

Letter to the Editor

Evidence for neuronal expression of functional Fc (ϵ and γ) receptors

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The Fc receptor family plays a key role in adaptive immunity through the binding of immunoglobulin antibodies that recognize an immune insult and elicit an inflammatory response leading to its clearance. Dysregulation of this receptor family may have untoward consequences that result in autoimmune and allergic diseases. Many of these diseases seem to involve the nervous system and are exacerbated by stress or other neurologic challenges. Recently, the presence of Fc receptors was uncovered on dorsal root ganglion neurons and suggested an IgG and possibly IgE-mediated activation of neurons.¹⁻³ We set out to explore more extensively which Fc receptors might be expressed in neurons, and whether they were functional and able to transmit signals to interconnected neurites *in vitro* and *in vivo*.

Messenger RNA was isolated from a highly pure culture of mouse superior cervical ganglion (SCG) neurons⁴ and expression of Fc receptor transcripts assessed by RT-PCR using specific primers for Fc γ and Fc ϵ family members. Fig 1, A, demonstrates the presence of transcripts for the immunoglobulin-binding α chain of Fc γ RI, II, III, and IV in 3 individual SCG neuron mRNA preparations. A small amount of the transcript for the low-affinity IgE receptor (Fc ϵ RII or CD23) was also detected relative to that seen in B cells, known to express this receptor. Fc γ RI transcripts were detected in bone marrow-derived mouse mast cells, but these levels were less than seen in the neurons. This observation and the inability to detect mouse mast cell protease (mMCP)-6 mRNA in either Balb/c or B16 mice together with the absence of CD23 transcripts in both neurons and mast cells provided confidence that the observed Fc receptor transcripts in neurons was not a result of mast cell contamination of cultures.

We also unexpectedly observed the presence of transcripts for the α , β , and γ chains of the high-affinity IgE receptor (Fc ϵ RI; Fig 1, B). Although the trimeric form ($\alpha\gamma_2$) of this receptor has been described in cells other than mast cells or basophils (such as in human Langerhans cells⁵), the expression of the tetrameric form ($\alpha\beta\gamma_2$) was previously thought to be limited to these proinflammatory cells. The trimeric Fc ϵ RI shows weak calcium signals compared with the tetrameric form because of the absence of the Fc ϵ RI β in the former.⁶ To determine whether the Fc ϵ RI was expressed on the cell surface of SCG neurons, cells were incubated with IgE and with an antibody to the neuronal specific protein gene product 9.5 (which encodes a neuronal ubiquitin C-terminal hydrolase not found on glia), and binding was visualized with a fluorescent secondary antibody. IgE was detected on the neuronal cell surface (Fig 2, A). Although the presence of protein gene product 9.5 was most evident in the cell body, IgE binding was detected in plasma membranes of both the cell body and neurite extensions. As shown in Fig 2, B to D, the expression of Fc ϵ RI was further confirmed by detection of the α , β , and γ chains of this receptor.

Scorpion venom is a known potent selective activator of neurons and elicits a rapid rise in intracellular Ca^{2+} (Fig 3, A). To test the functionality of Fc ϵ RI expressed on neurons, SCG neurons sensitized with Dinitrophenylated (DNP)-specific IgE were challenged with antigen (Ag) DNP-human serum albumin (HSA),

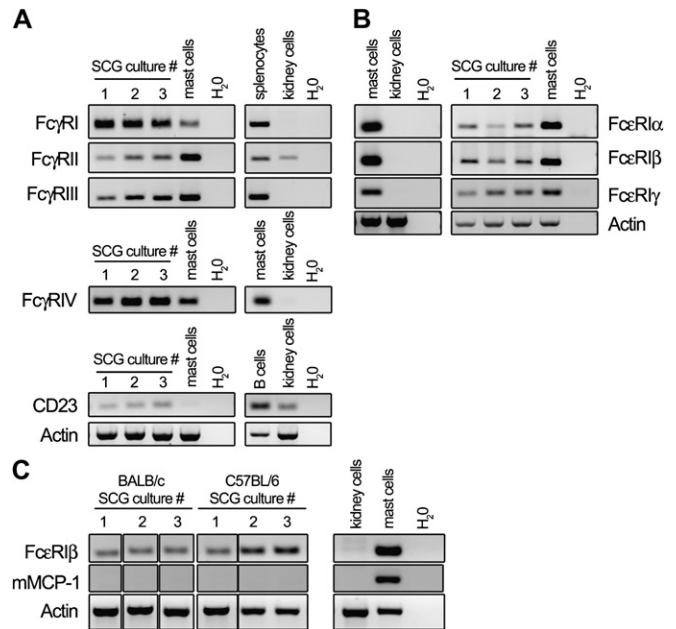


FIG 1. mRNA expression of the subunits of Fc ϵ RI, Fc γ RI-IV, and CD23 in SCG neurons. mRNA was collected from three (1-3) SCG neuronal cultures, and expression of the indicated mRNA was measured by RT-PCR. **A**, Expression of the α subunit of the low-affinity receptors for IgG (Fc γ RI-IV) and for IgE (CD23). **B and C**, Expression of the subunits of Fc ϵ RI and of mMCP-1 (**C**) in SCG neurons derived from Balb/c (**A-C**) or C57BL/6 (**C**) mice. Spleen cDNA and mRNA from bone marrow-derived mouse mast cells or from B cells were used as positive controls. The mRNA of the mouse IMCD-3 kidney epithelial cell line from the inner medulla collective duct was a negative control for Fc ϵ RI, mMCP-1, and Fc γ RI, III, and IV expression. A control for PCR contamination was performed by using H_2O .

and a rapid rise in intracellular Ca^{2+} was observed (Fig 3, A). No changes in intracellular Ca^{2+} were observed when serum albumin alone was used as Ag or when cells were not sensitized with IgE (data not shown). Fc γ RIV was recently described to bind the IgE^b allotype but does not recognize the IgE^a allotype,⁷ whereas Fc ϵ RI binds both allotypes. We excluded that IgE/Ag-mediated calcium response might occur through Fc γ RIV by use of both a IgE^a and IgE^b allotypes. Both IgE allotypes similarly elicited calcium responses (Fig 3, A). Addition of Ag elicited a relatively uniform Ca^{2+} response in the stimulated cell population (Fig 3, B). On the basis of the high-affinity binding of monomeric IgE (not removed by washing the cells) and the dose response to Ag (Fig 3, D), we could also exclude the involvement of CD23.

Thus, the findings demonstrate the presence of functional high-affinity Fc ϵ receptors on SCG neurons. Because Fc γ RIII is known to activate mast cells, its functionality was also tested. SCG neurons sensitized with DNP-specific IgG₁, which preferentially binds Fc γ RII and III but weakly to Fc γ RI and not to Fc γ RIV,⁸ showed modest rises in intracellular Ca^{2+} that increased with a large dose of Ag (Fig 3, C). Both IgE and IgG-mediated responses were concentration-dependent, and a Ca^{2+} response (Fig 3, D) was not elicited in all challenged neurons. Moreover, as expected (given the weak binding of monomeric IgG₁ to Fc γ receptors), increased responsiveness via IgG required much higher concentrations of Ag than for IgE.

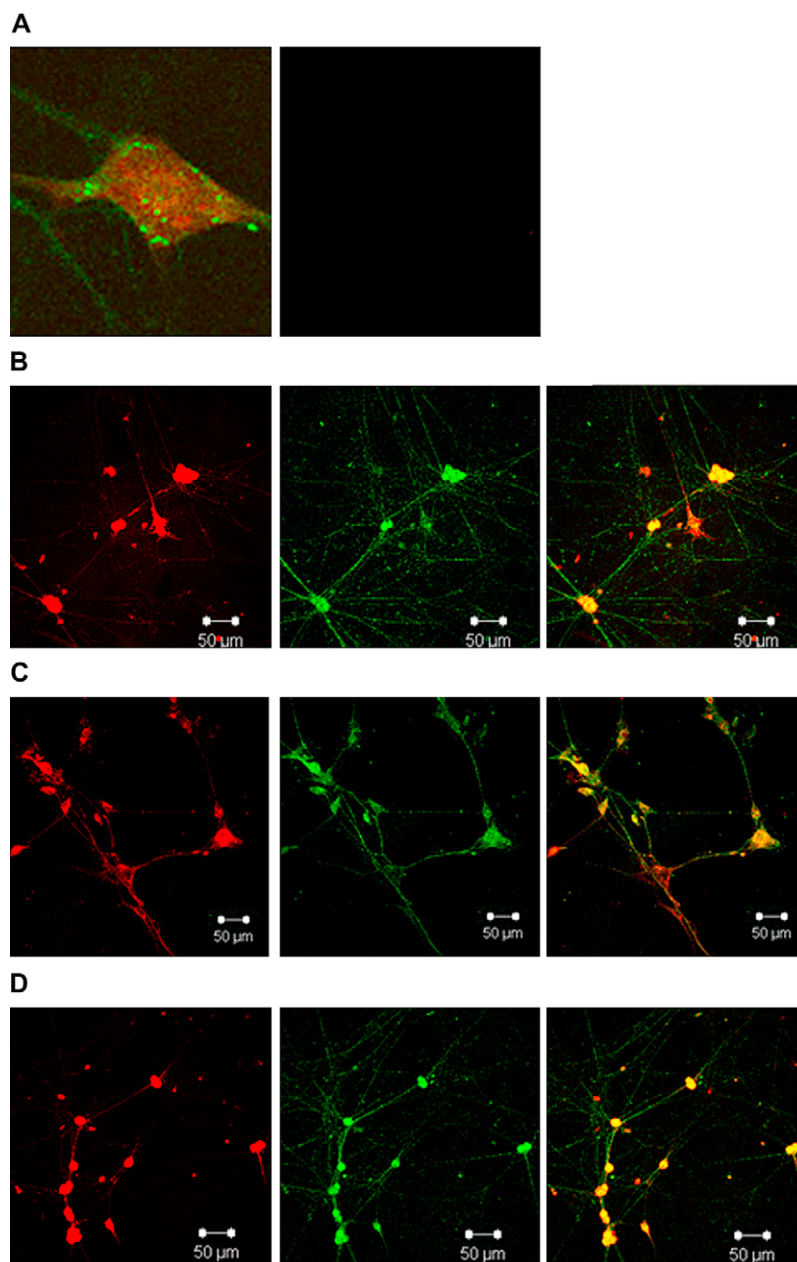


FIG 2. Neurons were sensitized with IgE and incubated overnight with antibody against IgE (**A**, left panel) or with an antibody of unknown specificity (**A**, right panel). Staining with the neuron specific marker protein gene product PGP9.5 (**B** and **C**, left panel in red) and FceRIα (**B**), β (**C**), or γ-chain (**D**, middle panel in green) is shown. Confocal images were overlain (**B-D**, right panel); yellow represents merging of red and green.

To test whether the Ca^{2+} signals elicited by FceRI stimulation could be transferred to interconnected neurites, we explored whether Ca^{2+} rises might be elicited in neighboring neurites after Ag challenge of an IgE-sensitized cell body or neurite. Using a spritzer micropipette, antigen was puffed directly onto a neuronal cell body, causing an instantaneous (<5 seconds) increased fluorescence in that cell body that moved from there to the connected neurites and propagated to the neighboring cell bodies and neurites (Fig 4). These findings showed that FceRI stimulation causes communication among interconnected neurites. To extend these findings to a more physiological setting, we explored whether neurons from the highly innervated intact jejunum⁹ would

respond to an FceRI stimulus. After placement of a micropipette spritzer (Fig 5, *A* and *G*, dotted lines) on a large myenteric plexus ganglion neuron (Fig 5, *A* and *G*, plain lines), the anti-DNP IgE sensitized plexus was challenged with Ag. Challenge with a spritz of 1 μg Ag gave robust calcium responses in adjacent neurons along the nerve fiber in sensitized (2/2) but not in nonsensitized mice (0/3). Repeated spritzes of nonconjugated HSA at this concentration elicited no responses (0/2; data not shown). To exclude possible mast cell involvement in the transmission of these robust signals, we conducted similar experiments in mast cell-deficient *W/W^v* mice and in their wild type control *WBB6F1*. In *W/W^v* mice (3/3), detectable intracellular calcium increases were observed on

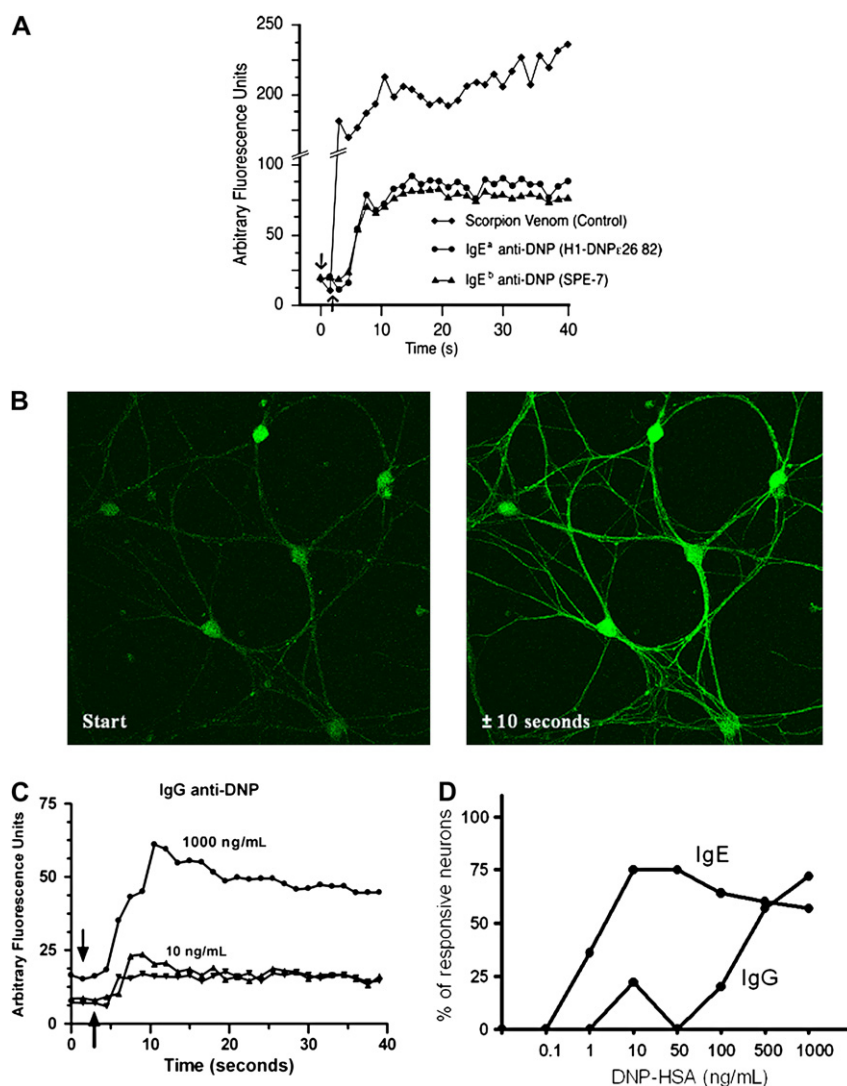


FIG 3. **A**, Kinetics of $[Ca^{2+}]_i$ increase on stimulation with scorpion venom (control) or with anti-DNP IgE^{a/b}-sensitized neurons after Ag stimulation (10 ng/mL). The arrows indicate the time of addition of the stimulus. **B**, Confocal image of calcium responses of IgE-sensitized neurons before and after Ag addition. **C**, Kinetics of $[Ca^{2+}]_i$ increase in anti-DNP IgG₁-sensitized neurons after Ag stimulation. **D**, Dose response of IgE and IgG-mediated neuron activation.

Ag challenge (Fig 5, A-C). No calcium signal was seen when the same ganglion was first challenged with HSA alone (Fig 5, D-F). The wild-type littermates (*WBB6F1*) responded positively (3/3) on challenge with specific Ag (Fig 5, G-I) but gave no response to HSA alone. These findings confirm that the observed signal transmission by FcεRI was not likely caused by mast cells and demonstrate the *in vivo* presence of functional FcεRI on jejunal neurons, because sham sensitization *in vivo* before an *ex vivo* challenge yielded no response to Ag challenge.

It is well known that sensory nerves may participate in hypersensitivity reactions, a process known as *neurogenic inflammation*, and several lines of evidence support the notion that sensory nerves may play an important role in cutaneous, lung, gastrointestinal, and joint inflammatory diseases. Here we now demonstrate that functionally active FcεRI is expressed on SCG and myenteric plexus neurons. The discovery of functional Fcε and Fcγ receptors on nerves clearly shows that this biological compartment is able to respond to the direct stimulus

of antibody-antigen interactions. Our findings define an independent neuronal (non-mast cell/non-basophil) compartment with probable involvement in allergic and possibly other diseases.

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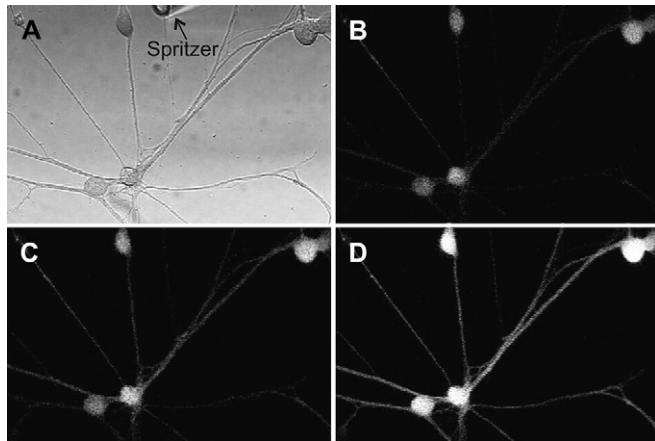


FIG 4. Activation of interconnected neurites by FcεRI stimulation of a single neuronal cell body. **A**, Bright field image of neuron network showing relation of Ag-containing spritzer to neuronal cell body sensitized with IgE anti-DNP. **B**, At time 0, beginning of 500-millisecond spritz with Ag (DNP-HSA). Shown are 0.5 seconds (**C**) and 1.8 seconds after onset of spritz (**D**).

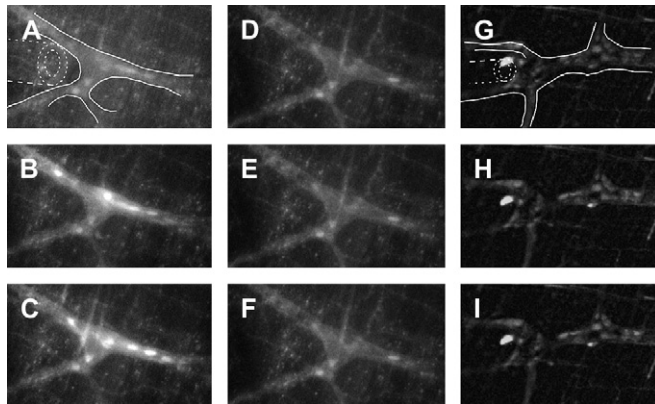


FIG 5. Myenteric ganglion calcium imaging. Spritzer (internal bore, 40 μ m) is indicated by *dotted lines* and myenteric plexus by *solid lines*. **A-C**, Anti-DNP IgE sensitized myenteric neurons were imaged in mast cell-deficient W/W^v mice. **A**, Resting condition. **B**, Fluorescent image captured 0.16 seconds after 20-millisecond spritz. **C**, Four seconds after spritz. **D-F**, No increase in calcium fluorescence was observed in non-haptenated HSA spritz. **G-I**, Positive calcium increases in WBB6F1 control littermates. Time sequence same as previous.

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