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Immuno-evasive tactics by schistosomes identifies an effective allergy preventative

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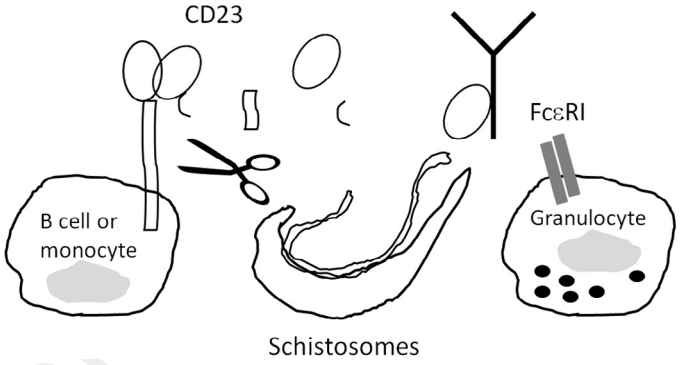
HIGHLIGHTS

- *Schistosoma mansoni* appears to cleave CD23 to release a 15 kDa soluble (s) CD23 fragment (SG-sCD23).
- SG-sCD23 can prevent IgE from binding to its receptors and prevent acute allergy.
- A point mutation in the IgE-binding head, D258E, increased efficacy of SG-sCD23.
- SG-sCD23 may be an effective allergy treatment.
- Immunity to calpain may inhibit CD23 cleavage and improve protection against schistosomiasis.

GRAPHICAL ABSTRACT



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ABSTRACT

Many chronic inflammatory diseases can be improved by helminth infection, but the mechanisms are poorly understood. Allergy and helminthiasis are both associated with Th2-like immune responses; thus, defining how infection with parasites leads to reduced allergy has been particularly challenging. We sought to better understand this conundrum by evaluating host–parasite interactions involved in Th2 immunity in human schistosomiasis. Immune cells were cultured with schistosomes and the effect on CD23, an IgE receptor associated with resistance in schistosomiasis, was evaluated. Cells treated with schistosomes demonstrated reduced surface CD23 levels with a parallel accumulation of soluble (s) CD23 suggesting this IgE receptor is proteolytically cleaved by the parasite. Consistent with this hypothesis, a schistosome-generated (SG)-sCD23 fragment of 15 kDa was identified. SG-sCD23 inhibited IgE from binding to CD23 and FcεRI, but lacked the ability to bind CD21. These results suggested that schistosomes target IgE-mediated immunity in immuno-evasive tactics. Based on its characteristics, we predicted that SG-sCD23 would function as an efficacious allergy preventative. Treatment of human FcεRI-transgenic mice with recombinant (r) SG-sCD23 reduced the ability of human IgE to induce an acute allergic response *in vivo*. In addition, an optimized form of rSG-sCD23 with an introduced point mutation at Asp258 (D258E)

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to stabilize IgE binding had increased efficacy compared to native rSG-sCD23. Schistosome infection may thus inhibit allergic-like protective immune responses by increasing soluble IgE decoy receptors. Allergy treatments based on this naturally occurring phenomenon may be highly effective and have fewer side effects with long-term use.

1. Introduction

Helminth infections are highly prevalent and cause significant morbidity worldwide (Hotez et al., 2006). Paradoxically, parasitic worm infections have been shown to protect against the development of many chronic diseases in animal models, including inflammatory bowel disease, diabetes, and allergy, but the mechanisms are unclear (Elliott and Weinstock, 2012). The effects of helminthiasis on allergy are particularly curious as both diseases are associated with a Th2-polarized immune response (Pulendran and Artis, 2012). Furthermore, the Th2 response is much more intense in helminth disease, though a higher concentration of parasite-specific IgE has not been shown to dilute functional allergen-specific IgE *in vitro* (Mitre et al., 2005). These observations raise the question of how worms might play a role in *reducing* allergic responses *in vivo*. In this vein, it has been postulated that chronic helminth infection may *dampen* global Th2 immune responses through immuno-regulatory processes that allow for parasitism (Broide, 2009). However, this idea is in conflict with multiple published observations that demonstrate people infected with parasitic worms exhibit extremely high levels of IgE and circulating eosinophils – much higher than that reported in allergy, indicating that this issue is highly complex (Dunne et al., 1992a; Ganley-Leal et al., 2006; Glovsky, 2007; Gould and Sutton, 2008; Hamilton et al., 2005; Satti et al., 2004).

IgE is thought to have an important role in protective immunity to parasitic schistosomes in humans, but the functionality is not clear (Dunne et al., 1992b; Jiz et al., 2009). The human immune system has numerous IgE receptor-bearing cells suggesting that IgE has multiple functions in schistosomiasis (Gould et al., 2003). Effector functions of IgE have been demonstrated and include increasing the larvacidal activity of granulocytes (Gounni et al., 1994). We previously reported that an increase in circulating FcεRII/CD23^{+} B cells was associated with the development of resistance in schistosome hyper-exposed populations from Kenya (Mwinzi et al., 2009). Our findings suggested that CD23+ B cells may utilize surface bound IgE to capture and shuttle antigens from the bloodstream to the splenic follicles to augment immune responses (Griffith et al., 2011; Onguru et al., 2011). Thus, IgE likely has diverse and unexpected roles in human immunity that remain to be defined.

CD23 has a broad cellular distribution in humans and is expressed by monocytes, resting eosinophils, and follicular dendritic cells in addition to B cells (Burton and Oettgen, 2011). CD23 is a type II integral membrane protein with a calcium-dependent lectin domain that binds IgE (Hibbert et al., 2005). A leucine zipper in the N-terminal stalk region allows CD23 molecules to form homotrimers, which increase the affinity for IgE to the same level as the high affinity IgE receptor, FcεRI (Hibbert et al., 2005). CD23 also contains a CD21-binding C-terminal tail on the lectin head that amplifies certain functions, particularly inflammatory cytokine production and augmentation of antibody production (Aubry et al., 1992). Cleavage of cell surface CD23 occurs in the N-terminal stalk by ADAM10 and other proteases to generate multiple soluble (s) forms of CD23 (Lemieux et al., 2007). Soluble fragments that are 29-, 33- and 37-kDa retain the ability to homo-trimerize and bind both IgE and CD21 (Bowles et al., 2011). Smaller sCD23 fragments, 17- and 25- kDa, bind IgE and CD21 and are released from the cleavage of larger soluble molecules by several host and microbial proteases. For example, neutrophils secrete an elastase, which cleaves the 37 kDa fragment into the 25 kDa sCD23 fragment, which can be visualized in the serum (Brignone et al., 2001). These smaller fragments lack the stalk region and generally exist as monomers.

The effects of sCD23 on the immune system depend on whether the fragment is an oligomer, large or small fragment, and to which ligand it binds (CD23-bound IgE, B cell receptor (BCR)ε, CD21) (Fremeaux-Bacchi et al., 1998; Kwon et al., 2012; McCloskey et al., 2007). Larger, trimeric fragments have high affinity for BCRε and stimulate IgE secretion by memory ε + B cells (McCloskey et al., 2007). The 25–29 kDa fragments of sCD23 have been shown to promote differentiation of germinal center B cells and secretion of TNF-α through ligation of CD21 (Cooper et al., 2012; Hibbert et al., 2005; Liu et al., 1991). In contrast, the smaller 17 kDa polypeptide may compete with larger fragments to reduce IgE production and has direct anti-inflammatory effects (Bowles et al., 2011; Hibbert et al., 2005).

In this report, we describe a potential mechanism by which *Schistosoma mansoni* targets CD23 and IgE in immuno-evasive tactics. Schistosomes induce the release of a small, 15 kDa isoform of sCD23 that both reduces the cell surface levels of the receptor and results in a soluble decoy receptor for IgE. These results suggest that schistosome infection may diminish protective immunity and by proxy, allergic responses, by regulating effector functions of IgE. We therefore developed the schistosome-generated (SG) sCD23 fragment into a potentially effective allergy treatment to regulate IgE in a physiologically relevant manner.

2. Materials and methods

2.1. Study area and helminth-infected 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. A portion of the study was conducted along the shores of Lake Victoria in western Kenya with adult males (aged 18–38) exposed to infectious cercariae working as car washers ($n = 23$) described in detail elsewhere (Karanja et al., 2002). Upon informed consent, peripheral blood was drawn into heparinized tubes for the assays to be outlined later.

Levels of resistance to reinfection are presented as the Index of Susceptibility/Resistance (IoS/R) and indicate the current history of resistance and exposure for each individual: (number of times reinfected) × 100/(amount of time followed [weeks]) × (mean number of cars washed per week [exposure]) = IoS/R (Ganley-Leal et al., 2006).

S. mansoni is the primary schistosome species to infect humans in the region. Stool samples were examined for *Schistosoma mansoni* eggs and 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 (PZQ); those positive for other helminth ova were treated with 400 mg of albendazole.

2.2. Flow cytometry on whole blood samples

B cells in fresh whole blood were evaluated for levels of surface CD23 by incubating 100 μl of heparinized blood with fluorescently labeled antibodies (anti-CD19; anti-CD23; BD Pharmingen, San Jose,

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