Antibodies and superantibodies in patients with chronic rhinosinusitis with nasal polyps

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Background: Chronic rhinosinusitis with nasal polyps is associated with local immunoglobulin hyperproduction and the presence of IgE antibodies against *Staphylococcus aureus* enterotoxins (SAEs). Aspirin-exacerbated respiratory disease is a severe form of chronic rhinosinusitis with nasal polyps in which nearly all patients express anti-SAEs.

Objectives: We aimed to understand antibodies reactive to SAEs and determine whether they recognize SAEs through their complementarity-determining regions (CDRs) or framework regions.

Methods: Labeled staphylococcal enterotoxin (SE) A, SED, and SEE were used to isolate single SAE-specific B cells from the nasal polyps of 3 patients with aspirin-exacerbated respiratory disease by using fluorescence-activated cell sorting. Recombinant antibodies with "matched" heavy and light chains were cloned as IgG_1, and those of high affinity for specific SAEs, assayed by means of ELISA and surface plasmon resonance, were recloned as IgE and antigen-binding fragments. IgE activities were tested in basophil degranulation assays.

Results: Thirty-seven SAE-specific, IgG- or IgA-expressing B cells were isolated and yielded 6 anti-SAE clones, 2 each for SEA, SED, and SEE. Competition binding assays revealed that the anti-SEE antibodies recognize nonoverlapping epitopes in SEE. Unexpectedly, each anti-SEE mediated SEE-induced basophil degranulation, and IgG_1 or antigen-binding fragments of each anti-SEE enhanced degranulation by the other anti-SEE.

Conclusions: SEEs can activate basophils by simultaneously binding as antigens in the conventional manner to CDRs and as superantigens to framework regions of anti-SEE IgE in anti-SEE IgE-FcεRI complexes. Anti-SEE IgG_1s can enhance the activity of anti-SEE IgEs as conventional antibodies through CDRs or simultaneously as conventional antibodies and as "superantibodies" through CDRs and framework regions to SEEs in SEE–anti-SEE IgE-FcεRI complexes. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Chronic rhinosinusitis with nasal polyps, aspirin-exacerbated respiratory disease,* Staphylococcus aureus *enterotoxin, superantigen, superantibody, basophil*

Staphylococcus aureus and its superantigens are implicated in the intense inflammatory processes of the upper and lower airways in patients with allergic diseases.[1] These superantigens, in particular *Staphylococcus aureus* enterotoxins (SAEs), are strongly associated with chronic rhinosinusitis with nasal polyps (CRSwNP), particularly in the subpopulation of patients with aspirin-exacerbated respiratory disease (AERD), as well as those with allergic rhinitis, asthma, and atopic dermatitis.[2-5] SAEs are a family of structurally related proteins comprising different serological types, such as staphylococcal enterotoxin (SE) A, SEB, SEC, SED, and SEE (up to SEU) and toxic shock syndrome toxin 1 (TSST-1).[6] SAEs are potent T-cell superantigens, causing polyclonal activation of up to 25% of certain T-cell populations by interacting with a common β-chain structural framework region in the T-cell receptor (TCR),[7,8] rather than the complementarity-determining region (CDR), which recognizes specific antigenic peptides bound to MHC. The activity of SEA and SED on B cells *in vitro* suggests that they can also act as B-cell superantigens by binding to the common structural framework regions in the immunoglobulin heavy-chain variable region (VH) domains shared by immunoglobulins with different CDRs,[9-11] as previously shown for staphylococcal protein A (SpA).[12-14] This might increase the polyclonality of the B-cell repertoire in patients with CRSwNP and allow *S aureus* to escape immune surveillance.[15,16]

Nasal polyps in patients with CRSwNP are inflammatory outgrowths of the paranasal sinus mucosa, which are generally characterized by T_H2 inflammation, local immunoglobulin production, and eosinophil infiltration driven by IL-5 and eotaxin.[17-20] Up to 100% of patients with AERD express anti-SAE IgEs in their nasal polyp homogenates and often have a higher prevalence of comorbid asthma and eosinophilic

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Abbreviations used
7-AAD: 7-Aminoactinomycin D
AERD: Aspirin-exacerbated respiratory disease
CDR: Complementarity-determining region
CRSwNP: Chronic rhinosinusitis with nasal polyps
Fab: Antigen-binding fragment
FACS: Fluorescence-activated cell sorting
SAE: *Staphylococcus aureus* enterotoxin
SE: Staphylococcal enterotoxin
SpA: Staphylococcal protein A
SPR: Surface plasmon resonance
TCR: T-cell receptor
TSST-1: Toxic shock syndrome toxin 1
VH: Heavy-chain variable region
VL: Light-chain variable region

inflammation,[17,21-23] and IgEs from nasal polyps activate basophils in response to allergens and SEB *in vitro*.[15] SEB acts as a human T-cell superantigen *in vivo* to cause the symptoms of atopic dermatitis.[24] Basophils isolated from patients with atopic dermatitis with anti-SAE IgE in their sera, but not those from healthy control subjects, were responsive to SAEs.[25] Although anti-SAE IgE can be detected in the circulation of patients with CRSwNP, B cells expressing this IgE are confined to the nasal mucosa, suggesting a role in sinonasal inflammation.[17] Whether the SAEs bind to CDRs, framework regions, or both is addressed in the present work.

Antibody production in nasal polyps of patients with CRSwNP is driven by local activation and differentiation into plasma cells of B cells on exposure to various aeroallergens and microbial antigens.[26,27] Thus nasal polyps removed from patients with AERD provide a unique source of tissue to study how SAEs can shape the local antibody repertoire. In the first study of this kind, we used an efficient single-cell RT-PCR method to clone and express antibodies from single SAE-specific B cells isolated from the nasal polyps of 3 patients with AERD and tested their primary function in effector cell activation.

METHODS

Patients' samples

Nasal polyps and sera were collected from 3 patients with AERD (HPK-014, HPK-016, and HPK-018) at the Royal National Throat, Nose and Ear Hospital, London, United Kingdom. The patients were male, with a mean age of 56 years. Only patient HPK-014 had positive skin prick test responses to aeroallergens. The ethical committee representing

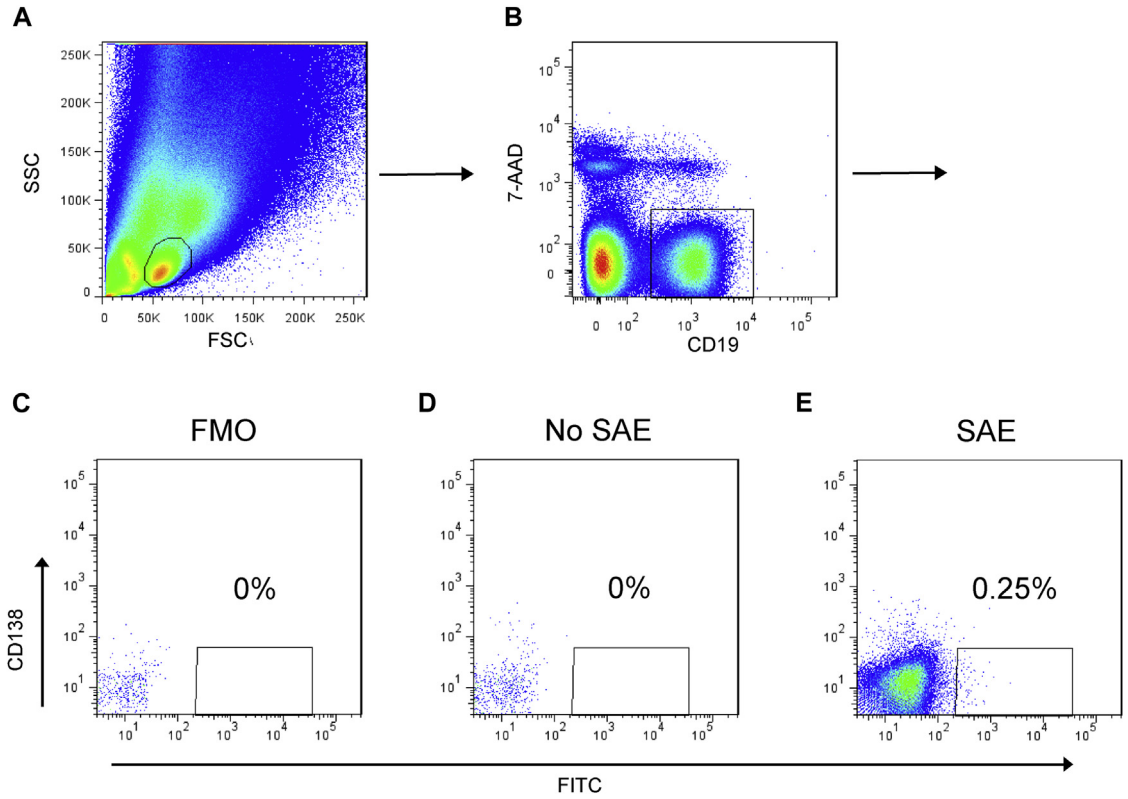

FIG 1. Isolation of SAE$^+$ B cells from nasal polyps by means of FACS. Cells were labeled with a mixture of biotinylated SEA, SED, and SEE and phycoerythrin-coupled anti-CD138, allophycocyanin-coupled anti-CD19, and 7-AAD. **A,** The lymphocyte population was selected for size and granularity. *FSC*, Forward scatter; *SSC*, side scatter. **B,** Live B cells were identified as CD19$^+$7-AAD$^-$ cells. **C-E,** Fluorescence minus one *(FMO)* control (Fig 1, *C*) and no SAE control (Fig 1, *D*) were used to select SAE$^+$CD19$^+$CD138$^-$7-AAD$^-$ cells, which constituted 0.25% of the total B-cell population (Fig 1, *E*). The *No SAE* control indicates that the cells were stained with all fluorescent antibodies and streptavidin–fluorescein isothiocyanate *(FITC)* but without biotinylated SAEs, whereas FMO control indicates cells stained with all antibodies but without streptavidin-FITC.