

# Images in Allergy and Immunology

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## Local isotype switching to IgE in airway mucosa

**Editor's note:** The feature, Images in allergy and immunology, is designed to highlight current concepts of the immunopathology of allergic diseases and other common immunopathologically mediated diseases. The presentation will appear as sets of images that involve cross-pathology, histopathology, and molecular pathology and will cover a range of topics of interest to allergists and immunologists.

Antibodies, while maintaining their antigen-binding site, can alter their effector function by switching the constant portion of their heavy chain. This process is termed *isotype switching* and allows the formation of the allergy-associated antibody isotype IgE. Classically, isotype switching to IgE has been considered an event restricted to lymphoid tissue, such as the regional lymph nodes and spleen, and the IgE-positive cells found within peripheral sites of allergic inflammation, such as the respiratory mucosa, have been thought to migrate from these centers. However, IgE has been detected at sites of allergic inflammation in the absence of positive skin test responses or increased serum IgE levels.<sup>1</sup> This indicates the possibility that B cells residing within the tissue might also participate in production of IgE by means of local isotype switching. IgE isotype switching involves the recombination of genomic DNA. Exons encoding the constant portion of the IgE antibody heavy chain (C $\epsilon$ ) are spliced downstream of the variable portion (VH) of the antibody, allowing for the transcription of IgE mRNA (Fig 1). Coordination of recombination is performed by specific genomic switch regions, S $\mu$

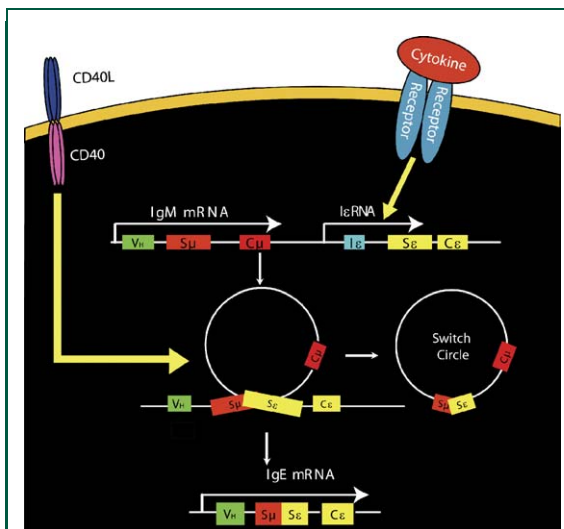
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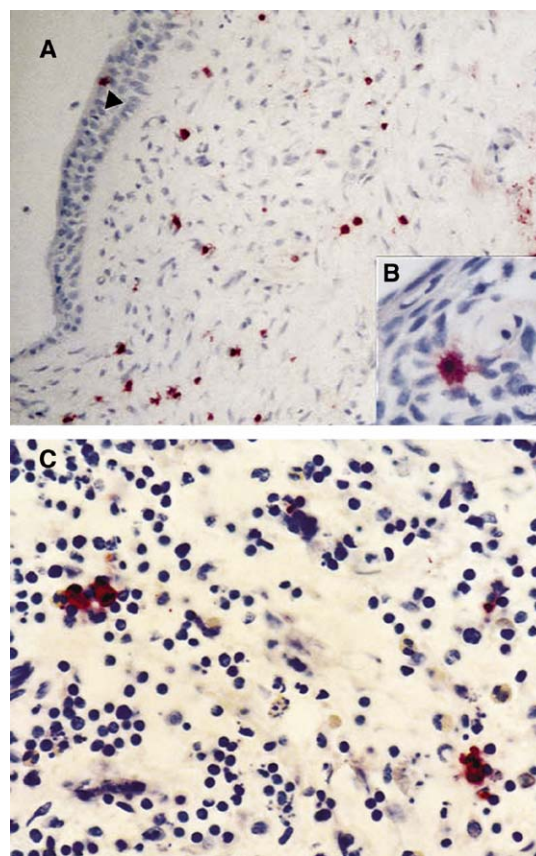
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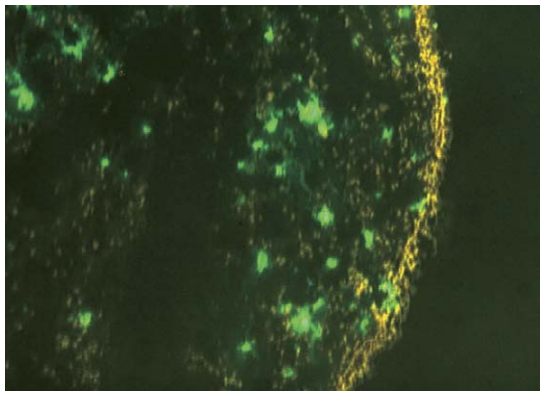
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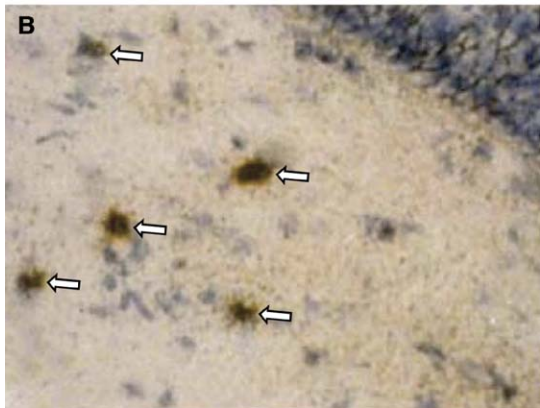
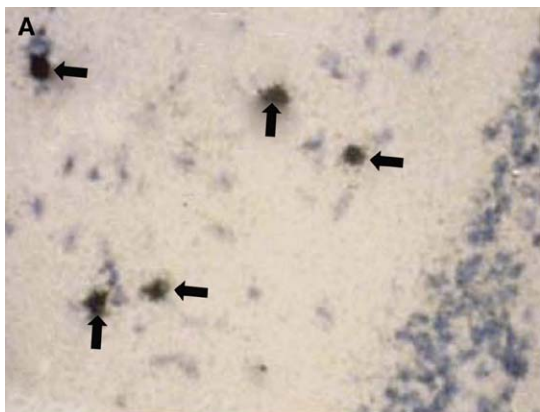
**FIG 1.** Isotype switching involves 2 steps: germline transcription and DNA recombination. Cytokines cause the transcription of I $\epsilon$  RNA, which is essential for switching. CD40 ligand allows recombination to proceed. Products of isotype switching are a gene for an immunoglobulin isotype (here IgE) and a switch circle, which is composed of the intermediate region that was deleted from genomic DNA.



**FIG 2.** B lymphocytes identified on the basis of CD20 immunoreactivity within allergic nasal mucosa cultured for 24 hours in the presence of specific allergen. B cells were observed just beneath the basement membrane (A), infiltrating the epithelial layer (B), and clustering within the submucosa in groups of 3 or 4 cells (C).<sup>4</sup>

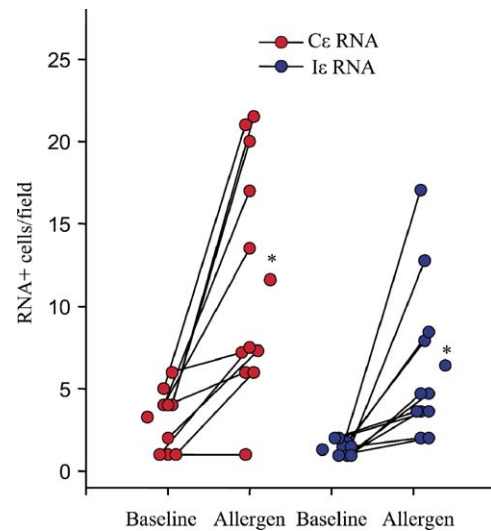


**FIG 3.** Immunofluorescence with anti-CD40 ligand antibody linked to FITC. Note the large number of positive cells in the submucosa of the upper airway.

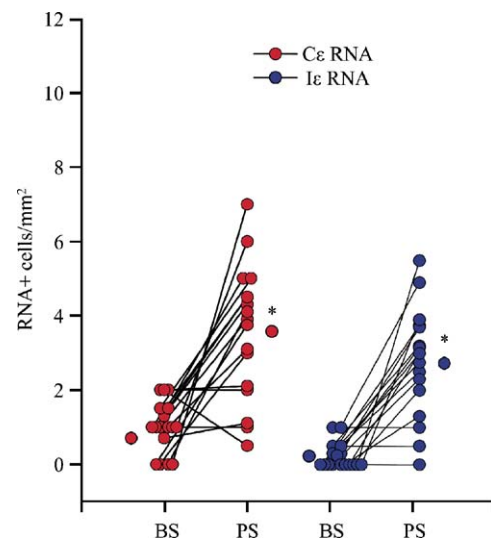


**FIG 4. A,** *In situ* hybridization for IgE mRNA ( $C\epsilon$  RNA) in the nasal mucosa of an allergic patient. *Black arrows* point to positive cells. **B,** Colocalization of IgE mRNA with resident CD20-positive B cells. *In situ* hybridization was performed with the same probe, whereas CD20-positive cells were determined with horseradish peroxidase immunocytochemistry. *White arrows* point to double-positive cells.

and  $S\epsilon$ , which are present upstream of  $C\mu$  and  $C\epsilon$ , respectively. B cells require 2 signals to undergo isotype switching. First, the cytokines IL-4 and IL-13 initiate germline transcription of a promoter upstream of  $S\epsilon$ ,  $I\epsilon$  RNA, composed of  $I\epsilon$  spliced directly to  $C\epsilon$ . This transcript is necessary but not sufficient for



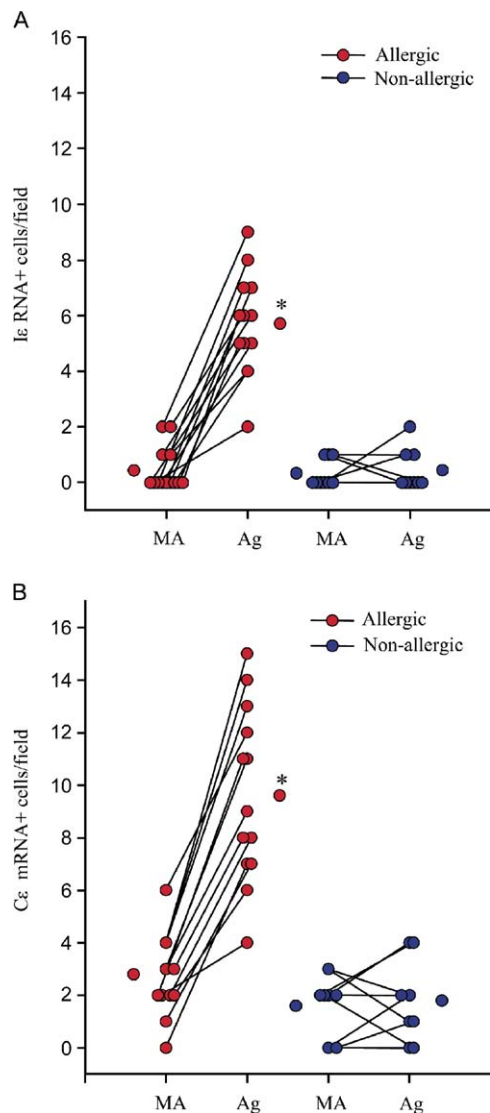
**FIG 5.** *In situ* hybridization-positive cells expressing  $C\epsilon$  RNA and  $I\epsilon$  RNA detected in patients with hay fever at baseline and 24 hours after an acute ragweed allergen challenge ( $P < .01$ , Student *t* test).<sup>2</sup>



**FIG 6.** *In situ* hybridization-positive cells expressing  $C\epsilon$  RNA and  $I\epsilon$  RNA detected in patients with allergic rhinitis before the allergen season (BS) and during the peak season (PS;  $P < .001$ , Student *t* test).<sup>3</sup>

recombination to take place. Second, activated T cells expressing CD40 ligand activate B-cell CD40, allowing class-switch recombination to continue. Recombinase activity aligns  $S\mu$  adjacent to  $S\epsilon$ . The DNA lying between is deleted,  $S\mu$ , and  $S\epsilon$  is removed, so that the  $C\epsilon$  region is placed adjacent to  $VH$ , forming the template for IgE heavy chain mRNA. The ends of the deleted DNA are spliced together at the S regions, giving rise to circular DNA products called *switch circles*.

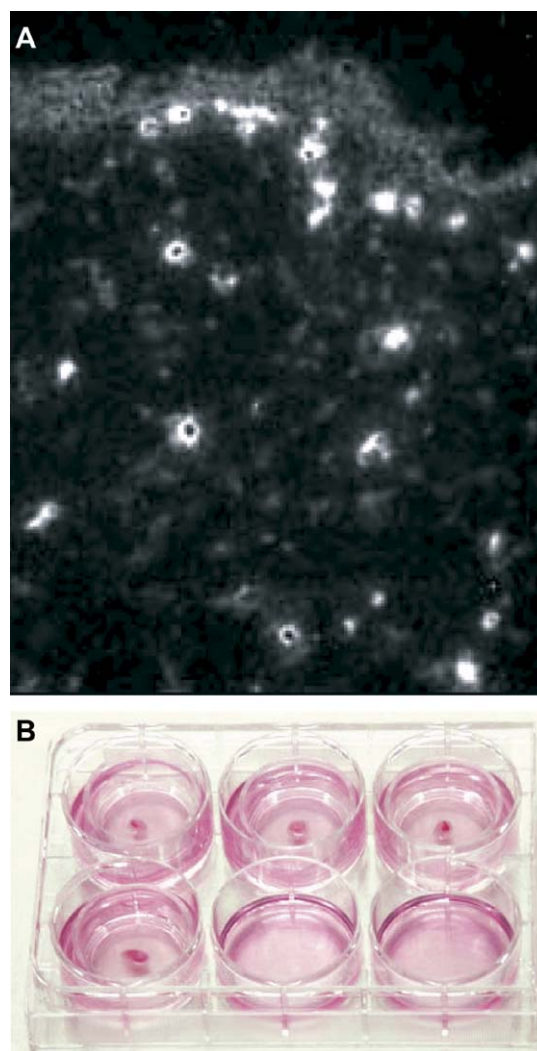
B cells can be identified in the nasal mucosa (Fig 2)<sup>4</sup> and can be provided with the necessary signals for



**FIG 7.** Expression of  $\epsilon$  RNA transcripts within explanted nasal mucosal tissue. Significantly higher numbers of I $\epsilon$  (A) and Ce (B) RNA-positive cells were observed in allergen-stimulated (Ag) compared with unstimulated (medium alone [MA]) allergic tissue but not within tissue obtained from nonallergic patients.<sup>4</sup>

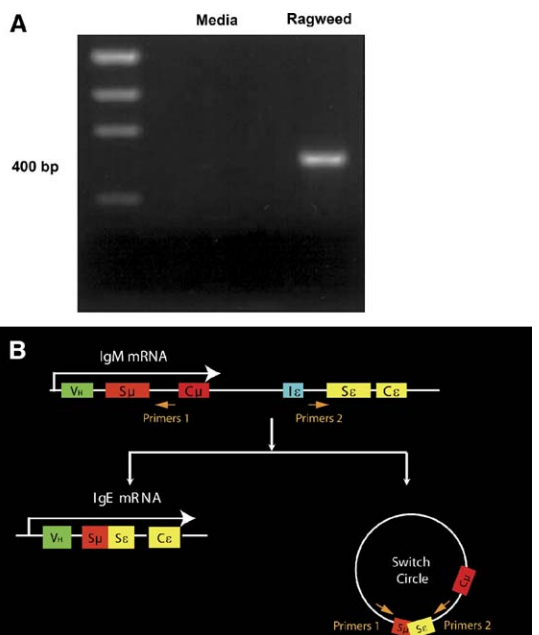
isotype switching. There are increased numbers of T cells expressing IL-4 and IL-13 mRNA. T cells, mast cells, basophils, and eosinophils express CD40 ligand, and within the mucosa of sensitized individuals, these cells are present in ample numbers (Fig 3). IgE-coated granulocytes, particularly mast cells, can release IL-4 and produce IL-13 on allergen cross-linking. This would lead to local B-cell isotype switching to IgE, in effect increasing mucosal IgE levels.

IgE mRNA-positive B cells have been identified in nasal mucosa of patients with symptoms of allergy (Fig 4). For some time, however, it was not clear whether these were truly resident B cells or simply those that infiltrated the nasal compartment after switching to IgE in lymphoid tissue. I $\epsilon$  RNA-positive



**FIG 8.** A, Representative example of I $\epsilon$  RNA *in situ* hybridization-positive cells found in an explant from nasal mucosa of a patient with rhinitis after *ex vivo* allergen challenge. B, Nasal explant model in which tissue biopsy samples taken from the inferior turbinate are cultured *ex vivo* and stimulated with different conditions. The tissue sits on a filter dish, which floats over the media and is exposed to air, thus simulating the *in vivo* nasal environment.

cells were identified in the nasal mucosa of patients with allergic rhinitis after acute allergen challenge (Fig 5)<sup>2</sup> after seasonal exposure (Fig 6).<sup>3</sup> Additionally, increased numbers of I $\epsilon$  RNA-positive cells and Ce RNA-positive cells were present in explanted nasal mucosa after allergen challenge (Figs 7 and 8).<sup>4</sup> Explanted tissue is devoid of cell recruitment, confirming that initiation of local isotype switching exists. Actual DNA recombination was also shown by means of PCR, in which positive S $\mu$ -S $\epsilon$  switch circle DNA was detected in explanted nasal mucosal tissue with *ex vivo* allergen challenge (Fig 9).<sup>5</sup> This work indicates that in addition to the lymph nodes and spleen, isotype switching to IgE can also occur locally within the respiratory mucosa. These findings suggest an



**FIG 9. A,** SeS $\mu$  switch circle DNA found within nasal mucosal tissue cultured for 24 hours with media alone or ragweed extract. **B,** When isotype switching occurs, the switch circles are looped out of the genomic DNA. Switch circles can be detected by means of nested PCR with primer sets that amplify the SeS $\mu$  junction of the circles but not genomic DNA.<sup>5</sup>

explanation for the presence of IgE within these tissues in the absence of positive skin test responses, serum IgE levels, or both.

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