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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)

Jagmohan Singh  
*Thomas Jefferson University*

Vaibhav Mehendiratta  
*Thomas Jefferson University*

Sergio A. Jimenez  
*Thomas Jefferson University*

Sidney Cohen  
*Thomas Jefferson University*  
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 Anthony J. DiMarino  
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**Authors**

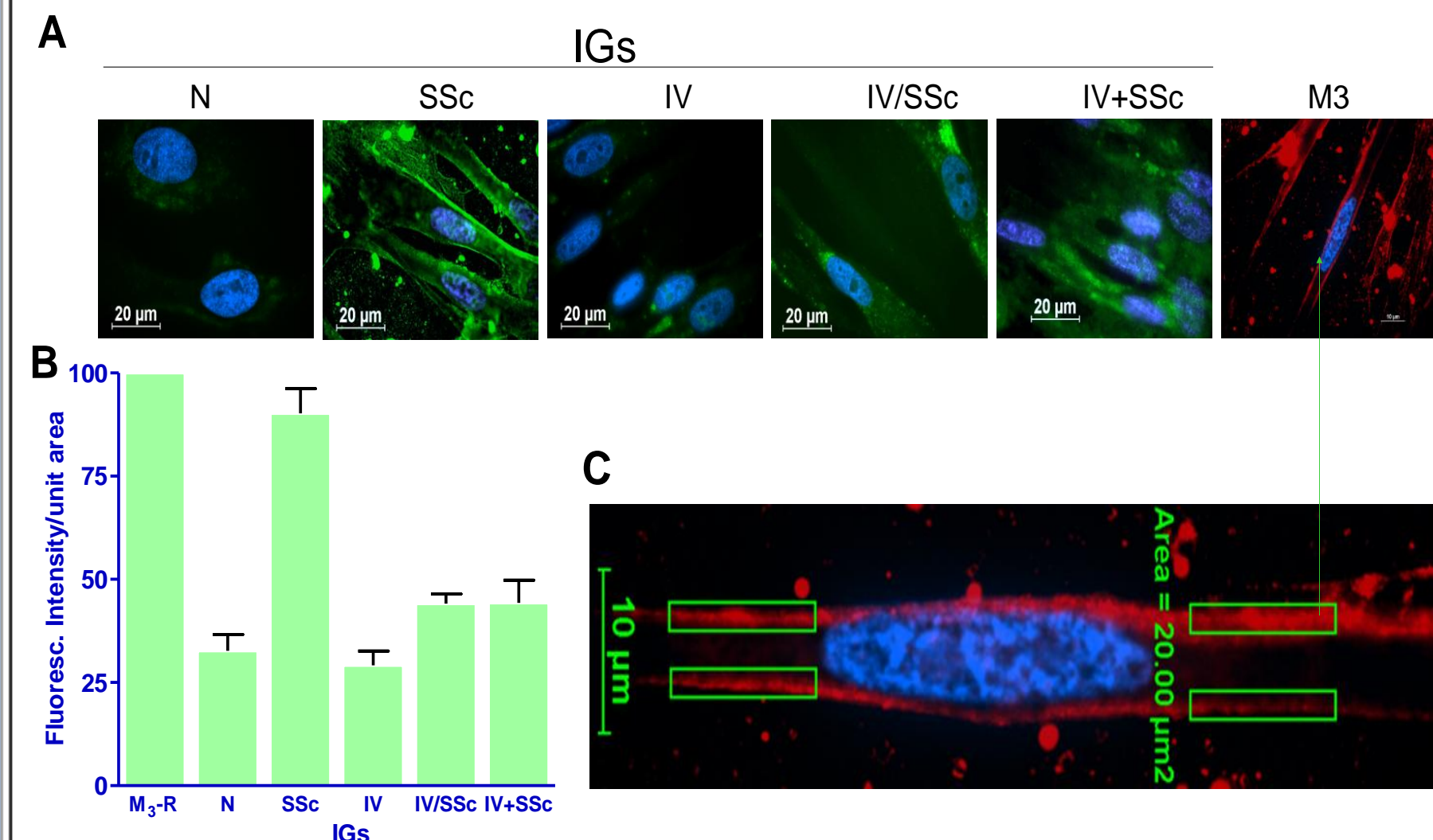
Jagmohan Singh, Vaibhav Mehendiratta, Sergio A. Jimenez, Sidney Cohen, Anthony J. DiMarino, and Satish Rattan

## 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 SSclgGs-caused  $M_3$ -R blockade by the pooled Intravenous immunoglobulins (IVIg).

## Results

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

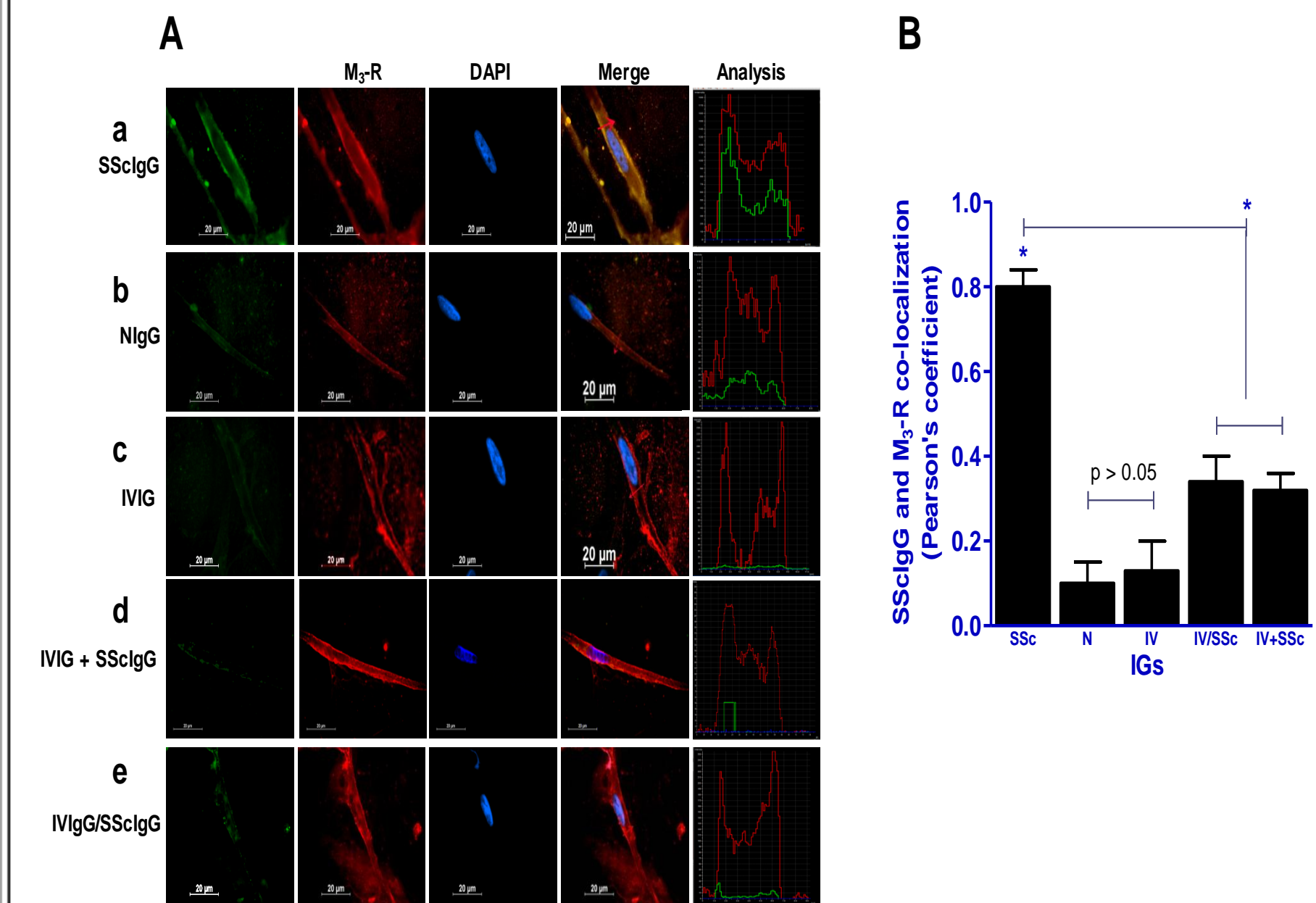


**Figure 1. (A)**  $M_3$ -R occupancy with SSclgG (vs. NlgG and IVIg) in the SMC membrane as determined by IFI/unit area. **(B)** Graph showing SSclgG binding to SMC membrane. **(C)** Schematics of membrane Intensity calculation.

## Methods

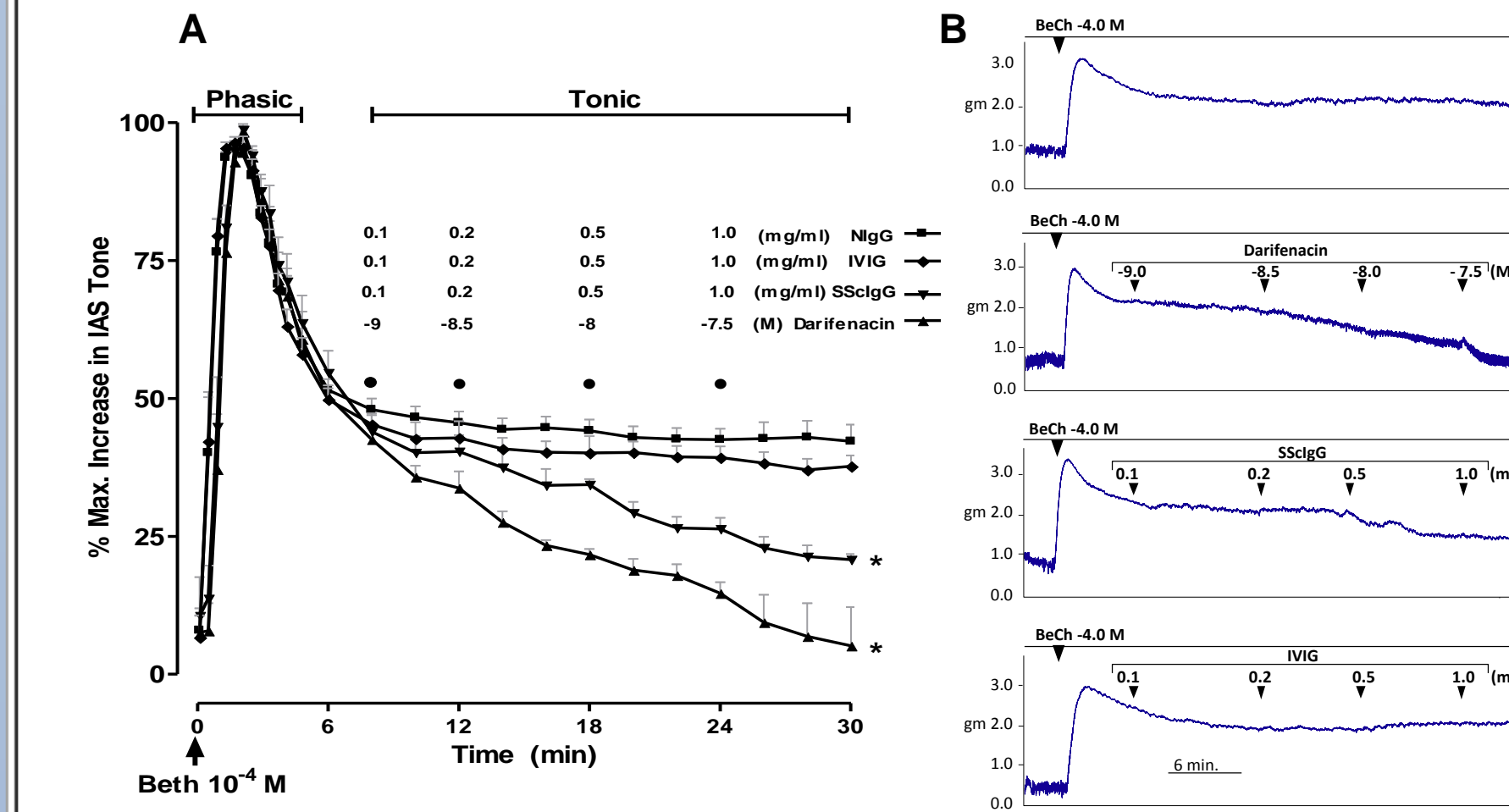
Effects of SSclgGs and IgGs from normal individuals (NlgGs) on  $M_3$ -R activation by bethanechol (BeCh) were determined in human internal anal sphincter (IAS) smooth muscle cells (SMCs), before and after IVIg.  $M_3$ -R occupancy and binding by the SSclgG was determined via immunofluorescence (IF),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

**Fig. 2** Co-localization of SSc IgGs with  $M_3$ -R is Blocked by IVIg



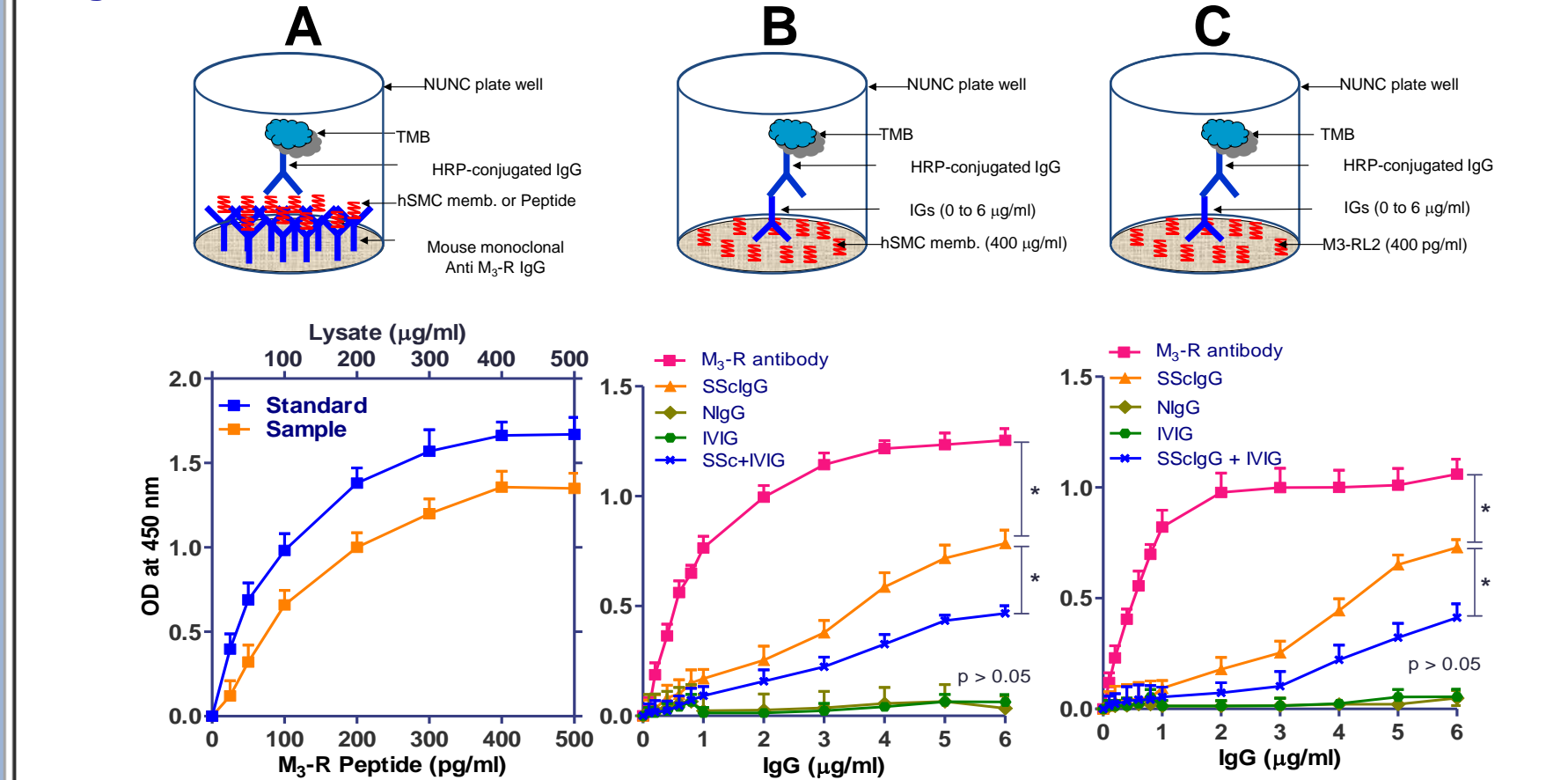
**Figure 2. (A)** Immunocytochemical co-localization of different IgGs (a,b,c,d,e) (FITC-conjugated; green) and  $M_3$ -R (TR-conjugated; red). **(B)** Pearson's coefficient shows significant colocalization of SSclgG and  $M_3$ -R.

**Fig. 3** SSc IgGs cause Functional Displacement of  $M_3$ -R: Reversed by IVIg



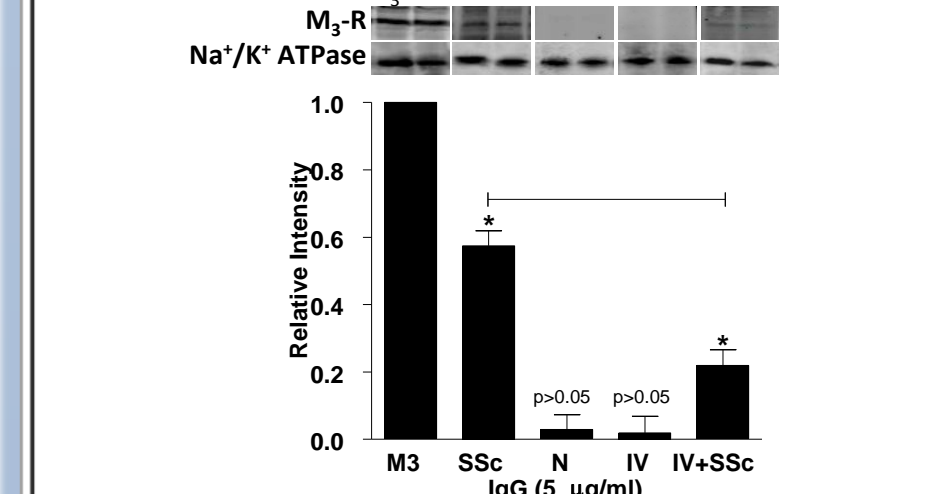
**Figure 3. (A)** SSclgG (and not NlgG and IVIg) in resemblance with darifenacin ( $M_3$ -R selective inhibitor) causes significant and conc.-dependent decrease in the BeCh-induced sustained contraction of IAS (\*;  $p < 0.05$ ;  $n = 6$ ). **(B)** Actual tracings of the above effects.

**Fig. 4** IVIg Reverses the binding of SSclgGs with second Loop of  $M_3$ -R ( $M_3$ -RL2)



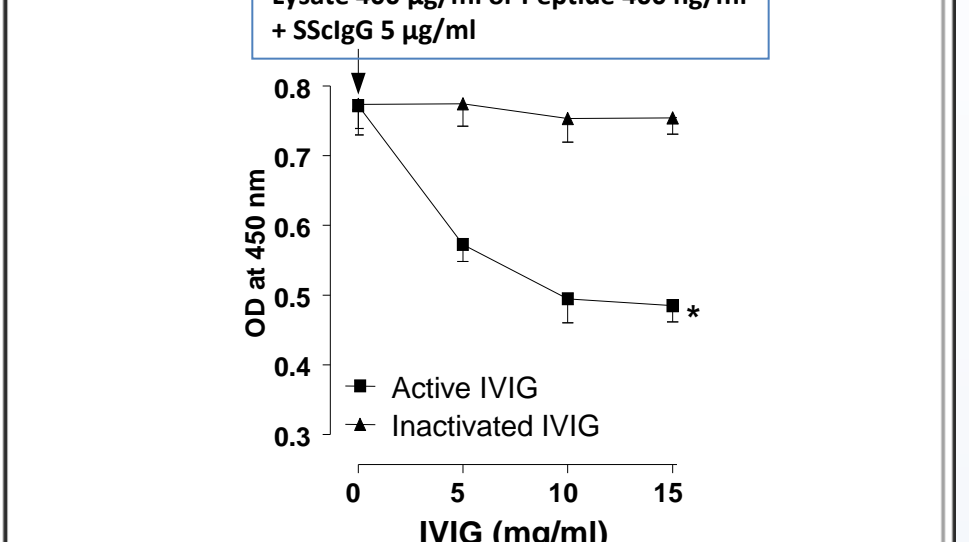
**Figure 4. (A)** ELISA binding studies for  $M_3$ -R peptide and human IAS SMC membrane fraction (HISMf): Standard binding curves with  $M_3$ -R and HISMf. **(B)** Data show  $M_3$ -R antibody and SSclgG bind with HISMf in a conc.-dependent manner (\*;  $p < 0.05$ ;  $n = 6$ ), and IVIg significantly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

**IVIg Blocks SSclgG Binding with  $M_3$ -R**  
**Fig. 5**



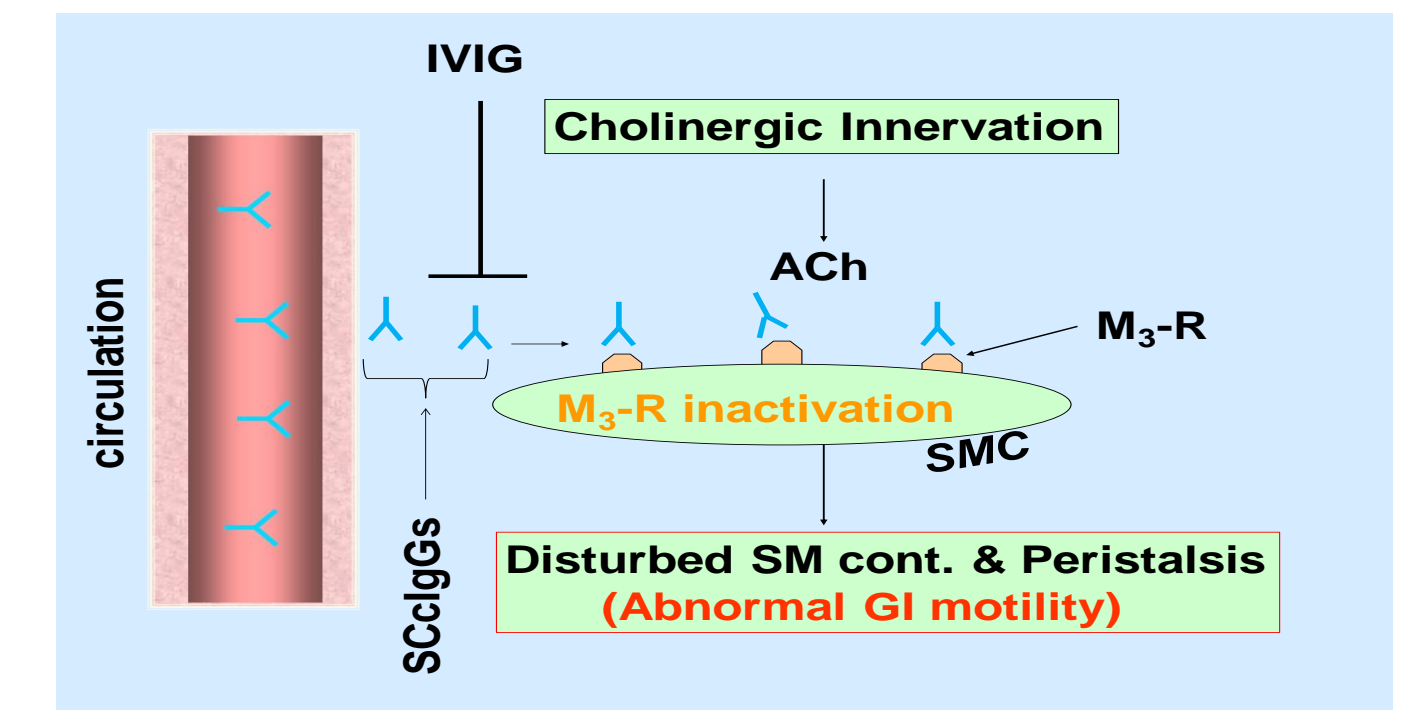
**Figure 5.** SSclgGs significantly bind with  $M_3$ -R (\*;  $p < 0.05$ ), in (HISMf). IVIg reverses this binding (\*;  $p < 0.05$ ).

**Fig. 6**



**Figure 6.** Active IVIg (not inactivated) neutralizes (\*;  $p < 0.05$ ) the binding of SSclgG with  $M_3$ -RL2 and the HISMf.

## Proposed Mechanism of Action of Pooled Human Immunoglobulin (IVIg)



## Summary

1. IgGs from scleroderma patients (SSclgGs) 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. SSclgGs 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 SSclgGs.

## Conclusions

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