

Identification of major human IgE-inducing parasite antigens: A path to therapeutic approaches?



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Type 2 immunity is a hallmark of allergic diseases, in which it plays a detrimental role, and the major contributor to anti-helminth immunity.^{1,2} It is characterized by the production of an expanding subset of alarmins (IL-25, IL-33, and thymic stromal lymphopoietin) and cytokines (IL-4, IL-5, IL-9, IL-13, IL-31, etc) and by the involvement of a specific group of cell subsets associated with innate and adaptive responses (group 2 innate lymphoid cells, dendritic cells, alternatively activated macrophages, mast cells, basophils, eosinophils, T_H2 lymphocytes, and B lymphocytes), ultimately leading to IgE production by B cells. IgE binds to and acts through 2 specific receptors. FcεRI is a high-affinity IgE receptor expressed by mast cells and basophils and, in humans, by antigen-presenting cells (dendritic cells, epidermal Langerhans cells, and monocytes/macrophages), eosinophils, and platelets.³ FcεRII/CD23 is a low-affinity receptor expressed mainly by B cells and regulating IgE production. IgE-dependent FcεRI-mediated cellular cytotoxicity (antibody-dependent cellular cytotoxicity) toward helminth larval stages carried out by eosinophils, macrophages, and platelets is a key mechanism in anti-helminth immunity. Mast cells and basophils contribute to anti-helminth immunity mainly through cytokine and protease production, whereas a specific role for IgE-mediated enhanced antigen presentation has not been formally demonstrated in the context of helminth infections. In line with the (fairly long) timing of IgE isotype switching and affinity maturation and with larval stages being the main targets, (antigen-specific) IgE concentrations are positively correlated with immunity to reinfection (eg, in schistosomiasis⁴) rather than with protection against a primary infection. Thus, identification of the major antigens promoting a strong IgE response is an important step both for establishing specific and sensitive diagnostic tools and for testing new vaccine candidates.

In an interesting study in this issue of the *Journal of Allergy and Clinical Immunology* that was conducted using filarial infections as a demonstrator, Hadadianpour et al⁵ identified major filarial antigens recognized by human IgE (hIgE) by using a labor-intensive technique previously applied for identification of *Aspergillus* antigens.⁶ This method is based on the generation of human IgE mAbs from hIgE-expressing B cells isolated from human PBMCs. From 7 patients with lymphatic filariasis, tropical pulmonary eosinophilia, loiasis, or onchocerciasis, 56 hIgE mAbs were generated regardless of their antigenic specificity and then screened for their reactivity toward a *Brugia malayi* extract by ELISA, Western blot, and specific ImmunoCAP assay. In all, 26 hIgE antibodies were positive in at least 1 assay. Specificity against other extracts from these human pathogens was not assessed. However, 13 antibodies cross-reacted with antigens from dog filaria *Dirofilaria immitis*. Importantly but surprisingly, these hIgE antibodies do not cross-react with 112 common allergens that might share some common glycan epitopes with parasite antigens, as reported in several studies.⁷ This suggests that an hIgE response during filarial infections is highly parasite specific. In all, 16 hIgE were able to immunoprecipitate filarial antigens that were analyzed by mass spectrometry, and 14 unique antigens were identified. All were excreted/secretory proteins, with a transthyretin-related (TTR) protein of unknown function being the dominant antigen for induction of hIgE response. Other proteins of interest were WbSXP-1, a nematode-specific secreted protein whose homolog is a vaccine candidate against *Ascaris*; a filarial homologue of human migration inhibitory factor; and an ubiquitously expressed 400-kDa polypeptide ladder-like protein (gp15/400). Production of recombinant antigens in a bacterial expression system (hence, lacking full native glycosylation) allowed further identification of 1 additional hIgE against *Wuchereria bancrofti*-specific antigens that were not selected on the basis of reactivity against *B. malayi*. Screening for reactivity against 15 other recombinant TTR family proteins identified multiple hIgEs with restricted or broad TTR cross reactivity, confirming TTR proteins as major antigens inducing an hIgE response against filariae. The functionality of hIgE cognate filarial (dimeric) antigen recognition was assessed in an IgE-mediated passive systemic anaphylaxis test using hFcεRIα transgenic mice expressing a humanized FcεRI with a structure and cell distribution comparable to that found in humans.⁸ Expectedly, cognate antigen injection induced hypothermia in hIgE-sensitized animals.

The advantages of the antigen-specific monoclonal hIgE approach chosen by Hadadianpour et al⁵ over direct IgE purification from serum or plasma are obvious. First, contamination with other isotypes might occur, as hIgG concentrations in serum are much higher than the hIgE concentrations, and hIgG can display overlapping antigen specificity with hIgE. Second, mAbs allow

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TABLE I. Comparison of human monoclonal IgE-based methods

Key step/sequence order	Per Hadadianpour et al ⁵	Per Croote et al ⁹	Comment(s)
PBMC isolation	1		
<i>In vitro</i> B-cell expansion	2		
hIgE ⁺ B-cell selection	3	1	I, ELISA; II, flow cytometry
Hybridoma generation	4		
Single-cell sorting	5	2	
Clone amplification-hIgE purification	6		
scRNA seq		3	II, Full transcriptome characterization
VDJ-C region sequencing and analysis	6'	(3)	II, Obtained from scRNA seq
Recombinant hIgE expression		3'	
Antigen reactivity screening	7		I, ELISA, Western blot, ImmunoCap
Antigen affinity purification	8		
Antigen identification	9		
Recombinant antigen expression	10		
hIgE cognate antigen recognition validation	11	4'	I, <i>In vitro</i> , ELISA, Western blot; <i>in vivo</i> , hIgE anaphylaxis in hFcεRIα Tg mice; II, ELISA
Method illustration	See Fig E1 in Hadadianpour et al ⁵	See Fig 1 in Croote et al ⁹	

ScRNA seq, Single-cell RNA sequencing.

precise identification of the epitope recognized. Third, the unlimited availability of hIgE mAbs allows a much more extensive biochemical characterization of not only the antibodies themselves but also, importantly, the identified antigens. Finally, together with the use of recombinant cognate antigens, hIgE mAbs can be used in functional assays *in vitro* or *in vivo*. A promising, yet complex, alternative approach is based on direct single-cell RNA sequencing of hIgE-expressing B cells from plasma followed by cloning and expressing all the IgE-relevant sequences as recombinant antibodies and has allowed the identification of clonal high-affinity hIgE from patients with peanut allergy.⁹ A major advantage of this recent method is that single-cell RNA sequencing additionally provides extensive information about individual hIgE-expressing B cells (see Table I for comparison). However, it has not yet been used in the context of helminth infections.

Surprisingly, besides gp15/400, none of the few known filarial antigens recognized by hIgE, such as tropomyosin, paramyosin, glutathione S-transferase, aspartic protease inhibitor, and venom allergen-like protein,¹⁰ were identified by Hadadianpour et al⁵ using monoclonal hIgE technology. A possible explanation for these barely overlapping helminth-specific antigen subsets identified by the 2 methods is that a monoclonal hIgE-based strategy for parasite antigen identification is able to select high-affinity epitope(s) even when the frequency of cognate IgE is very low. By contrast, procedures based on direct parasite antigen recognition by serum/plasma (polyclonal) IgE require a higher frequency of cognate IgE but are probably less stringent regarding IgE affinity for a given epitope.

Finally, although the induction of hIgE-mediated passive anaphylaxis in hFcεRIα Tg mice unambiguously demonstrates that hIgE-helminth antigen interactions are driving a functional mast cell response *in vivo*, a conclusion drawn by Hadadianpour et al⁵ from this experiment—namely, that hIgE, through mast cell activation, acts as an early sensor of infection, driving further eosinophil and basophil infiltration—appears speculative in the

absence of additional experiments. Indeed, IgE-mediated passive anaphylaxis is strictly dependent on mast cells and FcεRI. Thus, a role for any other FcεRI-expressing cell types, such as eosinophils or for CD23-expressing cells, cannot be established using this model. It is likely that during an actual helminth infection, all FcεRI- and CD23-expressing cell types bearing receptor-bound antigen-specific hIgE, rather than mast cells only, are directly and concomitantly activated following encounter with the cognate antigen and perform their respective function, including cytotoxicity, antigen presentation, cytokine, or IgE production. Therefore, the passive transfer of helminth antigen-specific hIgE before the infection of hFcεRIα Tg mice with *B malayi*, as proposed by Hadadianpour et al,⁵ remains the first key experiment to be performed for firmly establishing a protective role of antigen-specific monoclonal hIgE in *B malayi* infection. Use of mice also humanized for IgE and CD23 would further increase the relevance of such a demonstration. This would pave the way, on the one hand, for experiments aiming to determine which hFcεRI-expressing cell types contribute to this protective effect and, on the other hand, for testing the identified antigens in vaccinal strategies against deadly pathologies for which new therapeutic approaches are sorely needed. Should this experiment succeed, the monoclonal hIgE-based strategy described by Hadadianpour et al⁵ might be applied to identify new major antigens driving the IgE response in nonfilarial helminth infections.

I dedicate this editorial to Professor André Capron (1930-2020), a pioneer in anti-helminth immunity.

REFERENCES

- Devos M, Mogilenko DA, Fleury S, Gilbert B, Becquart C, Quemener S, et al. Keratinocyte expression of A20/TNFAIP3 controls skin inflammation associated with atopic dermatitis and psoriasis. *J Invest Dermatol* 2019;139:135-45.
- Haase P, Voehringer D. Regulation of the humoral type 2 immune response against allergens and helminths. *Eur J Immunol* 2021;51:273-9.
- Kraft S, Kinet JP. New developments in FcεRI regulation, function and inhibition. *Nat Rev Immunol* 2007;7:365-78.

4. Dunne DW, Butterworth AE, Fulford AJ, Kariuki HC, Langley JG, Ouma JH, et al. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur J Immunol* 1992; 22:1483-94.
5. Hadadianpour A, Daniel J, Zhang J, Spiller BW, Makaraviciute A, DeWitt AM, et al. Human IgE mAbs identify major antigens of parasitic worm infection. *J Allergy Clin Immunol* 2022;150:1525-33.
6. Wurth MA, Hadadianpour A, Horvath DJ, Daniel J, Bogdan O, Goleniewska K, et al. Human IgE mAbs define variability in commercial *Aspergillus* extract allergen composition. *JCI Insight* 2018;3:e123387.
7. Platts-Mills TA, Hilger C, Jappe U, van Hage M, Gadermaier G, Spillner E, et al. Carbohydrate epitopes currently recognized as targets for IgE antibodies. *Allergy* 2021;76:2383-94.
8. Dombrowicz D, Brini AT, Flamand V, Hicks E, Snouwaert JN, Kinet JP, et al. Anaphylaxis mediated through a humanized high affinity IgE receptor. *J Immunol* 1996;157:1645-51.
9. Croote D, Darmanis S, Nadeau KC, Quake SR. High-affinity allergen-specific human antibodies cloned from single IgE B cell transcriptomes. *Science* 2018;362:1306-9.
10. Fitzsimmons CM, Falcone FH, Dunne DW. Helminth allergens, parasite-specific IgE, and its protective role in human immunity. *Front Immunol* 2014;5:61.