



Original article

Allergen-specific IgE and IgG4 are markers of resistance
and susceptibility in a human intestinal nematode infectionJoseph D. Turner ^a, Helen Faulkner ^a, Joseph Kamgno ^b, Malcolm W. Kennedy ^c, Jerzy Behnke ^a,
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Abstract

IgG4 has been proposed to act as a 'blocking antibody' due to its ability to compete for the same epitopes as IgE thus preventing IgE-dependent allergic responses. IgG4 and IgE are both elevated in helminth infections and strong anti-parasite IgE responses are associated with resistance to infection. We wished to determine the relationship between anti-parasite IgG4 and IgE and *Ascaris lumbricoides* infection status. We examined anti-parasite responses, including antibody levels to recombinant *Ascaris* allergen-1A (rABA-1A), a target of serum IgE in endemic populations. Worm burden was indirectly estimated by measuring parasite egg output in a cross-sectional human population ($N = 105$). Levels of anti-parasite IgG4 and IgE in patients' plasma were quantified by immunoassay. Global anti-parasite antibody responses did not bear any significant relationships with intensity of *Ascaris* infection. Individuals who had detectable levels of IgE but not IgG4 to rABA-1A (11%) had lower average levels of infection compared with individuals who produced anti-rABA-1A IgG4 (40%) and sero-negative individuals (49%) ($P = 0.008$). The ratio of IgG4/IgE in rABA-1A responders positively correlated with intensity of infection ($P < 0.025$). IgG4 levels positively correlated with infection level in younger children (age 4–11) where average levels of infection were increasing ($P = 0.038$), whereas allergen specific IgE emerged as a correlate of immunity in older children and adults (age 12–36) where infection levels were decreasing ($P = 0.048$). Therefore, in a gastrointestinal helminth infection, differential regulation of anti-allergen antibody isotypes relate to infection level. Our results are consistent with the concept that IgG4 antibody can block IgE-mediated immunity and therefore allergic processes in humans.

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1. Introduction

Th2 responses, typified by the production of IgE, eosinophilia and mastocytosis, are the hallmarks of helminth infection, yet are also mediators of allergic disease [1]. It is thought that this arm of the immune response evolved in mammals to control helminth infections [2]. In murine models of infec-

tion, resistance to intestinal nematodes are dependent on Th2 responses [3]. By examining cytokine secretions in peripheral blood cells derived from gut helminth endemic communities, we and others have identified a link between strong Th2 activity and reduction in parasite burden [4] and resistance to re-infection following chemotherapy [5,6].

Both IgE and IgG4 are positively regulated by the Th2 cytokines, interleukin (IL)-4 and IL-13 [7–9]. However, whilst parasite-specific IgE, measured in patients' sera, has consistently been associated with natural resistance to infection [10,11] or re-infection [12–14] in a number of different human helminthiases, the role of elevated IgG4 in response to infection is less well defined. Evidence from lymphatic filariasis

Abbreviations: ABA, *Ascaris* body allergen; ABF, *Ascaris* body fluid; ANOVA, analysis of variance; EPG, eggs per gram; IL, interleukin; SIT, specific immuno-therapy; Th, T-helper; WHO, World Health Organisation.

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patients indicates that expression of this isotype is related to the asymptomatic state and that IgG4 can compete with IgE for the same epitopes within parasite molecules [15,16].

We therefore explored the relationships between parasite-specific IgE and IgG4 in a cross-sectional Cameroonian population where the human roundworm, *Ascaris lumbricoides*, is hyperendemic (as defined by the WHO based on the prevalence and quantity of parasite eggs passed in faeces of the sampled community [17]). We have previously reported that in this population, Th2 activity is related to a reduction in *Ascaris* infection levels [4]. We used an adult worm extract to assess global anti-parasite IgG4 and IgE antibody levels. In addition we examined responses to the recombinant form of *Ascaris* allergen-1A (rABA-1A), a member of the nematode polyprotein antigens/allergens [18] that appears to be restricted to nematodes. We selected to study IgG4 and IgE responses to ABA-1A because a high IgE response to this allergen is associated with resistance to *Ascaris* infection [11]. We report here that whilst antibody responses to whole, unfractionated parasite antigen do not relate to natural immunity in humans, the use of rABA-1A uncovers an underlying disassociation between IgE and IgG4 and natural resistance to infection.

2. Methods

2.1. Study population and clinical evaluation

The people of Ayéné in the central province of Cameroon kindly agreed to participate in the study. Participants were selected on the basis of (a) being resident in the area their entire lives and (b) not having received anthelmintic treatment within the previous year. The study group comprised 105 individuals aged 4–36 years. Stool specimens were collected on 2 consecutive days and samples from each were analysed using the Kato Katz Thick Smear Technique. The arithmetic mean number of helminth eggs per gram (EPG) faeces was determined. The percentage prevalence of *A. lumbricoides* was 70.5% and the mean intensity was 17,343 (range 0–99,264). *Trichuris trichiura* was also abundant in the study group with a percentage prevalence of 97% and a mean intensity of 7939 (range 0–110,700). No evidence of schistosomiasis, onchocerciasis or hookworm infection was found within the study group. All participants were subsequently offered mebendazole treatment. Informed consent was gained from the patients (or their parents/guardians) and the guidelines for human experimentation from the Ministry of Health, Cameroon, were followed in the conduct of clinical research. All studies received clearance from a Cameroonian Ethics committee.

2.2. Blood collection

Ten millilitres of venous blood (5 ml from small children) was drawn from each individual using vacutainers (BD Ltd.,

UK). The plasma portion was removed and aliquots stored and transported to the UK at -80°C . Each aliquot was subsequently thawed once to make glycerol stocks, stored at -20°C and used within 2 months.

2.3. Antigens

Adult *A. lumbricoides* were obtained by Oxantel (Pfizer, Hong Kong) mediated expulsion from infected individuals in Cameroon. Paralysed, intact worms appear in faeces from 24 h following treatment. The worms were washed thoroughly in distilled water, sexed and frozen before transport to the UK. A detergent extract was made, consisting of female worms only, as described previously [19]. The antigen preparation (AlAg) was sterile filtered, stored at -80°C and aliquots used only once. DNA encoding unit A of *Ascaris* ABA-1 Allergen was amplified from genomic DNA of adult parasites, expressed in *Escherichia coli* and affinity purified as described previously [11]. The antigen was stored at -80°C and aliquots used only once.

2.4. *Ascaris*-specific antibody ELISAs

The IgG subclass and IgE ELISA conditions were as described elsewhere [20] with the following modifications due to checkerboard optimisation using pooled European sera as a negative control. AlAg was used as the target antigen at an optimum concentration of 3 $\mu\text{g/ml}$ for IgG4 and 10 $\mu\text{g/ml}$ for IgE assays. ABA-1 was coated at 3 $\mu\text{g/ml}$ for IgG4 and 12 $\mu\text{g/ml}$ for IgE. Plasma samples were diluted 1:100 for AlAg-specific IgG4, 1:20 for AlAg-specific IgE, 1:50 for ABA-1-specific IgG4 and 1:5 for ABA-1-specific IgE. Human subclass specific mouse monoclonal antibodies were added at the following dilutions: anti-IgG4 (HP6009), 1:2000 (Skybio Ltd., UK) and anti-IgE (Calbiochem, USA), 1:4000. Horseradish peroxidase-conjugated rabbit anti-mouse antibody (Dako Ltd., UK) was diluted 1:1000. Antibody binding was detected by utilising *o*-phenylenediamine as chromogen (Sigma) and plates read, after 30 min, at 450 nm on an Anthos II ELISA reader operated by Stingray software (Wolf Lab. Ltd., UK). For all assays, the levels of antibody present in a given sample are expressed as arbitrary units according to the standard curve of pooled positives run on each plate and its corresponding dilution. The sensitivity of the assays was determined by taking the mean + 3 S.D. of eight control wells and the European cut-off value was determined by taking the mean + 3 S.D. of 10 European control subjects. Sensitivity and European control cut-off values were subtracted from each antibody measurement.

2.5. Statistical analysis

The analysis of parasitological data are described elsewhere [4]. The frequency distributions of all immunological measurements were scrutinised by the Kolmogorov–Smirnov test for normality. Because all parameters demon-

strated negative binomial distribution patterns after $\log_{10}$, natural log and square root – log transformations, analyses were undertaken using non-parametric tests or models with negative binomial error structures on untransformed data. The effects of host age and sex on anti-AIAG and anti-rABA-1A antibodies were initially compared between four age classes: age class 1: 4–7 years olds, age class 2: 8–11 years olds, age class 3: 12–14 years olds and age class 4: 15–36 years olds. Subsequently, the relationship between intensity of infection and antibody responses was examined in two age classes: age class 1, 4–11 years olds ($N = 46$) and age class 2, 12–36 years olds ($N = 59$). Median levels of infection were compared between those individuals who were AIAG or rABA-1A-specific IgG4/IgE antibody negative, AIAG or rABA-1A-specific IgG4 antibody positive, rABA-1A-specific IgE antibody positive and AIAG or rABA-1A-specific IgG4/IgE antibody positive using non-parametric ANOVA tests (Kruskal Wallis test). The effects of host age and sex on the ratio of AIAG IgG4/IgE or rABA-1A IgG4/IgE was examined using two-way ANOVA with age (four levels) and sex (two levels) fitted as factors in models with negative binomial error structures (General Liner Interactive Modelling, GLIM 4, Royal Statistical Society). Subsequently, IgG4/IgE ratios adjusted for the effects of host age or sex were fitted as covariates in separate GLIM models with negative binomial errors incorporating age (two levels) and sex (two levels) and with residuals of ANOVA of sex and age on EPG as the dependent variable. The relationships between each component of the ratio with intensity of infection in each age class were scrutinised post-hoc using Spearman's rank correlation tests.

3. Results

3.1. Parasitological analyses

The intensity of *Ascaris* infection, as defined by faecal egg output, within the entire study group (including uninfected individuals) was significantly related to the age of the patient ($\chi^2 = 19.878$, $P < 0.001$). Infection levels peaked in 8–11 years olds (Fig. 1A) and 12–36 years olds had significantly lower levels of *Ascaris* infection than 4–11 years olds (Fig. 1B). There were no effects of sex on the variation of faecal egg counts, either directly ($\chi^2 < 1$, $P > 0.05$) or as an interaction with age ($\chi^2 = 4.221$, $P > 0.05$).

3.2. *Ascaris*-specific serum IgG4 and IgE antibody levels

There was a marked heterogeneity in the antibody response toward both *Ascaris* antigens. Of the 105 individuals tested, 79% and 51% contained measurable levels of anti-AIAG or anti-rABA-1A specific antibody, respectively. All of the AIAG responders contained measurable levels of IgG4 whilst only 30% of the group also displayed measurable levels of AIAG-specific IgE. Of the individuals that recognised the recombinant allergen, 22.1% of the study group possessed both

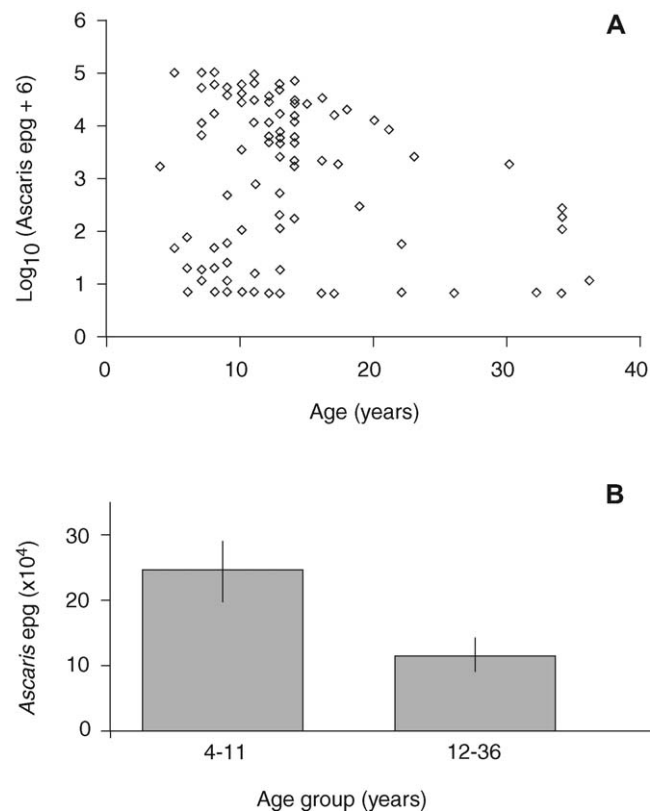


Fig. 1. **A:** The relationship between intensity of *Ascaris* infection and age. Patient age was determined to be significantly related to *Ascaris* faecal egg output (EPG) when analysed as a continuous variable in a two way ANOVA with age (four levels: 1 = 4–7 years olds; 2 = 8–11 years olds, 3 = 12–14 years olds and 4 = 15+ years olds) and sex (two levels) were added as factors [4]. In this model, *Ascaris* EPG initially rose from age class 1 to 2, peaked at age class 2 and was significantly reduced by age class 4. **B:** Mean levels of *Ascaris* infection in 4–11 and 12–36 years olds. For the purposes of antibody analysis, the study group was split into two age classes (1 = 4–11 years olds and 2 = 12–36 years olds) to broadly reflect the changes in EPG with age. Bars represent 1 S.E.M.

IgG4 and IgE, whilst another 18.3% and 10.6% of the study group displayed only sero-positive levels of anti-rABA-1 IgG4 or IgE, respectively.

3.3. Relationships between *Ascaris*-specific IgG4 or IgE and intensity of infection

The median levels of *Ascaris* infection (as defined by the number of eggs detected in 1 g of faeces, adjusted for age) were compared between the proportions of the study group who either did not possess detectable levels of *Ascaris* antibody or contained levels of IgG4, IgE or both isotypes. Whereas there were no significant differences between AIAG responder/non-responder groups ($P = 0.357$, data not shown), the level of *Ascaris* infection was significantly different between the individuals who were rABA-1A IgG4/IgE antibody negative, and those that contained detectable levels of IgE, IgE + IgG4 or IgG4 specific for the recombinant protein ($P = 0.008$, Fig. 2). The individuals who exhibited detectable levels of anti-rABA-1A IgE but not IgG4, had the lowest average infection levels, whereas the individuals who dis-

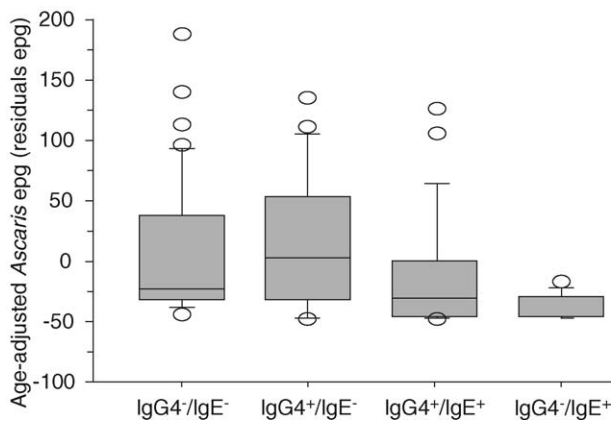


Fig. 2. The variance in intensity of *A. lumbricoides* infection between rABA-1A sero-negative (-), rABA-1A IgG4 sero-positive (IgG4+), rABA-1A IgG4 and IgE sero-positive (IgG4 IgE++) and rABA-1A IgE sero-positive (IgE+) individuals. Intensity of infection (y axis) is adjusted for the effect of age (residuals of ANOVA EPG $\times$ age) and negative values are transformed by addition of 48 to all values. Box plots illustrate 25th, median and 75th percentiles. Bars indicate 5th and 95th percentiles. Outliers are plotted as individual points.

played detectable levels of IgG4 but not IgE to the allergen, or did not respond by producing either anti-ABA-1 isotype, had higher average levels of infection. Interestingly, the IgE/IgG4 double positive group had a median level of *Ascaris* infection intermediate between those individuals producing exclusively IgE or IgG4.

These differences were explored in more detail by calculating ratios of AIAg or rABA-1A IgG4/IgE in each responder and correlating the ratio of IgG4/IgE with intensity of *Ascaris* infection for the whole group. AIAg and rABA-1A IgG4/IgE ratios were first analysed for relationships with patient age and sex (Table 1). Because these potentially confounding variables significantly influenced ratios, residual data derived from these models were employed in subsequent analyses. Whereas no significant correlation was evident between intensity of infection and the ratio of AIAg IgG4/IgE ($P < 0.95$), the ratio of rABA-1A IgG4/IgE was positively associated with the level of *Ascaris* infection ($P < 0.025$, Fig. 3), illustrating that differential expression of these isotype responses toward ABA-1 are linked with variation in the degree of natural resistance to infections.

Following analysis of the IgG4/IgE ratio in relation to intensity of infection, the study group was divided into two age classes, based on whether there was a net increase

Table 1
Analysis of the effects of age or sex on parasite-specific IgG4/IgE ratios

Continuous ^a variable	Interaction (age $\times$ sex)		Sex		Age	
	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
Anti-AIAg IgG4/IgE	14.48	< 0.005**	0.89	ns	8.13	< 0.025*
Anti-rABA-1A IgG4/IgE	25.17	< 0.005**	0.14	ns	12.91	< 0.005**

^a The ratio of ABA-1A specific IgG4/IgE was assessed for the effects of patient age and sex by being entered as the continuous variable in a two way ANOVA incorporating age (four levels) and sex (two levels).

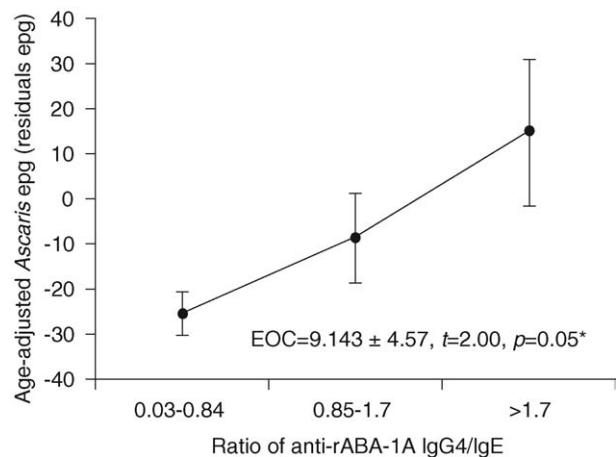


Fig. 3. The relationship between anti-rABA-1A IgG4/IgE ratios and intensity of *A. lumbricoides* infection. Intensity of infection (*Ascaris* EPG, y axis) is adjusted for the effect of age (residuals of ANOVA EPG $\times$ age) and negative values are transformed by addition of 48 to all values. Estimates of coefficients (EOC) $\pm$ 1 S.E. indicate the gradient of the linear relationship. Tau (*t*) values are the ratio of EOC to the standard error of EOC, the significance of the *t* ratio was assessed by comparison with percentiles of the *t* distribution. Significance is indicated at or below the 5% level (*).

(4–11 years olds) or decrease (12–36 years olds) in faecal egg counts (Fig. 1B). Levels of anti-rABA-1A IgG4 and IgE responses were examined independently against the intensity of *Ascaris* infection in each class (Fig. 4). From this it was determined that a significant, positive relationship existed between IgG4 and *Ascaris* EPG in the younger age class only ($P = 0.038$). However, in the older age class, where the net accumulation of worms had ceased, a negative association between ABA-1-specific IgE and the intensity of infection was observed ($P = 0.048$).

4. Discussion

We have illustrated that whilst antibody levels to crude, unfractionated worm extract do not demonstrate associations with *Ascaris* infection, IgE and IgG4 responses to a defined helminth allergen are disassociated in terms of their relationship with levels of infection. In accordance with earlier work [11], we have observed that high ABA-1 IgE levels in patients' plasma are linked to resistance to *Ascaris* infection in terms of reduced or absent parasite egg output. In addition, we have provided evidence that anti-allergen IgG4 is positively related to infection level. Moreover, we indicate that the contrasting relationships between anti-ABA-1 IgG4 and IgE responses are related to patient age and, crucially, to age categories where average levels of infection are increasing or decreasing, respectively.

The observational evidence presented here may be indicative of an age- (and/or exposure-) related, functional role for anti-parasite IgE in mediating an immuno-protective response in the face of sustained and continuous exposure to *Ascaris* and a transient preferential expression of allergen-specific IgG4 that successfully delays the onset of IgE-mediated

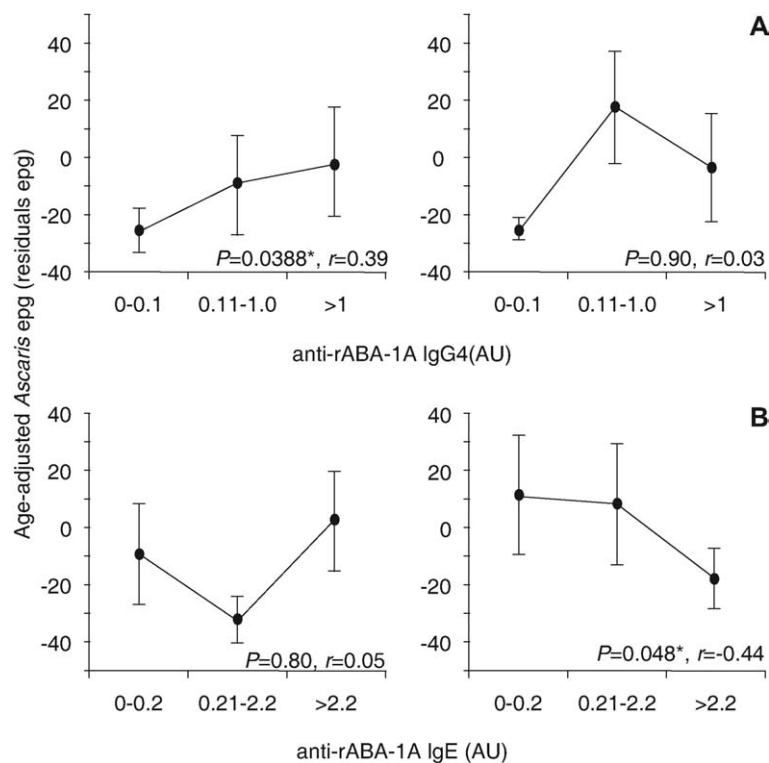


Fig. 4. Relationships between anti-rABA-1A IgG4 (A) and IgE (B) levels and intensity of *A. lumbricoides* infection in 4–11 years olds (left hand panels) and 12–36 years olds (right hand panels). Mean levels of adjusted egg output are plotted $\pm$ 1 S.E.M. *P* values and Spearman's rho (*r*) values are calculated from Spearman's rank correlation tests. Significance is indicated at or below the 5% level (*).

immunity. Alternatively, these isotype responses may represent dynamic, underlying immunological processes that determine whether an individual will be successful in limiting infection. Certainly, we have already demonstrated that in this population, Th2 cytokine responses, induced during ex vivo recall assays to parasite antigen, are quantitatively related to a reduction in infection levels in the 12–36 years old age group only [4]. In either case, we provide evidence that the relative degree of natural resistance, represented by the level of parasite egg output, tallies with a dichotomy in antibody isotype responses.

Parasite-specific IgE has been associated with resistance (or at least a less susceptible phenotype) in a number of human helminth infections including the ceacum dwelling gut nematode *T. trichiura* [10] and blood dwelling *schistosoma* species [12,13,21]. More recently, IgE has been demonstrated to be necessary for optimum clearance of *Trichinella spiralis* in a murine model of infection [22] supporting a mechanistic role for this immunoglobulin in gut parasite immunity. The life cycle of *Ascaris* spp. includes a convoluted, larval, tissue-migratory phase to an eventual adult residence in the lumen of the small intestine. If parasite-specific IgE plays a direct role in limiting infection, ABA-1 could be targeted at a number of parasite developmental stages and in various sites in the body. The allergen is abundantly expressed within the pseudocoelomic fluid of the parasite [23], but is also secreted [24], is present within the body wall [24] and is expressed by both tissue invasive and adult stages [25]. The tissue-migratory path of *A. lumbricoides* and the expression of ABA-1 by early

larval stages would suggest that IgE-mediated responses could target the initial stages of infection, impeding the development and maturation of the tissue-migratory larvae and ultimately reducing egg output. If this were the case then one could envisage the lung as an ideal site where IgE receptor binding could initiate an allergic reaction capable of damaging the parasite. The lung has been identified as the tissue where immune killing proceeds following mucosal vaccination of a recombinant *Ascaris* protein, rAS16, in a swine model of *Ascaris* infection [26]. Interestingly, large amounts of anti-rAS16 immunoglobulins were produced in vaccinated pigs and purified anti-rAS16 gamma-globulins were shown to have direct larvicidal activity against *Ascaris* larvae in vitro. Whilst it is more unlikely that IgE could operate in the lumen of the small intestine, the possibility remains that this isotype could enter a severely inflamed gut to directly target adult stages as it has been shown that several markers of acute inflammatory processes have been observed in children predisposed to light worm burdens [11].

An unexpected finding was a lack of correlation between IgE antibody responses to a crude, unfractionated, antigen preparation from *Ascaris*, but a clear association when only the (recombinant) ABA-1A protein was used as target antigen. In assays using a heterogeneous antigen such as *Ascaris* body fluid (ABF), considerable individuality in antibody repertoires (both IgG and IgE) has been observed [27,28]. Such diversity is probably due to immune repertoire restriction by (class II) MHC, which is clearly the case in rodents infected with *A. suum* [29,30]. The fact that some infected individuals

respond to a particular set of antigens of the parasite, and others respond to a different set, serves to confuse the analysis and obscure an association between (IgE) antibody responses and relative resistance or susceptibility. Focussing on an antigen that is particularly abundant in *Ascaris* and is recognised by a large proportion of infected individuals appears, however, to uncover an association between IgE antibody responses and naturally acquired immunity in a human population. This effect may also be explained by the fact that nematode polypeptide antigens/allergens appear to preferentially induce IgE and Th2 responses in naturally infected humans or experimentally infected animals [31,32]. Thus, focussing on a particular allergen to the exclusion of other protein antigens of the parasite that may not be preferential targets of IgE antibody responses will reveal associations with putative IgE-based protective immunity. The effect means that a balanced mixture of the parasite's allergens may be generally useful in immunoepidemiological surveys, and that this will presumably apply to other species of helminth parasite in which genetic restriction of the immune response is observed.

There are two putative mechanisms whereby preferential expression of IgG4 over IgE could occur which would subsequently lead to negation of IgE-mediated immunity and therefore enhancement of parasite survival. Firstly, it has been established that antibody-producing plasma cells switch sequentially from IgM via IgG4 to IgE [33]. Therefore, cessation of class switching of anti-ABA-1 clones at IgG4 would consequently mean a blockage of IgE expression at the level of germline transcription. Secondly, allergen-specific IgG has been described as 'blocking antibody' owing to its ability to effectively compete for IgE epitopes in both helminth and non-helminth derived allergens [15,34]. Because IgG4 displays a weak affinity for Fc γ receptors [35,36], an effective competition with IgE for ABA-1 epitopes expressed on the surface or secreted in close proximity of migratory larvae would prevent IgE sensitised basophil, eosinophil, or mast cell degranulation. IgG4-mediated allergen epitope blockade has been cited as a putative mechanism of specific-immunotherapy (SIT) for allergic disease as increased anti-allergen IgG4 correlates with the onset of hyporesponsiveness following treatment [37]. Therefore, IgG4 may enhance parasite survival by inhibiting IgE-mediated effector responses.

Whilst both IgG4 and IgE are positively regulated by IL-4, IL-13 and CD40 ligation, less is known about the precise signals required to differentially regulate these isotypes. IL-10 has been proposed as one such regulatory signal that can enhance IgG4 whilst down-regulating IgE [38]. Interestingly, IL-10 has also been implicated in the dampening of allergic responses and increased production of allergen-specific IgG4 in patients receiving SIT [39]. Because chronic helminth infection induces a modified Th2/T regulatory environment with concomitant high-level secretion of IL-10 [40], elevated levels of IgG4 may reflect, or indeed be directly involved in an underlying regulatory response to *Ascaris*

infection in susceptible individuals. Certainly, in this study population, the younger, more susceptible age class demonstrate a positive relationship between levels of IL-10 secreted in response to whole worm antigen in recall assays [4], which may explain the observed positive association between ABA-1A-specific IgG4 and intensity of infection in this age class. However, in the older age class, more individuals displayed measurable Th2 (IL-9 and IL-13) recall responses compared with the younger age class and both levels of IL-10 and IL-13 were negatively associated with levels of *Ascaris* infection. The presence of heightened Th2 responses in the older age class may override the effects of IL-10 and induce switching from IgG4 to IgE.

Although ABA-1 is an extremely potent allergen, immediate hypersensitivity reactions are seldom seen in individuals residing in endemic areas and pulmonary *Ascaris* syndrome is relatively rare [41]. However, in areas of seasonal transmission, cases of *Ascaris*-mediated pulmonary pneumonitis are more commonly encountered [42]. Therefore, there would appear to be mechanisms in place preventing wide-scale anaphylaxis that are selectively initiated following sustained, chronic exposure. The correct balance of anti-ABA-1 IgG4 and IgE expression may be important in limiting immunopathology whilst allowing for the expression of a necessary degree of IgE-mediated control of infection to halt the acquisition of pathophysiological worm burdens.

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References

- [1] M. Yazdanbakhsh, A. van den Biggelaar, R.M. Maizels, Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease, *Trends Immunol.* 22 (2001) 372–377.
- [2] R.M. Maizels, D.A. Bundy, M.E. Selkirk, D.F. Smith, R.M. Anderson, Immunological modulation and evasion by helminth parasites in human populations, *Nature* 365 (1993) 797–805.
- [3] F.D. Finkelman, J.F. Urban Jr., The other side of the coin: the protective role of the TH2 cytokines, *J. Allergy Clin. Immunol.* 107 (2001) 772–780.
- [4] J.D. Turner, H. Faulkner, J. Kamgno, F. Cormont, J. Van Snick, K.J. Else, R.K. Grencis, J.M. Behnke, M. Boussinesq, J.E. Bradley, Th2 cytokines are associated with reduced worm burdens in a human intestinal helminth infection, *J. Infect. Dis.* 188 (2003) 1768–1775.
- [5] R.J. Quinnell, D.I. Pritchard, A. Raiko, A.P. Brown, M.A. Shaw, Immune responses in human necatoriasis: association between interleukin-5 responses and resistance to reinfection, *J. Infect. Dis.* 190 (2004) 430–438.
- [6] J.A. Jackson, J.D. Turner, L. Rentoul, H. Faulkner, J.M. Behnke, M. Hoyle, R.K. Grencis, K.J. Else, J. Kamgno, M. Boussinesq, J.E. Bradley, T helper cell type 2 responsiveness predicts future susceptibility to gastrointestinal nematodes in humans, *J. Infect. Dis.* 190 (2004) 1804–1811.

- [7] D. Vercelli, H.H. Jabara, K. Arai, R.S. Geha, Induction of human IgE synthesis requires interleukin 4 and T/B cell interactions involving the T cell receptor/CD3 complex and MHC class II antigens, *J. Exp. Med.* 169 (1989) 1295–1307.
- [8] H. Gascan, J.F. Gauchat, M.G. Roncarolo, H. Yssel, H. Spits, J.E. de Vries, Human B cell clones can be induced to proliferate and to switch to IgE and IgG4 synthesis by interleukin 4 and a signal provided by activated CD4+ T cell clones, *J. Exp. Med.* 173 (1991) 747–750.
- [9] J. Punnonen, G. Aversa, B.G. Cocks, A.N. McKenzie, S. Menon, G. Zurawski, R. de Waal Malefyt, J.E. de Vries, Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells, *Proc. Natl. Acad. Sci. USA* 90 (1993) 3730–3734.
- [10] H. Faulkner, J. Turner, J. Kamnogo, S. Pion, M. Boussinesq, J. Bradley, Age and infection intensity dependent cytokine and antibody production in human trichuriasis: the importance of IgE, *J. Infect. Dis.* 185 (2002) 665–672.
- [11] C. McSharry, Y. Xia, C.V. Holland, M.W. Kennedy, Natural immunity to *Ascaris lumbricoides* associated with immunoglobulin E antibody to ABA-1 allergen and inflammation indicators in children, *Infect. Immun.* 67 (1999) 484–489.
- [12] P. Hagan, U.J. Blumenthal, D. Dunn, A.J. Simpson, H.A. Wilkins, Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*, *Nature* 349 (1991) 243–245.
- [13] D.W. Dunne, A.E. Butterworth, A.J. Fulford, H.C. Kariuki, J.G. Langley, J.H. Ouma, A. Capron, R.J. Pierce, R.F. Sturrock, Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection, *Eur. J. Immunol.* 22 (1992) 1483–1494.
- [14] I. Hagel, N.R. Lynch, M.C. Diprisco, E. Rojas, M. Perez, N. Alvarez, *Ascaris* reinfection of slum children—relation with the IgE response, *Clin. Exp. Immunol.* 94 (1993) 80–83.
- [15] R. Hussain, E.A. Ottesen, IgE responses in human filariasis. IV. Parallel antigen recognition by IgE and IgG4 subclass antibodies, *J. Immunol.* 136 (1986) 1859–1863.
- [16] R. Hussain, R.W. Poindexter, E.A. Ottesen, Control of allergic reactivity in human filariasis. Predominant localization of blocking antibody to the IgG4 subclass, *J. Immunol.* 148 (1992) 2731–2737.
- [17] A. Montresor, D.W.T. Crompton, D.A.P. Bundy, A. Hall, L. Savioli, Guidelines for the Evaluation of Soil-Transmitted Helminthiasis and Schistosomiasis at Community Level, World Health Organisation, 1998 (WHO/CTD/SIP/98.1).
- [18] M.W. Kennedy, The nematode polyprotein allergens/antigens, *Parasitol. Today* 16 (2000) 373–380.
- [19] D.R. Palmer, A. Hall, R. Haque, K.S. Anwar, Antibody isotype responses to antigens of *Ascaris lumbricoides* in a case control study of persistently heavily infected Bangladeshi children, *Parasitology* 111 (1995) 385–393.
- [20] H. Faulkner, J. Gardon, J. Kamnogo, P. Enyong, M. Boussinesq, J. Bradley, Antibody responses in onchocerciasis as a function of age and infection intensity, *Parasite Immunol.* 23 (2001) 509–516.
- [21] C.E. Demeure, P. Rihet, L. Abel, M. Ouattara, A. Bourgois, A.J. Dessein, Resistance to *Schistosoma mansoni* in humans: influence of the IgE/IgG4 balance and IgG2 in immunity to reinfection after chemotherapy, *J. Infect. Dis.* 168 (1993) 1000–1008.
- [22] M.F. Gurish, P.J. Bryce, H. Tao, A.B. Kisselgof, E.M. Thornton, H.R. Miller, D.S. Friend, H.C. Oettgen, IgE enhances parasite clearance and regulates mast cell responses in mice infected with *Trichinella spiralis*, *J. Immunol.* 172 (2004) 1139–1145.
- [23] J.F. Christie, B. Dunbar, I. Davidson, M.W. Kennedy, N-terminal amino acid sequence identity between a major allergen of *Ascaris lumbricoides* and *Ascaris suum*, and MHC-restricted IgE responses to it, *Immunology* 69 (1990) 596–602.
- [24] M.W. Kennedy, F. Qureshi, Stage-specific secreted antigens of the parasitic larval stages of the nematode *Ascaris*, *Immunology* 58 (1986) 515–522.
- [25] Y. Xia, H.J. Spence, J. Moore, N. Heaney, L. McDermott, A. Cooper, D.G. Watson, B. Mei, R. Komuniecki, M.W. Kennedy, The ABA-1 allergen of *Ascaris lumbricoides*: sequence polymorphism, stage and tissue-specific expression, lipid binding function, and protein biophysical properties, *Parasitology* 120 (2000) 211–224.
- [26] N. Tsuji, T. Miyoshi, M.K. Islam, T. Isobe, S. Yoshihara, T. Arakawa, Y. Matsumoto, Y. Yokomizo, Recombinant *Ascaris* 16-kDa protein-induced protection against *Ascaris suum* larval migration after intranasal vaccination in pigs, *J. Infect. Dis.* 190 (2004) 1812–1820.
- [27] E.M. Fraser, J.F. Christie, M.W. Kennedy, Heterogeneity amongst infected children in IgE antibody repertoire to the antigens of the parasitic nematode *Ascaris*, *Int. Arch. Allergy Immunol.* 100 (1993) 283–286.
- [28] M.W. Kennedy, L.A. Tomlinson, E.M. Fraser, J.F. Christie, The specificity of the antibody response to internal antigens of *Ascaris*: heterogeneity in infected humans, and MHC (H-2) control of the repertoire in mice, *Clin. Exp. Immunol.* 80 (1990) 219–224.
- [29] L.A. Tomlinson, J.F. Christie, E.M. Fraser, D. McLaughlin, A.E. McIntosh, M.W. Kennedy, MHC restriction of the antibody repertoire to secretory antigens, and a major allergen, of the nematode parasite *Ascaris*, *J. Immunol.* 143 (1989) 2349–2356.
- [30] M.W. Kennedy, E.M. Fraser, J.F. Christie, MHC class II (I-A) region control of the IgE antibody repertoire to the ABA-1 allergen of the nematode *Ascaris*, *Immunology* 72 (1991) 577–579.
- [31] M. Yazdanbakhsh, W.A. Paxton, A. Brandenburg, R. Van Ree, M. Lens, F. Partono, R.M. Maizels, M.E. Selkirk, Differential antibody isotype reactivity to specific antigens in human lymphatic filariasis: gp15/400 preferentially induces immunoglobulin E (IgE), IgG4, and IgG2, *Infect. Immun.* 63 (1995) 3772–3779.
- [32] H. Tezuka, S. Imai, S. Hidano, S. Tsukidate, K. Fujita, Various types of *Dirofilaria immitis* polyproteins selectively induce a Th2-type immune response, *Infect. Immun.* 71 (2003) 3802–3811.
- [33] D. Vercelli, One cytokine, two isotypes: a Trojan horse, Pandora's box, and an evolving paradigm, *Am. J. Respir. Crit. Care Med.* 162 (2000) S86–S90.
- [34] V. Visco, C. Dolecek, S. Denepoux, J. Le Mao, C. Guret, F. Rousset, M.T. Guinneeppain, D. Kraft, R. Valenta, A. Weyer, J. Banachereau, S. Labecque, Human IgG monoclonal antibodies that modulate the binding of specific IgE to birch pollen Bet v 1, *J. Immunol.* 157 (1996) 956–962.
- [35] R.C. Aalberse, R. van der Gaag, J. van Leeuwen, Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response, *J. Immunol.* 130 (1983) 722–726.
- [36] J.S. van der Zee, P. van Swieten, R.C. Aalberse, Serologic aspects of IgG4 antibodies. II. IgG4 antibodies form small, nonprecipitating immune complexes due to functional monovalency, *J. Immunol.* 137 (1986) 3566–3571.
- [37] S. Flicker, R. Valenta, Renaissance of the blocking antibody concept in type I allergy, *Int. Arch. Allergy Immunol.* 132 (2003) 13–24.
- [38] P. Jeannin, S. Lecoanet, Y. Delneste, J.F. Gauchat, J.Y. Bonnefoy, IgE versus IgG4 production can be differentially regulated by IL-10, *J. Immunol.* 160 (1998) 3555–3561.
- [39] C.A. Akdis, T. Blesken, M. Akdis, B. Wuthrich, K. Blaser, Role of interleukin 10 in specific immunotherapy, *J. Clin. Invest.* 102 (1998) 98–106.
- [40] R.M. Maizels, M. Yazdanbakhsh, Immune regulation by helminth parasites: cellular and molecular mechanisms, *Nat. Rev. Immunol.* 3 (2003) 733–744.
- [41] D.W. Crompton, *Ascaris* and ascariasis, *Adv. Parasitol.* 48 (2001) 285–375.
- [42] A.P. Gelpi, A. Mustafa, Seasonal pneumonitis with eosinophilia. A study of larval ascariasis in Saudi Arabia, *Am. J. Trop. Med. Hyg.* 16 (1967) 646–657.