Epitopes, Specificity, IgG Subclasses and Functional Effects of Anti-Calcium-Sensing Receptor Autoantibodies in Patients with Autoimmune Polyendocrine Syndrome Type 1

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Background
- Autoimmune polyendocrine syndrome type 1 (APS1) is a rare disorder caused by mutations in the autoimmune regulator (AIRE) gene [1].
- Major diseases are chronic mucocutaneous candidiasis (100% of APS1 patients), hypoparathyroidism (80%), and Addison’s disease (70%).
- Pathology includes chronic inflammation of internal organs and organ-specific and anti-cytokine (e.g., IFN-α and IFN-ω) antibodies.
- Autoantibodies against the calcium-sensing receptor (CaSR) (Figure 1), which is highly expressed on the parathyroid, are found in 36% of patients with APS1 [2].

Details of APS1 patients
AIRE mutations: 15 R257X homozygotes; 1 R257X/967-979del13 compound heterozygote.
Antibodies against IFN-α, 15/16 APS1 patients; IFN-ω, 15/16; IFN-α, 2/16; IL-22, 16/16; IL-17F, 14/16; IL-17A, 13/16; CaSR, 16/16.
Disease components: chronic mucocutaneous candidiasis, 16/16 APS1 patients; hypoparathyroidism, 15/16; Addison’s disease, 16/16; alopecia 6/16; vitiligo, 2/16; keratitis, 5/16; hypogonadism, 6/16; type 1 diabetes mellitus, 4/16; autoimmune thyroid disease, 2/16.

Anti-CaSR autoantibody functional effects
- Investigated in CaSR peptide ELISAs with IgG subclass-specific secondary antibodies.
- Anti-CaSR autoantibodies recognising epitope 1 (41-69), epitope 2 (171-195), and epitope 4 (260-340) were of the IgG1 subclass.
- Anti-CaSR autoantibodies recognising epitope 2 (114-126) were of the IgG1 and IgG3 subclasses.

Aims
- To characterise anti-CaSR autoantibodies in APS1 patients in relation to:-
  • Epitopes (binding sites)
  • Specificity
  • IgG subclass
  • Effects on CaSR function

Anti-CaSR autoantibody IgG subclass
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Anti-CaSR autoantibody specificity
Figure 3: APS1 patient sera were pre-absorbed with a panel of CaSR peptides prior to measuring CaSR binding reactivity in a specific CaSR peptide ELISA. The results are shown for antibody binding in a CaSR peptide 114-126 ELISA which indicated that antibodies against epitope 2 were specific for that binding site. Similar results were obtained for all four identified epitopes.

Figure 4: Effect of APS1 patient IgG on the response of the CaSR to Ca
2+ stimulation by measuring inositol phosphate (IP1) accumulation in HEK293-CaSR cells. The results showed that IgG from two patients stimulated significantly IP1 accumulation when compared with control IgG at [Ca
2+] of 0.5, 1.5 and 3 mM (P values < 0.05).

Figure 5: Effect of APS1 patient IgG on the response of the CaSR to Ca
2+ stimulation by measuring inositol phosphate (IP1) accumulation in HEK293-CaSR cells. The results showed that IgG from two patients stimulated significantly IP1 accumulation when compared with control IgG at [Ca
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Conclusions
- Anti-CaSR autoantibody binding sites are located in the surface accessible ECD of the receptor.
- Anti-CaSR autoantibodies are mainly of the IgG1 subclass. This subclass of antibody can activate complement and bind to Fcy receptors and therefore cause cellular damage. This aspect requires further investigation in relation to the parathyroid.
- A minority of APS1 patients have anti-CaSR autoantibodies that can activate the CaSR. Further studies are required to determine if these CaSR-stimulating antibodies can prevent PTH secretion from parathyroid cells.

References

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