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Effect of Pooled Human Intravenous Globulin (IVIG) on the Reversal of Cholinergic Inhibition of Smooth Muscle by Immunoglobulins (IgGs) from Patients with Scleroderma (SSc)

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Backgrounds & Aims

The gastrointestinal (GI) tract is the most common internal organ system affected in SSc. We and others have shown before that the SSc immunoglobulins (IgGs) cause selective blockade of muscarinic type-3 cholinergic (M_3-R) in the GI tract. Presently, there is no effective treatment for SSc although numerous cytotoxic and immunomodulatory agents have been employed with limited success and are marred with serious side effects. Present studies investigated the reversibility of SScIgGs-caused M_3-R blockade by the pooled Intravenous immunoglobulins (IVIG).

Methods

Effects of SScIgGs and IgGs from normal individuals (NIgGs) on M_3-R activation by bethanechol (BeCh) were determined in human internal anal sphincter (IAS) smooth muscle cells (SMCs), before and after IVIG. M_3-R occupancy and binding by the SScIgG was determined via immunofluorescence (IF), Western blotting, and ELISA, respectively. Functional displacement of M_3-R occupancy by the SScIgGs was determined employing different concentrations of the IgGs during the sustained phase of the BeCh-induced contraction of rat IAS smooth muscle strips.

Results

Fig. 1 SSc IgGs Occupy M_3-R thus Reducing M_3-R Immunofluorescence, Reversed by Pooled Human Immunoglobulin (IVIG)

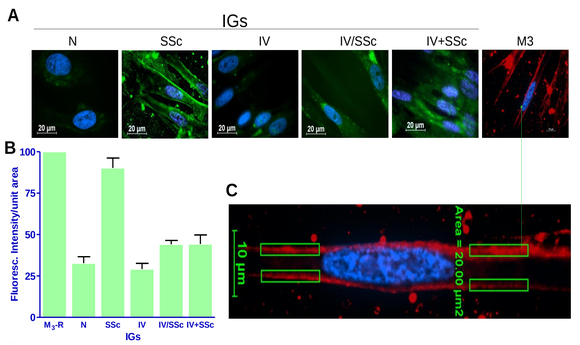


Figure 1. (A) M_3-R occupancy with SScIgG (vs. NIgG and IVIG) in the SMC membrane as determined by IFI/unit area. **(B)** Graph showing SScIgG binding to SMC membrane. **(C.)** Schematics of membrane Intensity calculation.

Fig. 2 Co-localization of SSc IgGs with M_3-R is Blocked by IVIG

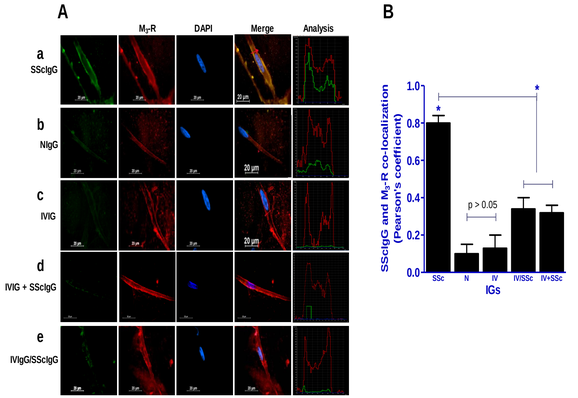


Figure 2. (A) Immunocytochemical co-localization of different IgGs (a,b,c,d,e) (FITC-conjugated; green) and M_3-R (TR-conjugated; red). **(B)** Pearson's coefficient shows significant colocalization of SScIgG and M_3-R.

Fig. 3 SSc IgGs cause Functional Displacement of M_3-R: Reversed by IVIG

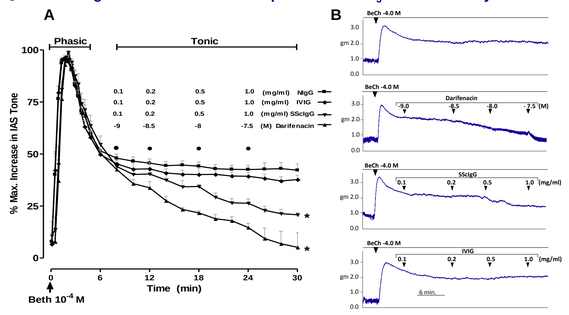


Figure 3. **(A)** SScIgG (and not NIgG and IVIG) in resemblance with darifenacin (M_3-R selective inhibitor) causes significant and conc.-dependent decrease in the BeCh-induced sustained contraction of IAS (*; $p < 0.05$; n = 6). **(B)** Actual tracings of the above effects.

Fig. 4 IVIG Reverses the binding of SScIgGs with second Loop of M_3-R (M_3-RL2)

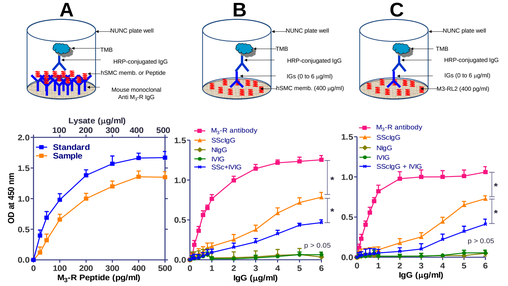


Figure 4. (A) ELISA binding studies for M_3-R peptide and human IAS SMC membrane fraction (HISMF): Standard curves with M_3-R and HISMF. **(B)** Data show M_3-R antibody and SScIgG bind with HISMF in a conc.-dependent manner (*; $p < 0.05$; n = 6), and IVIG significantly decreases this binding. **(C)** Similar data were obtained when M_3-R peptide (M_3-RL2) instead of HISMF was used.

Fig. 5 IVIG Blocks SScIgG Binding with M_3-R

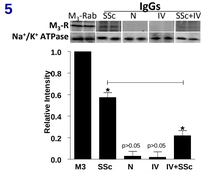


Figure 5. SScIgGs significantly bind with M_3-R (*; $p < 0.05$), in (HISMF). IVIG reverses this binding (*; $p < 0.05$).

Fig. 6

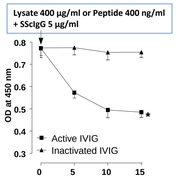
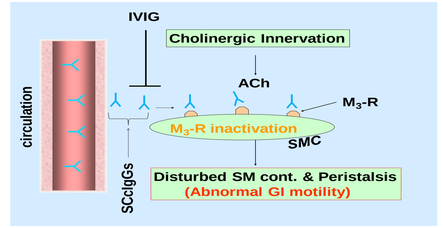


Figure 6. Active IVIG (not inactivated) neutralizes (*; $p < 0.05$) the binding of SScIgG with M_3-RL2 and the HISMF.

Proposed Mechanism of Action of Pooled Human Immunoglobulin (IVIG)



Summary

1. IgGs from scleroderma patients (SScIgGs) inhibit muscarinic type-3 cholinergic (M_3-R) activation, as shown by the data in human IAS smooth muscle cells and rat smooth muscle strips.
2. SScIgGs inhibit M_3-R occupation as shown by immunocytochemistry and Elisa-binding studies.
3. Pooled intravenous globulin (IVIG) reverses the M_3-R occupancy and activation primarily by neutralizing circulating the SScIgGs.

Conclusions

- IVIG abrogates SScIgGs-mediated block of M_3-R by blocking the circulating SScIgGs.
- This mechanism may be partly responsible for the restoration of M_3-R-mediated cholinergic dysfunction in SSc-related GI manifestations.