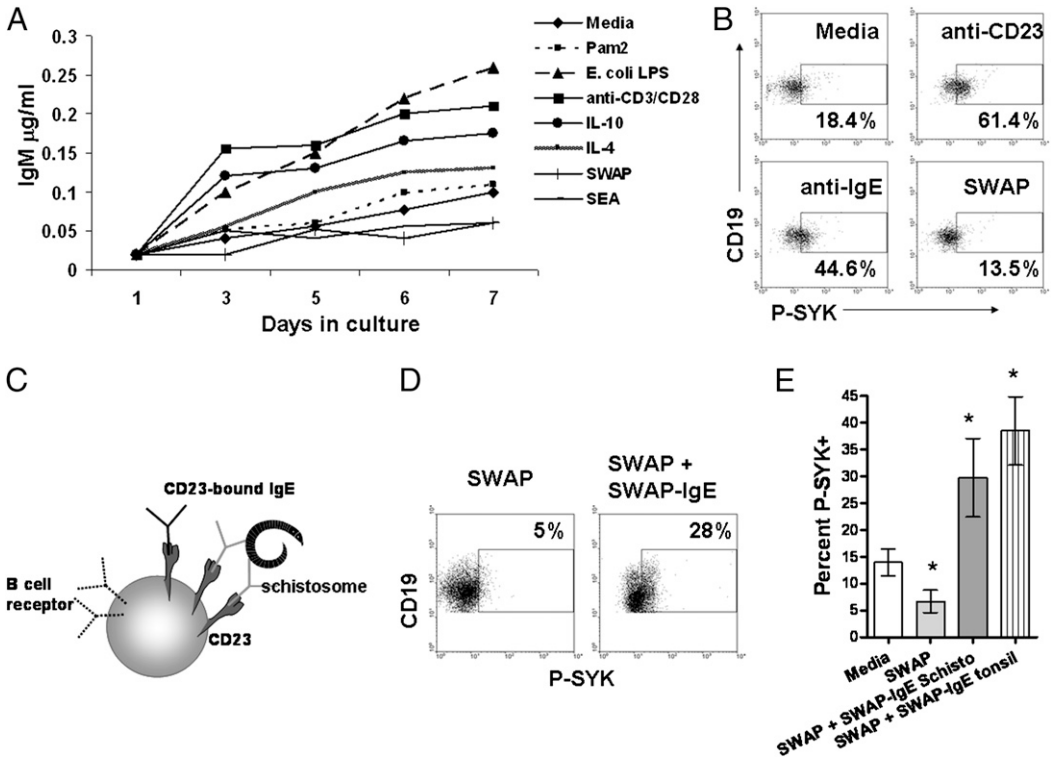

FIGURE 3. CD23-bound parasite-specific IgE recovers B cell responses to schistosomes. *A*, Reduced B cell responses to schistosome Ags. Splenic mononuclear cells were cultured with the stimuli indicated for up to 7 d, and IgM was measured in cell-free supernatants. SWAP and schistosome egg Ag (SEA) did not induce IgM production above untreated cells (Media). Positive controls include TLR4 (*E. coli* LPS) and TLR2 ligands (Pam2CSK4; Pam2) as well as T cell activation (anti-CD3/CD28). Representative data from purified B cells and mononuclear cells from multiple tissues, including tonsils, peripheral blood, and spleen, are shown. *B*, Cross-linking CD23 via anti-CD23 (*top right*) and anti-IgE (*bottom left*) results in phosphorylation of SYK. SWAP (schistosome Ags) reduce basal levels of P-SYK (*bottom right*). Peripheral blood B cells from an uninfected/unexposed donor. *B*, Schematic demonstrating that B cells were loaded with SWAP-specific IgE^{high} Ab from resistant Kenyan car washers and stimulated with schistosome Ags. *C*, Addition of SWAP IgE^{high} Ab to B cells recovers the ability of CD23 cross-linking to phosphorylate SYK in response to schistosome Ag. Shown are B cells from occupationally exposed Kenyans stimulated for 10 min. *D*, Composite data demonstrating the effect of schistosome Ags on basal P-SYK levels. Data are shown as means ± SE, and Media control includes B cells from susceptible Kenyan car washers ($n = 4$), unexposed/uninfected patients ($n = 5$), and tonsil B cells ($n = 8$). SWAP IgE^{high} recovers responses of B cells from susceptible car washers ($n = 3$; gray bar) and tonsils ($n = 6$; striped bar). $^*p < 0.05$ compared with Media.

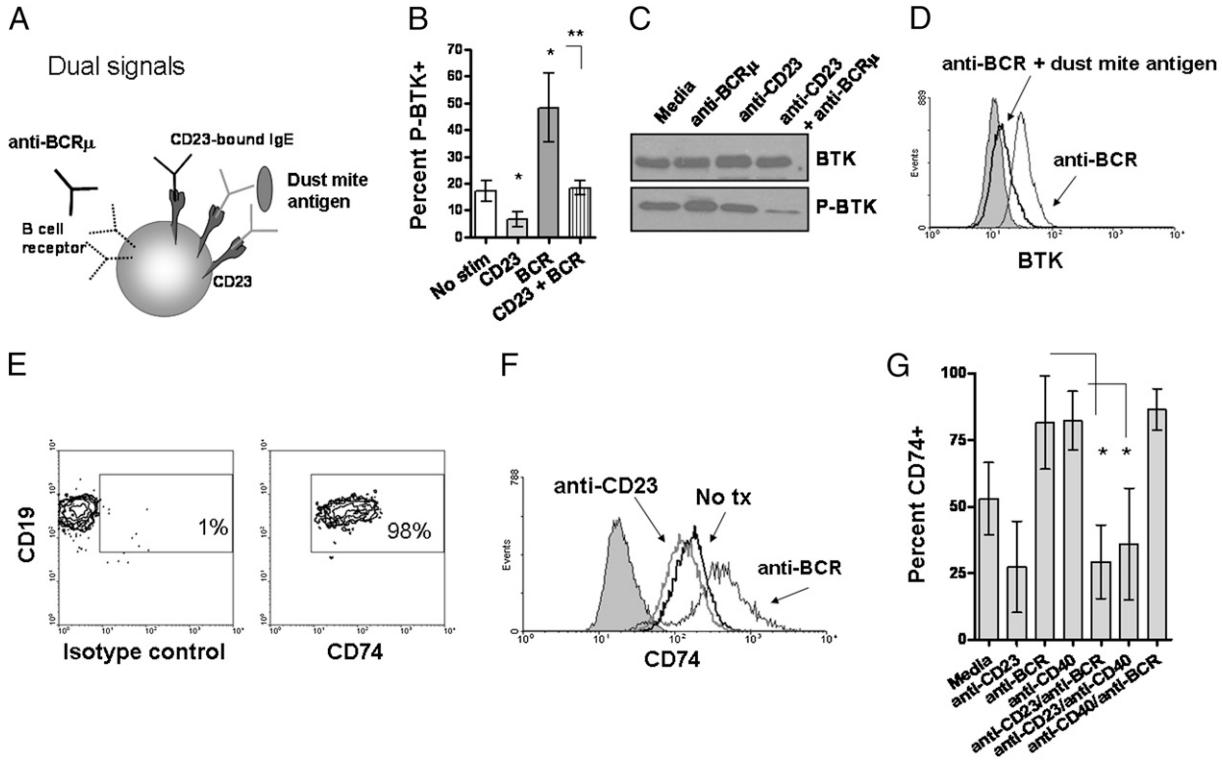

FIGURE 4. CD23-mediated signaling dominates over BCRμ. *A*, Schematic demonstrating experimental protocol to induce dual (CD23 and BCR) signaling though B cells. *B*, Cross-linking of CD23 (anti-CD23) reduces P-BTK, whereas anti-BCRμ induces a strong P-BTK response. Dual stimulation results in the reduction of BCR-mediated induction of P-BTK. Ramos B cells ($n = 4$; 10 min posttreatment): $*p < 0.05$ compared with media control; $**p = 0.01$. Data are shown as means ± SE. *C*, Reduction of P-BTK in dual-stimulated B cells as confirmed by Western blot. Peripheral blood B cells from uninfected/unexposed subject. *D*, CD23-mediated activation trumps the BCRμ under more physiological conditions. Ramos B cells were bound by dust-mite specific IgE^{high} (gray fill) and stimulated with anti-BCRμ (thin black line) or anti-BCRμ plus dust mite Ag (thick black line). The addition of dust mite Ag reduces BCR-mediated phosphorylation of BTK. Representative of eight experiments with tonsil and Ramos B cells. *E*, Level of CD74 on circulating B cells. Representative of 10 blood samples. CD74 expression ranged from ~25 to 99% of $CD19^+$ B cells. *F*, CD23 cross-linking reduces CD74 expression on Ramos B cells. In contrast, BCRμ cross-linking increased surface expression of CD74. Gray fill indicates isotype control. B cells were cultured for 18 h. *G*, Composite data demonstrating the effects of dual signals on CD74 expression by Ramos B cells. CD23 cross-linking reduces the effects of BCR and CD40 stimulation on CD74 expression; $n = 3$ separate experiments. $*p < 0.05$. B cells were cultured for 18 h.

and stimulatory anti-CD40 increased surface expression, anti-CD23 reduced levels of CD74 (Fig. 4*F*, 4*G*). Furthermore, dual stimulation through CD23 in combination with BCRμ or CD40 resulted in reduction of CD74 levels (Fig. 4*G*). These results suggest that Ag presentation pathways involving HLA-DR/CD74 are dominated by Ag capture through CD23-bound IgE when compared with the BCR pathway.

CD23 is a rheostat for the level of Ag-specific IgE

The innate function of the naive $BCR\mu^+$ B cell is seemingly sacrificed for the presumably higher specific and more critical response mediated by CD23-bound IgE. This possibility is intriguing because memory B cells, which would also have high affinity BCRε, might perform the same function and they may also stimulate naive T cells. This suggests that the mix of CD23-bound IgE specificities plays an important role in dictating the appropriate immune responses as memory B cells, even within pools specific for schistosomes, and may not represent overall systemic memory, whereas CD23-bound IgE might (26).

Circulating B cells likely carry IgE specificities that are reflective of free IgE in the serum, but they differ from the BCR. Furthermore, there is feasibly a dynamic mix of CD23-bound IgE specificities, which reflects the status of the host's immunity. We thus asked how B cells would respond to Ag in the face of changing concentrations of Ag-specific IgE bound by CD23. Responses by $CD23^+$ B cells bound by dust-mite specific IgE^{high} were compared with B cells bound by polyclonal IgE. As above, CD23-bound dust mite IgE reduced P-BTK in response to dust mite Ag (Fig. 5*A*, *left*). Surprisingly, however, B cells bound by polyclonal IgE demonstrated a robust P-BTK response following stimulation with dust mite Ag (Fig. 5*A*, *left*) or SWAP (not shown). Interestingly, endogenous dust mite Ag responses were apparent in B cells from some tonsil donors (Fig. 5*A*, *right*) and our results suggest the addition of polyclonal IgE to B cells augmented the P-BTK response to dust mite Ag (Fig. 5*A*, compare *right* and *left panels*). Furthermore, there was a dose effect of polyclonal IgE in the presence of Ag-specific IgE (Fig. 5*B*). Thus, a potentially undetectable level of Ag-specific IgE is responsible for augmenting this response. Dust mite Ag had a null effect on B cells bound by myeloma IgE, supporting this possibility (Fig. 5*C*). Polyclonal IgE plus Ag-mediated signaling was not associated with activation of BLNK or p38 (Fig. 5*D*) or other BCR downstream signaling that CD23 cross-linking did not activate.

Discussion

Although it has been long recognized that increased IgE is associated with protection against parasitic helminths in humans, the immunological function of IgE remains undefined (27). Animal models have not been informative in this aspect, primarily due to the differential cellular distribution of IgE receptors (FcεRI and FcεRII/CD23) (28, 29). Hypotheses for the role of IgE in helminthiasis have centered on effector functions of $Fc\varepsilon RI^+$ myeloid

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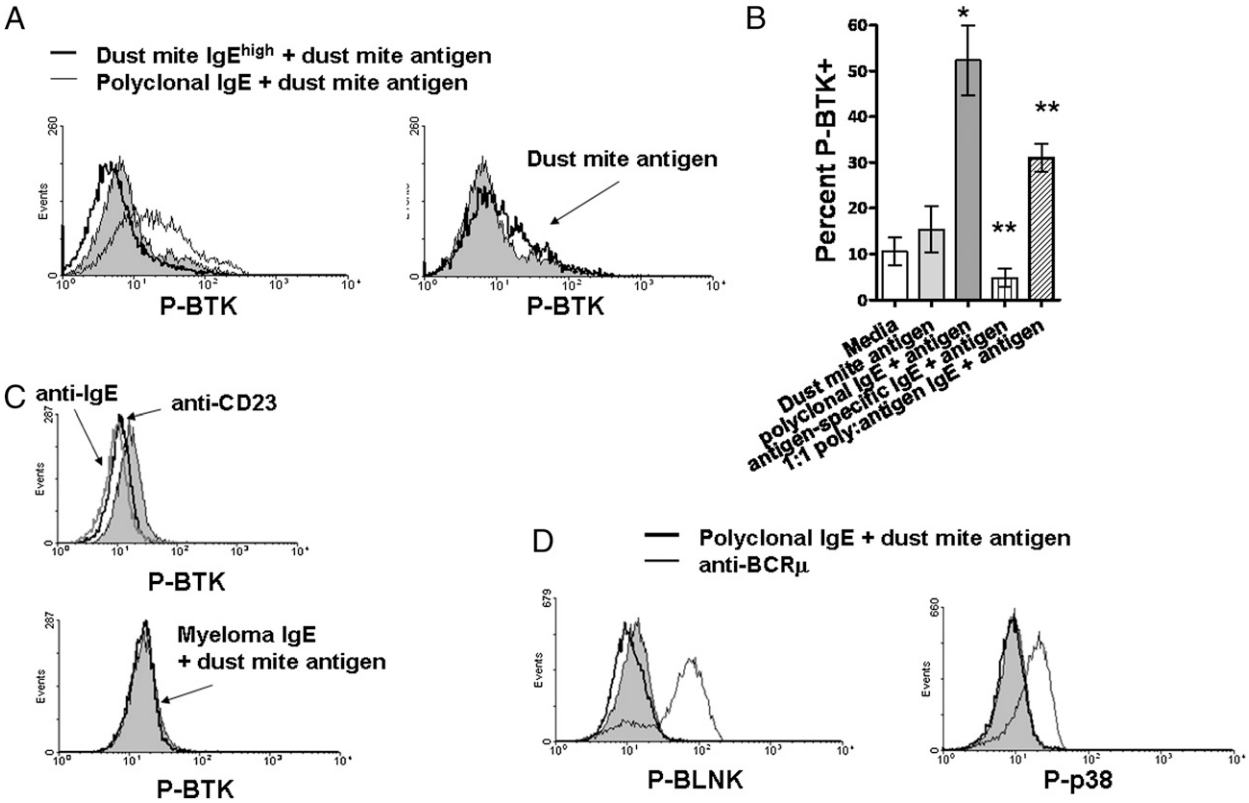

FIGURE 5. CD23-bound polyclonal IgE acts as a rheostat for the concentration of Ag-specific IgE. *A*, Polyclonal IgE induces BTK phosphorylation in the presence of Ag. Tonsil B cells were bound by dust mite IgE^{high} or polyclonal IgE (no detectable dust mite- or schistosome-specific IgE) and stimulated with dust mite Ag for 10 min. As above, CD23 activation (dust mite Ag plus dust mite-specific IgE; thick black line) reduces P-BTK. In contrast, CD23-bound polyclonal IgE (thin black line) recovered P-BTK compared with media control (gray fill); representative of eight separate experiments with two separate pools of IgE (*left*). Endogenous dust mite Ag responses (black line) in B cells from a tonsil (representative of 3 of 10 donors) compared with media (gray fill; *right*). *B*, Composite data (mean ± SE) demonstrating the effect of the concentration of Ag-specific IgE on responses to dust mite Ag. $*p < 0.05$ compared with media; $n = 8$; $**p < 0.05$ compared with polyclonal IgE plus Ag. Serum containing polyclonal IgE was mixed 1:1 with serum containing Ag-specific IgE prior to IgE-binding assay. *C*, Ramos B cells bound by myeloma IgE do not respond to dust mite Ag (black line; *bottom*). *Top panel* depicts control responses to anti-IgE or anti-CD23. Gray fill indicates media control; 10 min. *D*, Polyclonal IgE plus Ag (thick black line) does not induce phosphorylation of BLNK (10 min) or p38 (30 min) compared with Media (gray fill) and anti-BCRμ (thin black line). Representative of four separate experiments.

cells that cooperate with IgE to kill parasitic larvae; however, in vivo evidence is lacking in humans (30). Another conundrum in schistosome human immunology is the length of time and exposure required for individuals to demonstrate evidence of resistance (31). This is best illustrated in age-acquired immunity where young children demonstrate susceptibility to infection/reinfection, heavy parasite burden, and low levels of schistosome-specific IgE (32). Resistance begins to develop around the time of puberty onset when schistosome-specific IgE is apparent (33, 34), but the mechanisms remain unclear and are the subject of debate (35, 36).

Most of the cohort examined in this study was not exposed to schistosome as children, and IgE-associated immunity has been shown to take up to 6 y of adult hyperexposure to the parasite and repeated drug treatments (5, 37). Nevertheless, some of the study participants never develop measurable resistance (38). Our results may partly explain these observations. We demonstrate that B cells are inherently susceptible to suppression by schistosome Ags. Although we did not define the mechanism, our preliminary results suggest a role for schistosome TLR4 ligands (L. Ganley-Leal, unpublished observations) (39). This potent immunosuppressive effect on B cells may elucidate why, for example, susceptible individuals develop IgE toward other pathogens but lack sufficient schistosome-specific IgE to develop resistance (40). $CD23^+$ B cells were found to be more prominent during the development of immunity in the occupationally exposed car washers, suggesting an important role for CD23-bound IgE in generating the necessary threshold of IgE required for protection (5). As the development of resistance to schistosomiasis is tied to CD23 expression by B cells, failure to upregulate CD23 may represent a bona fide barrier in the development of immunity. Indeed, it was recently shown that more resistant children demonstrate higher levels of $CD23^+$ B cells, illustrating that lack of CD23 expression is correlated with susceptibility in endemic regions (41). These previous reports are supported by our observations in this study that an overall dominance of CD23-mediated B cell activation results in the sacrifice of the innate function of the BCRμ for the presumably more specific and critical response mediated by CD23-bound IgE. Interestingly, in mice, IgE production can occur in IgM-deficient μMT mice upon helminth infection, suggesting the existence of a BCRμ-independent pathway for the production of IgE as well as the dispensability of the BCRμ in this respect (42).

Fig. 6 depicts our model of CD23-bound IgE-mediated amplification of immunity by B cells in schistosomiasis. We hypothesize that rare, schistosome-specific B cells are present during a primary infection and are likely highest in individuals who eventually develop resistance. These B cells recognize schistosomes through their BCR and eventually differentiate to produce a small amount

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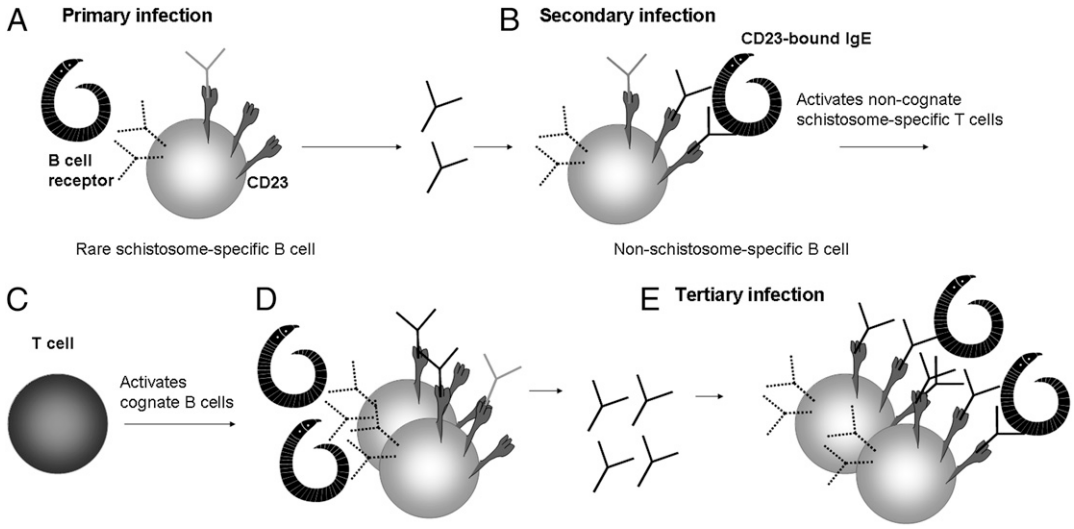

FIGURE 6. Model of acquired resistance in schistosomiasis. *A*, Rare schistosome-specific B cells produce a low level of IgE during a primary schistosomiasis. *B*, The schistosome-specific IgE is bound by more naive B cells that express CD23 to patrol the host. Upon secondary infection, the CD23-bound IgE allows more B cells to respond to schistosomes and reduces the immunosuppressive effects of schistosome Ags on humoral immunity. *C*, CD23-activated B cells present Ag to activate noncognate, but schistosome-specific, T cells. *D*, The expanded schistosome-specific T cells subsequently activate rare schistosome-specific, cognate B cells, which differentiate to produce IgE. *E*, Amplification of the immune response continues as levels of schistosome-specific IgE arm more naive $CD23^+$ B cells with the ability to respond to parasite infection. Finally, the necessary threshold of Ag-specific IgE is met following several rounds of infection, which may take several years.

of IgE. This nascent IgE arms more $CD23^+$ B cells to patrol the host with parasite-specific receptors. Upon secondary infection, schistosomes are immediately recognized by a higher number of B cells, which endocytose Ag from the CD23-bound IgE, rather than the BCR. The CD23-bound schistosome-specific IgE also plays an important role in removing the inherent suppressive effects of the parasite on the B cells. Ag presentation by CD23-bound IgE-activated B cells to noncognate, but schistosome-specific, T cells allows subsequent cognate B cell responses to increase the production of parasite-specific IgE and maintain the continual slow amplification of the immune response (10).

Recent evidence suggests that the BCR can sense the affinity of an Ag to direct the appropriate response of the B cell (43). CD23 may also act as a rheostat for mediating the appropriate immune response in the context of IgE concentrations, or affinities, for specific Ags. The function of the robust BTK response to Ag in the presence of CD23-bound polyclonal IgE remains unclear. It is likely that the response is due to undetectable Ag-specific IgE, and it is possible that the response may help to reduce the threshold for specific, but undefined, BCR-mediated responses, similar to the roles of CD19 or CD21 (44, 45). This possibility raises the question of how pre-existing IgE to other pathogens would affect the development of immunity to schistosomes. This question is currently under investigation by our group, as efforts in vaccine strategies for schistosomiasis may be affected by pre-existing CD23-bound IgE specificities in schistosome endemic regions. Furthermore, the robust response mediated by CD23-bound polyclonal IgE to dust mite Ag has more global clinical relevance, particularly in the treatment of allergy. Strategies that reduce IgE by altering the isotype via immunotherapy or vaccines may unintentionally contribute to the perpetuation of allergen-specific IgE and allergies because a lower level of Ag-specific IgE appears to increase some B cell responses. A better understanding of the bona fide role(s) of IgE in human immunity should lead to improved treatments for parasitic diseases as well as acute and chronic allergic diseases.

In conclusion, our results present a possible explanation for the association of high levels of parasite-specific IgE with resistance to schistosomiasis and why IgE-mediated immunity requires years of exposure to schistosomes (1). Previous results have demonstrated that IgE production appears to be tightly regulated and may be coupled to cell division (46). In the mouse, B cells require at least three divisions to produce IgG, whereas B cells require five divisions to produce IgE. Although this has not been clarified in the human system, IgE production has been notoriously difficult to induce (47). The propensity to require additional cellular divisions to produce IgE coupled with the indirect or secondary step mediated by $CD23^+$ B cells may explain why immunity to schistosomes develops over several years. Thus, while one effector function of IgE in schistosomiasis may be to work through cells, such as eosinophils, to damage or kill worms, the major role of IgE may be to augment recall responses by naive B cells, which then lead to other effector mechanisms not yet elucidated in the human host.

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Disclosures

The authors have no financial conflicts of interest.

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