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Changes in IgE- and Antigen-dependent histamine-release in peripheral blood of *Schistosoma mansoni*-infected Ugandan fishermen after treatment with praziquantel

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Abstract

Background: Parasite-specific IgE levels correlate with human resistance to reinfection with *Schistosoma spp*. after chemotherapy. Although the role of eosinophils in schistosomiasis has been the focus of a great deal of important research, the involvement of other Fcε receptor-bearing cells, such as mast cells and basophils, has not been investigated in relation to human immunity to schistosomes. Chemotherapy with praziquantel (PZQ) kills schistosomes living in an *in vivo* blood environment rich in IgE, eosinophils and basophils. This releases parasite Ags that have the potential to cross-link cell-bound IgE. However, systemic hypersensitivity reactions are not induced by treatment. Here, we describe the effects of schistosomiasis, and its treatment, on human basophil function by following changes in total cellular histamine and *in vitro* histamine-release induced by schistosome Ags or anti-IgE, in blood samples from infected Ugandan fishermen, who are continuously exposed to *S. mansoni* infection, before and 1-day and 21-days after PZQ treatment.

Results: There was a significant increase in the total cellular histamine in blood samples at 1-day post-treatment, followed by a very significant further increase by 21-days post-treatment. *In vitro* histamine-release induced by *S. mansoni* egg (SEA) or worm (SWA) Ags or anti-IgE antibody, was significantly reduced 1-day post-treatment. The degree of this reduction correlated with pre-treatment infection intensity. Twenty-1-days post-treatment, SEA-induced histamine-release was

still significantly lower than at pretreatment. Histamine-release was not correlated to plasma concentrations of total or parasite-specific IgE, nor to specific IgG4 plasma concentrations.

Conclusion: The biology of human blood basophils is modulated by *S. mansoni* infection and praziquantel treatment. Infection intensity-dependent suppression of basophil histamine-release, histamine-dependent resistance to infection, and similarities with allergen desensitisation are discussed as possible explanations of these observations.

Background

High levels of circulating IgE are characteristic of both parasitic helminth infections and hypersensitivity conditions such as asthma and allergy. IgE and other Th2 mediated responses have been shown to be important in immunity to helminth infections. In human populations living in schistosomiasis endemic areas, high levels of IL-4, IL-5 [1,2], eosinophilia [3] and parasite-specific IgE are associated with resistance to reinfection after chemotherapy [4-6]. In previous studies in Kenya, levels of IgE specific for the adult *Schistosoma mansoni* worm, when measured after PZQ treatment but before re-infection, negatively correlated with subsequent reinfection intensities [7]. Specific IgE responses against Ags present in the outer tegument of the adult worm were also significantly associated with resistance to reinfection after treatment [8]. Human IgE and eosinophils have been shown to combine in antibody-dependent, cellular cytotoxicity mechanisms (ADCC) to kill early schistosome larvae *in vitro*[9]. However, this mechanism may not be as effective *in vivo* as, on penetration of its vertebrate host, the parasite rapidly disguises its outer tegumental surface by absorbing host Ag [10] and also becomes innately refractory to ADCC killing [11]. The roles of other major Fcε receptor-bearing effector cells such as mast cells and basophils has yet to be defined in human immunity to schistosomiasis. *In vitro* basophil studies have suggested a secretagogue potential of some *S. mansoni* Ag [12,13] or of plasma factors from infected patients [13], but the relationship between *S. mansoni* infection and basophils, and its relationship with human susceptibility to infection/reinfection, is not known. The role of basophils in allergy is an active area of research. Interestingly, it is suggested that allergic diseases are less prevalent in areas that are endemic for helminth infections and, when they are present, the manifestations of these diseases are less severe in helminth-infected individuals [14]. Various immune regulatory processes have been put forward as candidate mechanisms for the control of the potentially adverse effects of IgE responses in connection to both potential hypersensitivity to helminth Ags themselves and allergy in general [15].

Chemotherapy to kill schistosome worms whilst they are living in an intravenous environment that is rich in IgE, eosinophils and basophils, would seem to have the potential to induce a systemic hypersensitivity reaction. Orally administered PZQ, the drug of choice, is rapidly absorbed into the blood, where it can be metabolised within 90 minute[16]. Within one hour of contact with PZQ, the outer tegument of the worm is severely disrupted [17]. This rapid disruption of the worm tegument would lead to the exposure of worm Ags, some known to be recognised by IgE [7], directly to the blood. Despite this, only a very few heavily infected older individuals have transient hypersensitivity responses, usually within a few hours of treatment, such as urticaria and oedema[18]. This suggests that some aspect(s) of infection or reactions between infection and host response to infection, circumvents the most potentially damaging effects of systemic interactions between specific-IgE, mass-released parasite Ags and immune effector cells such as mast cells, eosinophils and basophils.

Here we describe the effects of schistosomiasis and the intravenous killing of the parasite on basophil function by following the changes in total cellular histamine content and *in vitro* basophil histamine-release induced by schistosome Ags or anti-IgE Ab. The studies were carried out using washed blood from infected Ugandan fishermen, before and at 1-day and 21-days after they were treated with PZQ.

Results and discussion

Increases in total cellular histamine content of blood in S. mansoni *infected individuals 1-day and 21-days after treatment*

Washed blood, as defined in the Methods, was used to determine the total cellular histamine content of *S. mansoni*-infected individuals. By using total cellular histamine, rather than basophil number, we were able to compare the amount of histamine released with different stimuli with the amount of cellular histamine available in the blood at each of the times of sampling, irrespective of basophil number and degree of basophil activation. As most platelets are believed to be depleted during the washing steps and as basophils are the major contributor to blood cellular histamine, the most significant measured histamine was of basophil origin. Figure 1 shows that, between pre-treatment and 1-day post-treatment, there was a significant increase in total cellular histamine content of the blood of infected individuals ($P = 0.019$, $n = 25$), and a very significant increase by 21-days post-treat-

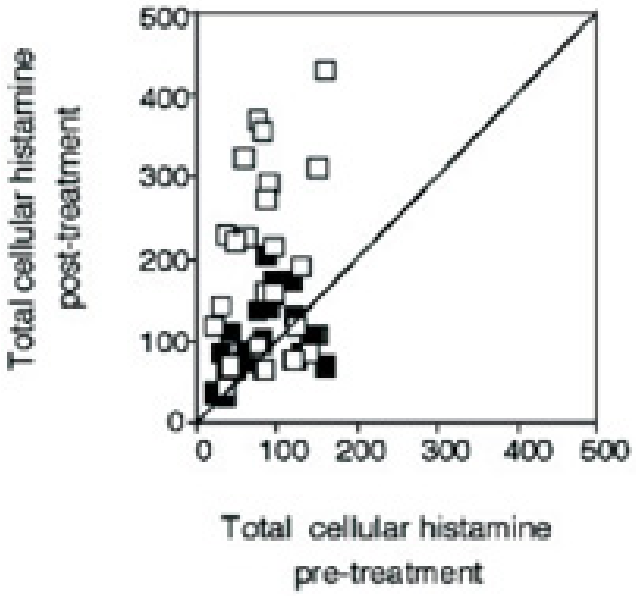

Figure 1

Increases in total cellular histamine content of blood from *S. mansoni*-infected individuals pre-treatment, 1- and 21-days post-treatment. The changes in total cellular histamine content (ng/ml) from washed blood of *S. mansoni*-infected individuals (n = 25) from pre-treatment levels to 1-day (black square) or 21-days (open square) after treatment with praziquantel. Any point that plots on the diagonal line is unchanged from the pre-treatment level. The differences in the levels of total cellular histamine were statistically significant between all time points and increased pre-treatment<1-day post-treatment<21-days post-treatment.

Table 1: Total cellular histamine content in the blood of *S. mansoni*-infected individuals, pre- and post-treatment, and in non infected individuals.

	Median total cellular histamine content (ng/ml)
pre-treatment (N = 32)	81.0 (43.5 – 97.0)
1 day post treatment (N = 29)	108.0 (85.5 – 145.5)
21 days post-treatment (N = 25)	160.5 (83.8 – 278.8)
non-infected (N = 8)	179.0 (163.0 – 248.5)

Median total cellular histamine content, expressed as ng/ml blood, from *S. mansoni*-infected individuals, and from non-infected individuals. Total cellular histamine content was measured in whole washed blood of each *S. mansoni*-infected individuals at pre-treatment, 1-day or 21-days post-treatment, and in each non-infected individuals at the same time as 21-days post-treatment. Numbers in brackets are 25% and 75% percentiles, respectively.

ment (p = 0.001, n = 25). The greatest increase occurring between 1-day and 21-days post-treatment time points (p = 0.005, n = 25). Total cellular histamine content was also measured in the washed whole blood cells from eight individuals who had not been exposed to schistosomiasis at the same time as the 21-days post-treatment samples were processed. Values of median cellular histamine content for the infected study cohort at the three time-points and for the non-exposed group are summarised in Table 1. This shows that the median histamine content in the non-exposed group was close to the increased median histamine content found in the blood from the *S. mansoni*-infected group 21-days post-treatment. The non-exposed donors were not matched for age and sex with the infected cohort (and therefore are not considered as a control group), however, it is possible that the observed augmentation of total histamine after treatment may represent a return to a normal, uninfected, steady state rather than a treatment-related up-regulation. A classical interpretation of these observations would be that infected individuals have lowered basophil counts and after treatment their basophil counts return to levels comparable to those found in non-infected individuals. Alternatively, the low total cellular histamine content could be attributable to lowered histamine content per cell.

Changes in maximal histamine-release from the blood of S. mansoni*-infected individuals 1-day after treatment*

The maximal level of histamine released when washed whole blood cells were cultured in the presence of either anti-IgE Ab, or SEA or SWA schistosome Ag is shown in Table 2. It is expressed either as a percentage of total cellular histamine released, and thereafter called histamine-releasability, or as the absolute amount of released histamine in ng/ml. Histamine-releasability data reflect how much of the total cellular histamine present in a standard volume of blood can be released in response to either anti-IgE Ab or schistosome Ag. The absolute amount of released histamine reflects how much histamine a particular stimulant could release from cells in a standard volume of blood, whether this is a function of basophil numbers and/or their histamine-releasability state. Potential spontaneous release was subtracted from the final assay value during histamine measurement, thus only stimuli effects were measured.

Figure 2 shows that before treatment, the basophils of most infected individuals were able to release histamine via an IgE-dependent pathway and after SEA or SWA stimulation. Basophils from individuals that had not been exposed to schistosomiasis showed no significant release upon stimulation with SEA or SWA used at the same concentrations under the same conditions (data not shown). Circulating specific and non-specific IgE is greatly elevated in chronically infected schistosomiasis patients [19,20]

Table 2: Histamine released from the blood of *S. mansoni*-infected individuals, pre- and post-treatment, after *in vitro* stimulation with anti-IgE or schistosome Ag.

	anti-IgE		SEA		SWA	
	ng/ml	%	ng/ml	%	ng/ml	%
pre-treatment (N = 32)	**24.3** (20.5;38.0)	**35.0** (22.6;51.8)	**32.8** (23.4;44.5)	**49.7** (26.9;61.6)	**31.5** (23.9;44.1)	**51.3** (21.5;66.9)
1-day post-treatment (N = 29)	**16.5** (9.8, 26.0)	**14.5** (7.5;28.5)	**20.0** (9.0;36.0)	**19.1** (8.0;38.1)	**20.0** (9.8;31.0)	**17.1** (8.2;37.8)
21-days post-treatment (N = 25)	**35.5** (22.8;51.0)	**20.5** (15.2;31.3)	**37.5** (29.0;63.3)	**25.7** (15.5;39.9)	**48.0** (37.0;68.3)	**33.2** (21.7;44.6)

Median histamine-release, expressed as absolute amount (ng/ml blood) or as histamine-releasability (% of total cellular histamine content of the blood) from each *S. mansoni*-infected individual, induced by *in vitro* stimulation with either anti-IgE, SEA or SWA, pre-treatment, 1-day or 21-days post-treatment. Numbers in brackets are 25% and 75% percentiles, respectively. Differences in absolute amounts between pre-treatment and either post-treatment time-points are statistically significant ($p < 0.05$). Differences in absolute amounts between SEA-stimulated and anti-IgE-stimulated histamine-release and between SWA-stimulated and anti-IgE-stimulated histamine-release are statistically significant ($p < 0.05$) at all time-points. The difference in absolute amounts between SEA-stimulated and SWA-stimulated histamine-release is statistically significant($p < 0.05$) at 21-days post-treatment. Statistically significant differences in histamine-releasability are mentioned in the text. (Wilcoxon's ranks tests).

and this was verified in the present study. Median total IgE measured in the plasma of *S. mansoni*-infected individuals at pretreatment, 1-day and 21-days post-treatment was 587.5 UI (range 2603), 404.1 UI (range 2276.7) and 1616.5 UI (range 2850.4), respectively. The values are in the same range as previously reported in *S. mansoni*-infected patients [20,21] and are comparable to what has been reported in highly allergic individuals[22]. Noticeably, a rise in total IgE for a few months after the release of antigens in the blood has also been reported in allergic patients after desensitisation, but total IgE decreased in the longer term [23]. Our study did not include time-points later than 21-days post-treatment but an elevation of serum total IgE has been reported 4 months after an anti-schistosomiasis treatment [21]. As might be expected, non-Ag-specific anti-IgE stimulation induces histamine-release from the basophils from the study cohort. As shown in Figure 3, anti-SEA and anti-SWA IgE was detected in the plasma of the study participants at all time points and would, therefore, have been able to occupy basophil FcεR1.

At 1-day post-treatment, most individuals showed decreased histamine-releasability (Figure 2). Histamine-releasability induced by SEA ($p = 0.002$, $n = 21$); SWA ($p = 0.004$, $n = 23$); and anti-IgE ($p = 0.001$, $n = 23$) dropped very significantly, as did the absolute amount of released histamine (Table 2). This reduced ability to release cellular histamine, including after anti-IgE stimulation, suggests a general desensitisation, similar to that reported in the desensitization of individuals to specific allergens by either sub-optimal or super-optimal IgE receptor activation [24-26]. Praziquantel is a short acting drug that quickly causes the release of Ags from damaged worms and eggs. This may result in a rapid, *in vivo* basophil degranulation that desensitises the basophils to further Ag stimulation. Since total cellular histamine is low, the extent of such an early release would be limited, as shown in Table 2 by the absolute amount of released histamine lower at pre-treatment than at 21 days post-treatment, thus preventing the onset of hypersensitivity reactions after treatment. Additionally, any such early *in vivo* release of histamine may down-regulate further release through an autocrine mechanism involving H2 receptors [27].

A alternative explanation of this down-regulated histamine-releasability, in the presence of increased cellular histamine content, may be that after the worms and eggs destruction and subsequent release of parasite Ags by Praziquantel treatment, immature bone marrow basophils were freshly released into the blood. This mechanism has been suggested as the cause of increased basophil counts associated with reduced histamine-release in allergic patients 5 days after treatment with rhG-CSF [28].

Changes in maximal histamine release from the blood of S. mansoni*-infected individuals 21-days after treatment*

At 21-days post-treatment, SEA or anti-IgE stimulated histamine-releasability by the blood of most patients was still significantly lower than it was before treatment (Figure 2; SEA: $p = 0.017$, anti-IgE: $p = 0.059$). Thus, it appeared that the blood basophils were desensitized 1-day post-treatment and that, in relation to anti-IgE and SEA at least, this desensitised state was still evident 21-days after treatment. However, the absolute amount of released histamine was significantly higher at 21-days post-treatment compared with pre-treatment levels with SEA ($p = 0.009$), SWA ($p = 0.000$) or anti-IgE Ab ($p = 0.008$) stimulation (Figure 2 and Table 2). At 21-days post-treatment, histamine-releasability was not higher than pre-treatment % histamine-releasability, suggesting

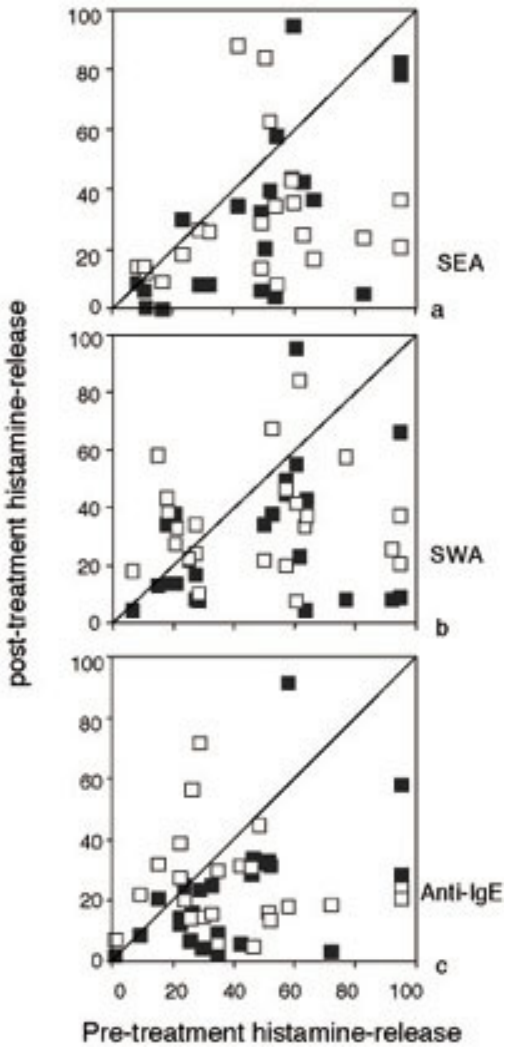

Figure 2

The percentage of histamine released from the blood of *S. mansoni*-infected individuals, pre- and post-treatment, after *in vitro* stimulation with anti-IgE or schistosome Ag. Histamine-releasability (the maximum level of histamine-release, expressed as the % of total cellular histamine content of the blood of each individual), from the washed blood cells of *S. mansoni*-infected individuals after *in vitro* stimulation with either SEA, SWA or anti-IgE. Histamine-releasability *in vitro* for each infected individual at the pre-treatment time point is compared with that 1-day (black square) and 21-days (open square) after treatment after *in vitro* stimulation with SEA (Fig 2a), SWA (Fig 2b) and anti-IgE (Fig 2c). Any point that plots on the diagonal line is unchanged from the pre-treatment level. The differences between the levels of histamine-releasability between pre-treatment and 1-day post-treatment were statistically significant for all stimuli. The differences between the levels of histamine-releasability between pre-treatment and 21-days post-treatment were statistically significant and nearly significant for SEA-stimulated and anti-IgE-stimulated histamine release, respectively.

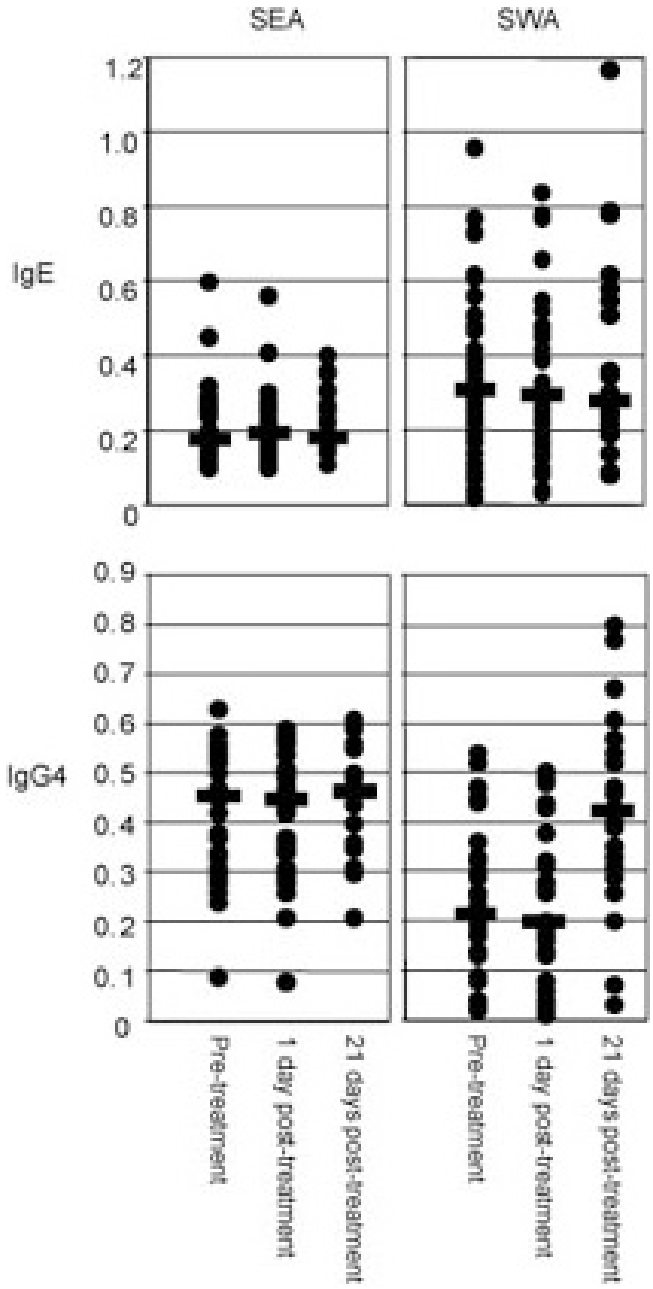

Figure 3

Plasma levels of Ag-specific IgE and IgG4 measured at each time point in the plasma of *S.-mansoni*-infected people. Antibody levels measured by ELISA at pre-treatment (N = 32), 1 day post-treatment (N = 32) and 21 days post-treatment (N = 25) are expressed as OD values. Individual measurements are represented by dots. Medians are represented by horizontal bars.

that the observed higher absolute amount of released histamine was the consequence of the up-regulation of total cellular histamine content.

At 21-days post-treatment there would have been few, if any, parasites remaining in the blood, and little or no parasite Ag in the circulation [29]. For example, at this time-point CAA, a diagnostic schistosome gut-associated circulating Ag, could not be detected in the plasma of 98% of the treated individuals (data not shown). Thus, basophils present at 21-days post-treatment would not have been subject to *in vivo* Ag challenge prior to *in vitro* culture. In these circumstances the basophils would have reacted normally to *in vitro* Ag stimulation via surface receptor bound Abs, including IgE. However, the continued reduction in histamine-releasability 21-days post-treatment, compared with the infected pre-treatment state, suggests the presence of a, yet to be identified, infection-associated priming factor that is removed or becomes ineffective after treatment.

We examined the possibility that, alternatively, an Ag-specific desensitisation, comparable to the immuno-therapeutic desensitisation of allergic patients, could have occurred. A role for IL-10 and TNFα of T-cell origin has been postulated in basophil desensitisation after wasp venom immunotherapy [30], and these cytokines were detectable in the plasma at all time-points. We found however no correlation between the plasma concentration of these cytokines and histamine-releasability after treatment in this study cohort (data not shown). The mechanism down-regulating histamine-releasability at 21 days post-treatment is therefore likely to be different from the one involved in therapeutic immuno-desensitisation.

Comparison of SEA-, SWA- and anti-IgE-induced histamine-releasability

We assumed that anti-IgE would induce the highest possible IgE-dependent histamine-releasability and compared it to that of SEA and SWA, to assess the secretagogue potential of the parasite Ag. As shown in Figure 2, both SWA and SEA induced significantly higher histamine-releasability than anti-IgE Ab at all study time-points: SEA Vs anti-IgE pre-treatment p = 0.002 (n = 32); 1-day post-treatment p = 0.002 (n = 29), 21-days post-treatment p = 0.004 (n-25); SWA Vs anti-IgE pre-treatment p = 0.004 (n = 32), 1-day post-treatment p = 0.002 (n = 29), 21-days post-treatment p = 0.000 (n = 25). There was no statistically significant difference between histamine-releasability induced by SEA and that induced by SWA at pre-treatment and at 1 day post-treatment, but at 21 days post-treatment, SWA-induced histamine-releasability was significantly higher than SEA-induced histamine-releasability (p = 0.005, N = 25). Parasite factors, such as a *S. mansoni* analogue to human translationally controlled tumour protein (TCTP), may induce an additional IgE-independent histamine release, or enhanced non Ag-specific, IgE-dependent release [13,31]. These non-classical pathways could therefore be involved in the additional histamine-release. However we didn't detect histamine-release from basophils from non-infected individuals after SEA or SWA stimulation under the same conditions. This enhanced histamine-releasability could then appear to be specifically induced by the experience of infection. We however have to consider the fact that our methodology may have been less sensitive than those used in the description of these non-classical pathways and that low levels of histamine-release in non-infected individuals may not have been detected. Thus, it is possible that parasite factors may have directly induced additional or enhanced histamine-releasability.

Post-treatment changes in **in vitro** *basophil sensitivity to anti-IgE and parasite Ags*

The sensitivity of blood cells to parasite Ag or anti-IgE stimulation was assessed by determining the lowest of the 9 concentrations of Ag or of the 3 concentrations of anti-IgE used in the assay, that was capable of triggering a significant histamine-release. The lower the concentration required, the higher the blood cell sensitivity. At 1-day post-treatment, the sensitivity of blood cells from most individuals was either unchanged (anti-IgE:15 out of 32, SEA:17 out of 32, SWA:12 out of 32) or had decreased (anti-IgE:13 out of 32, SEA:13 out of 32, SWA:13 out of 32), compared with the number of individuals with increased sensitivity for each stimuli (SEA p = 0.006, SWA p = 0.030 or anti-IgE Ab p = 0.026). This reduced sensitivity was parallel to the decline in histamine-release at 1-day post-treatment shown in Figure 2. This general decline in basophil sensitivity during the first 24-hours after treatment contrasted with an increase in sensitivity from 1-day to 21-days post-treatment. During this period, the sensitivity of blood cells from most individuals increased (anti-IgE:9 out of 25, SEA:12 out of 25, SWA:13 out of 25) or was unchanged (anti-IgE:14 out of 25, SEA:9 out of 25, SWA:8 out of 25) for SEA, SWA or anti-IgE Ab stimulation (p= 0.021, p = 0.010 and p = 0.024, respectively), mirroring the re-establishment of histamine-release at 21-days post-treatment shown above. When the changes in sensitivity between consecutive time-points were compared, significant or highly significant negative associations were found between the changes from pre-treatment to 1-day post-treatment and the changes from 1-day to 21-days post-treatment (r = -0.636, p = 0.001 for anti-IgE; r = -0.568, p = 0.003 for SEA; r = -0.465, p = 0.019 for SWA; n = 25, for all conditions). Thus, the individuals whose basophil sensitivity decreased from pre-treatment to 1-day post-treatment tended to be the same individuals whose basophil sensitivity had increased by 21-days post-treatment.

Whereas histamine-releasability shows the potential strength of histamine-release, sensitivity reflects the Ag concentration required to induce degranulation. In a mouse experimental model, sensitivity, contrarily to histamine-releasability, was shown to be correlated to affinity of the IgE for the Ag [32]. This reduced sensitivity to Ag or anti-IgE Ab could reflect qualitative rather than quantitative changes in plasma antibodies. The parallel changes in histamine-releasability and in basophil sensitivity show that reduced basophil sensitivity could be partly responsible for the post-treatment reduction in histamine-releasability in blood culture after treatment.

The lack of relationship between anti-IgE- and Ag-induced histamine-releasability, and plasma total IgE, specific IgE and specific IgG4

Plasma levels of total IgE, anti-SEA and anti-SWA IgE and IgG4 were measured at the 3 study time points. Histamine-releasability was not associated with level of total IgE at any time-point. Indeed, despite a statistically significant increase in total IgE between pre-treatment and 21-days post-treatment ($p = 0.024$, $N= 24$), anti-IgE stimulated histamine-releasability was lower in the blood of most patients at 21-days post-treatment. Histamine-releasability was associated with parasite-specific plasma IgE levels only for SEA-induced histamine-release with anti-SEA IgE at 1-day post-treatment ($r = 0.513$, $p = 0.012$, $n = 23$), when histamine-releasability was at its lowest. There was no statistically significant change in plasma anti-SEA IgE between pre-treatment and 1-day post-treatment, as shown in Figure 3, while there was a very significant decrease in SEA-induced histamine-releasability. No significant correlation between IgG4 levels and SEA or SWA Ag-stimulated histamine-releasability was found. Thus it would appear that post-treatment changes in the level of histamine-releasability *in vitro* were regulated either at the cellular level or by serum factors affecting the *in vivo* sensitisation, priming or neutralisation of the basophils or of other surface-bound factors, other than ELISA-detectable parasite-specific IgE or IgG4. A similar lack of relationship between plasma Ab and histamine-release has been reported from studies of cord-blood basophils passively-sensitized with plasma from *S. mansoni* infected adults [18] and RBL-2H3 cells transfected with FcεRI sensitized with plasma from allergic patients [33].

The influence of pre-treatment infection intensity on post-treatment anti-IgE- and Ag-induced histamine releasability

The intensity of *S. mansoni* infection pre-treatment, expressed in eggs per gram of faeces, was compared with the changes in histamine-releasability pre- and post-treatment. Figure 4 shows that at 1-day post-treatment, the time when histamine-releasability was lowest, the pre-treatment intensity of infection correlated negatively with SEA-, SWA- and anti-IgE-stimulated % histamine-release. Thus, the greater the intensity of *S. mansoni*-infection pre-treatment, the lower the releasability of blood cell histamine at 1-day post-treatment. This clearly demonstrates that the decline in histamine-releasability seen after treatment was dependent on the *S. mansoni*-infection rather than on the direct action of the drug itself. As the degree of 1-day post-treatment suppression of histamine-releasability was dependent on the intensity of infection, but is triggered by treatment, this suggests that suppression of % histamine-release is related to the amount of Ag that is released *in vivo* by the drug-induced disruption of different numbers of infecting parasites. This desensitized state persists until 21-days post-treatment in relation to SEA (Figure 2). Interestingly, a negative association between level of Ag exposure and subsequent reactivity has been reported in bee-keepers allergic to bee venom, in whom the number of stings was negatively correlated to skin sensitivity and who showed less side reaction to venom desensitisation than other bee venom allergic patients who have been less exposed to bee stings before treatment [34].

Although the relationship between infection and low post-treatment histamine-releasability could result from an infection intensity-dependent down-regulation mechanism there is a particularly interesting alternative explanation. It has been suggested from studies in the murine model of schistosomiasis that parasite induced histamine-release may regulate the intensity of schistosome infection by triggering inflammation reactions that prevent super-infection [35]. In relation to the present study, it is possible that a high histamine-releasability response to parasite Ag might be protective against infection/re-infection. If this were true, the association between low infection intensity and high histamine-releasability would result in a restriction of parasite numbers by an IgE-dependent histamine-release, not intense infection suppressing histamine-release. With regard to this hypothesis, it would be interesting to test if the magnitude of *in vitro* histamine-releasability in response to treatment was predictive of the subsequent resistance or susceptibility of individuals to re-infection with *S. mansoni*.

Conclusions

The present work describes changes in basophils biology and the modulation of basophil function, induced by the treatment of *S. mansoni* infection. Some of these changes may be a return to steady non-infected state. *S. mansoni* treatment-induced human basophil immune modulation is associated with pre-treatment infection intensity. The mechanisms involved in post-treatment basophil desensitisation at 1-day post-treatment may have similarities to those that induce desensitisation to allergens with immunotherapy. Analysis of histamine-release from naïve

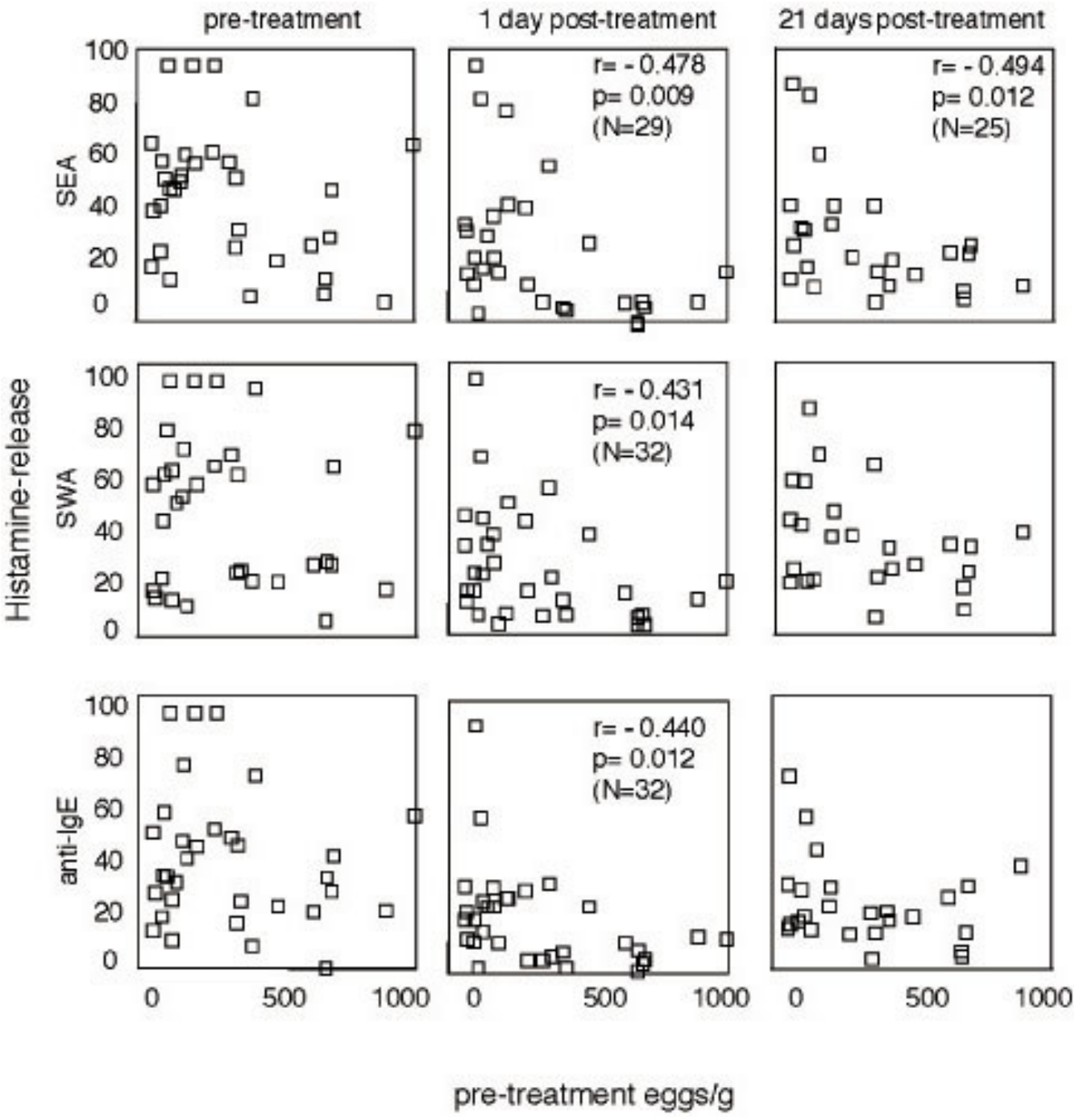

Figure 4

The relationship between *S. mansoni* pre-treatment infection intensity and the histamine-releasability from the blood of infected individuals, pre- and post-treatment, after stimulation with anti-IgE or schistosome Ag. Histamine-relasability (the maximum level of histamine-release, expressed as the % of total cellular histamine content of the blood of each individual), from the washed blood cells of *S. mansoni*-infected individuals after *in vitro* stimulation with either SEA (n = 29), SWA (n = 32) or anti-IgE (n = 32), compared with the pre-treatment levels of *S. mansoni*-infection (eggs per gramme of stool, epg). Statistically significant correlations between epg and histamine-releasability are shown (Spearman's rank correlations).

basophils passively sensitised with the plasma from the same individuals should provide data on the plasma factors involved in the regulation of the basophil response. It is hoped that such studies, coupled to the monitoring of reinfection after treatment, will provide detailed information about the role of basophils in IgE mediated immunological protection against reinfection after treatment and mechanisms by which the potential adverse effects of IgE-mediated immune effector mechanisms are down-regulated.

Methods

Study cohort selection

A cohort of forty individuals was selected in the fishing village of Bugoigo, on the Eastern Shore of Lake Albert, Masindi District, Uganda. Vegetation in the shallow parts of the lake was an ideal habitat for snails of the genus *Biomphalaria*, particularly *B. sudanica* and *B. stanleyi*, the two intermediate host species of *S. mansoni* in this area. Adult men were, through their occupation, the part of the population the most exposed to infection. The select study group comprised males aged between 18 and 45 years old (mean age 34.5 years), having resided in Bugoigo for at least three years and consenting to participate. The selection was made after parasitological examination of three stool samples per individual, with two Kato thick smears per sample using 50 mg of faeces per slide [36]. All the selected individuals had detectable *S. mansoni* eggs, but those with over 70 eggs per slide were excluded to reduce the number of outliers. The mean pre-treatment egg count for the selected cohort was 282 (range = 983) eggs per gram of faeces.

Non-infected group

A group of 8 healthy volunteers, members of the sample collection team, was bled at the same time as the third bleed of the study group. This control group comprised 5 Africans (2 females and 3 males) and 3 Europeans (1 female and 2 males).

Blood collection and PZQ treatment

Informed consent was obtained from all those who participated in this study, in line with the National guidelines of the Ugandan Ministry of Health, whose ethical review committees approved all the protocols used, and the US Department of Health and Human Services. Thirty ml-blood samples taken by venipuncture in heparinised syringes (10 U/ml heparin Na salt, Sigma, UK) were collected from the 33 participants before they received a single dose of 40 mg/kg body weight of PZQ. The participants were asked to come back to donate blood samples a second time (1-day post-treatment) exactly 24 hours after having been treated. A third sample was taken 21-days post-treatment from the 28 participants who came back to donate blood. Only 3 ml of the collected blood was used for histamine assays. The rest of the blood was used for other assays that were parts of the same main study. At the completion of this study the whole Bugoigo community was treated with PZQ.

Antigen coating of microtitre plates used for histamine release

Glass fibre microtitre plates from Ref Lab, Denmark were coated with 25 μl of the following substances:

Histamine (50 ng/ml) added to four wells; α-IgE from DAKO, Denmark, used in three concentrations (1:200 (7 μg/ml), 1:1000, 1:2000), each concentration was added in two wells; SEA and SWA, used in nine concentrations with the dilution factor 3.5 and each concentration was added into two wells. The highest concentration of SEA and SWA was 50 μg/ml. All dilutions were made in distilled water containing 5% glycerol. Subsequently the allergen coated microtitre plates were dried for 6 hours at 37°C, and thereafter the plates were packed and sealed until use in Bugoigo. Preliminary experiments using blood from 3 *S. mansoni*-infected patients showed that histamine-release performed in Ag-coated plates (stored for 3 months at 20°C) varied less than 5% from histamine release induced by freshly prepared Ag. Control experiments using blood from 5 non-infected individuals showed no histamine-release to SEA and SWA (data not shown).

Histamine release assay

Histamine-release was performed using the glass fibre assay described elsewhere [37], which allows the capture of released histamine, irrespective of basophil source, after stimulation. The assay was however simplified for direct release of histamine by peripheral blood without passive sensitisation. In brief 3 ml of each blood sample was washed twice in PIPES buffer (Ref Lab, Denmark) at room temperature to eliminate platelets and plasma factors not already cell-bound. The samples were reconstituted to the initial volume with PIPES buffer and thereafter referred to as "washed blood", with the addition of IL-3 (5 ng/ml washed blood). Substances in the Ag-coated plates were dissolved by adding 25 μl PIPES buffer to each well prior to addition of 25 μl washed blood per well. Histamine was released and subsequently bound to glass fibres in the microtitre plate. Thereafter the plate was washed with distilled water. The plates were stored in the dark at room temperature during the 21-days study period in Bugoigo and subsequently shipped to the Ref Lab for histamine analysis.

Histamine analysis

Histamine was measured by spectrofluorometry as described elsewhere[37]. Spontaneous release is automat-

ically subtracted with plate background from measured data by the analysis software.

Antigen preparation

S. mansoni worms and eggs for Ag preparation were obtained from mice infected with 250 *S. mansoni* cercariae. Forty-two days after infection mice were injected s.c. with hydrocortisone acetate (2.5 mg/mouse), to reduce granuloma formation around the eggs in the livers. 49-days post-infection, the portal blood of infected mice were perfused to recover the worms, as described previously [38]. Recovered worms were frozen in droplets in liquid nitrogen and stored in liquid nitrogen until use. PBS soluble extract of adult worm Ag (SWA) was prepared by recovering the lipid-free supernatant fraction of the finely crushed frozen worms after centrifugation for one hour at 10,000 g at 4°C as described previously [39]. Soluble egg Ag (SEA) was the supernatant fluid obtained after *S. mansoni* eggs were homogenised in PBS as described previously [40] except that the homogenisation step was done by sonication. Both SWA and SEA were filtered through sterile 0.22 μm pore-size filters and endotoxin content was measured using the Limulus Amebocyte Lysate Kit (QCL-1000, Biowhittaker Inc, Walkersville, MD, USA). The levels of endotoxin in the native Ag used in these studies were as follows 10.7 ng endotoxin /mg SEA and 25 ng endotoxin /mg SWA. Whole blood cultures in the presence of these levels of endotoxin induced neither cell activation, nor cytokine release (data not shown).

Specific antibody determination

A semi-quantitative measurement of SEA- and SWA- specific IgE and IgG4 isotypes was carried out on the plasma of the study individuals by ELISA as described previously [7]. Briefly, SEA-specific and SWA-specific antibodies were captured in Immulon-2 flat bottom plates (Dynex) coated with SEA and SWA, respectively. Detection of IgG4 and IgE was done by use of mouse anti-human IgG4 clone RJ4 (Skybio Ltd, UK) and mouse anti-human IgE clone HP 6029 (CN Biosciences, UK), respectivelyThe assays were developed using biotinylated sheep anti-mouse Ig followed by streptavidin-biotin-HRP complex (both reagents from A P Biotech) and by incubation with O-phenylenediamine (OPD). Optical densities (OD) were read at 490 nm. Samples from the 3 time-points were processed at the simultaneously to allow for comparison of OD.

Total IgE determination

Total IgE were quantified in the plasma of the study individuals by in-house sandwich ELISA. Both antibodies were obtained from Pharmingen. Mouse anti-human IgE clone G7-18 was used for capture in Immulon 2 flat bottom plates (Dynex). Detection was made with biotinylated mouse anti-human IgE clone G7-26, with poly-HRP (CLB) amplification and incubation with OPD. Sample plasmas and human IgE myeloma standards (Calbiochem) were diluted in 10% animal sera (an equivolume mix of complement-inactivated sera from mouse, rat, goat and fetal bovine) to block heterophilic antibodies.

CAA measurement

CAA was quantified in the plasma of the study individuals by ELISA, after modification of the method described by Deelder and colleagues [41]. The modification consisted in using different anti-CAA antibodies as those reported in the original article. Both capture and detection antibodies were provided by Dr G.J. van Dam and Prof. A.M. Deelder, University of Leiden, the Netherlands. In summary, trichloroacetic acid-treated samples or standards (TCA soluble SWA) [42] were incubated in Immulon 2 HB plates (Dynex) coated with mouse anti-CAA antibody clone 147-39A and captured CAA was detected with biotinylated mouse anti-CAA clone 147-3G4. Incubations with alkaline phospatase-conjugated streptavidin (Dako) and the chromogenic substrate pNPP (Sigma) allowed reading at 405 nm.

Data treatment

For each Ag or anti-IgE Ab stimulus, the amount of histamine released at the stimulus concentration that induced the highest release was defined as the maximal histamine-release. The sensitivity was defined as the lowest stimulus concentration required to induce a histamine-release equal or greater to 10 ng/ml. This threshold represents 2 standard deviations from the average histamine quantification using a blank sample. Statistical analysis with non parametric tests was done with SPSS 10 for Macintosh: comparisons of related variables were made using Wilconxon's ranks test, independent variables were compared using Mann-Whitney U test and correlations between variables were made using Spearman's test.

Abbreviations

Ab: antibody; Ag: antigen; CAA: circulating anodic antigen; PZQ: praziquantel; SEA: *S. mansoni* Soluble Egg Antigen; SWA: *S. mansoni* Soluble Worm Antigen.

Authors' contributions

MZS adapted and performed the histamine release assay in the field conditions. PC analysed the data and wrote the manuscript. PSS set-up the plate histamine release assay, performed the histamine measurement from the plates and blood samples and wrote the manuscript, SJ set up and organised the field study of which this work was a part, FM.J prepared the antigens, processed the samples and performed the plasma measurement of total and specific antibodies, as well as CAA assays, CF prepared the antigens and processed samples in the field, KH, CR, CHK, FK, JKM and GK processed samples in the field, BJV, JHO, and NBK set up the larger study of which this work

was part, DWD set up the larger study of which this work was part and wrote the manuscript.

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