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Downsizing Proteins Without Losing Potency or Function

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Introduction

Human complement protein C3a (Figure 1) is produced after activation of a complex network of plasma and membrane proteins that constitute the complement system, named for their combined capacity to complement antibody-mediated immune defense [1,2]. C3a itself is an inflammogen, probably best known for its ability to attract (chemotaxis) and degranulate certain immune cells which contain granules that release inflammatory stimuli like histamine, tryptase, heparin and other enzymes most commonly associated with allergies, asthma and acute inflammatory responses [3-8]. We have used this inflammatory protein, which is rapidly degraded in plasma, as a test case to downsize a protein to plasma-stable small molecules that mimic the potent and selective functions of the full length C3a protein. Here we summarize the principle and effectiveness of this idea, which starts with a functionally important amino acid in C3a and rationally grows it into functional surrogates for C3a. We compare activity profiles for the resulting peptidomimetics versus human C3a, all compounds binding to a specific G protein coupled receptor (C3aR) expressed on the plasma membrane surface of human immune and other cell types [9,10].

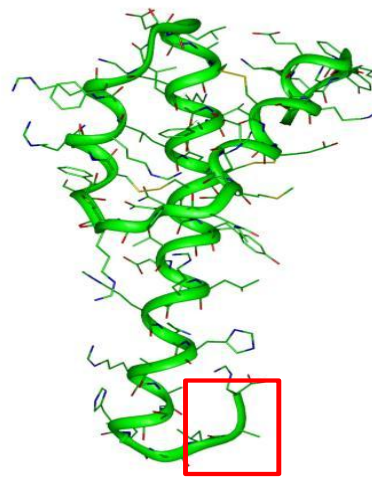

Fig. 1. Human C3a is 77 amino acids with its GPCR-activating C-terminus boxed.

Results and Discussion

The *C*-terminal arginine residue of C3a has been reported to be important for binding to and activating human C3aR [11]. Receptor mutagenesis is consistent with C3a interacting through its guanidinium side chain of Arg77 contacting Asp417 while its *C*-terminal carboxylate contacts Arg161 and Arg340 [12]. Moreover removal of this Arg77 residue from C3a dramatically reduces C3aR binding affinity and agonist efficacy. Our novel approach, inspired by our previous work with ascidiacyclamide and thiazole peptides [13-15], incorporates different dipeptide mimics (Figure 2) into the *C*-terminal tripeptide segment (Leu-Ala-Arg) of C3a, using a range of heterocycles as conformational constraints (Figure 3). These heterocyclic dipeptide mimics confer potent C3aR agonist or antagonist potencies.

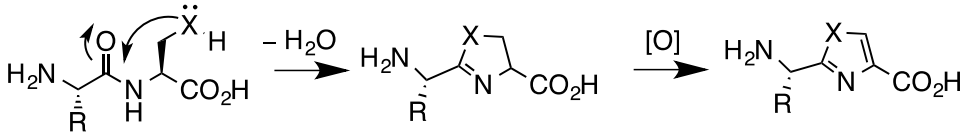

Fig. 2. Heterocyclic dipeptide mimetics derived from dipeptides.

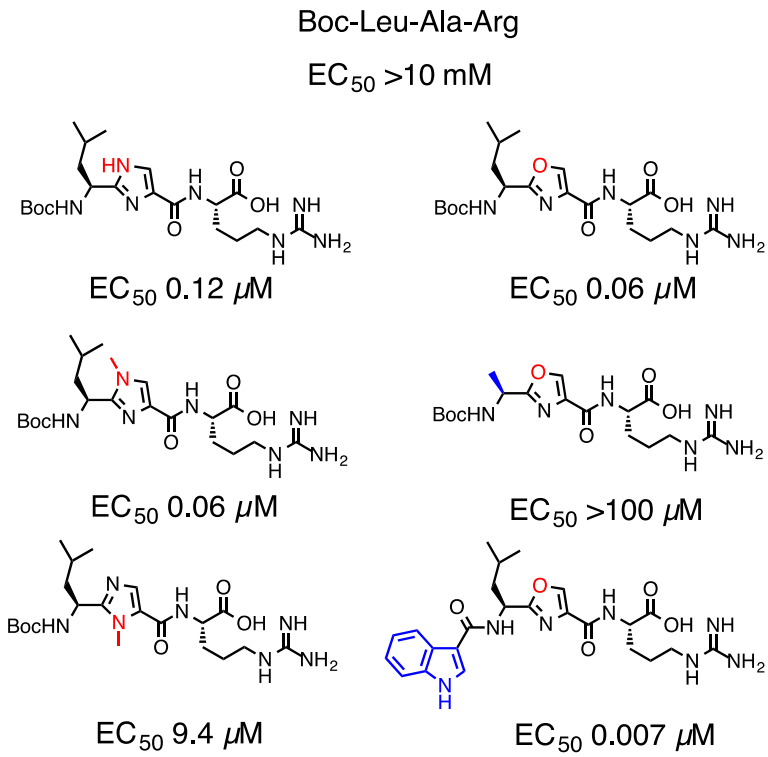

Fig. 3. Agonist potency of peptidomimetics measured by Ca^{2+} release in human monocyte-derived macrophages relative to the tripeptide Boc-Leu-Ala-Arg and hC3a (EC_{50} 40 nM).

We found that the nature of the heterocycle profoundly affected compound activity. For example, a hydrogen-bond accepting nitrogen conferred agonist activity, with much greater potency for imidazoles and oxazoles (Figure 3) than for oxadiazoles, furans and other heterocycles. Interestingly, there was a linear correlation [9,10] between the C3aR-binding affinity (measured by competition with ^{125}I-C3a) and the calculated hydrogen-bond acceptor interaction energy (kcal mol^{-1}) between water and heteroatom of heterocycles compared with the water dimer (determined using *ab initio* methods MP2/6-311++G(3d,3p) and corrected for the basis set superimposition error within Gaussian 09). This enabled us to tune agonist potency by rational variation of the heterocyclic component incorporated into the dipeptide mimetics, coupled with changes to other substituents. All of these compounds were far more stable in rat plasma (unchanged after 2h) than C3a (undetectable after 10 mins), suggesting their use as agonist surrogates for C3a *in vitro* and possibly *in vivo*.

However, the above information only relates to one functional measure of comparable agonist activity for these ligands compared to human C3a. The most potent agonists were therefore further examined for other agonist properties typically exhibited by human C3a. We found that the most potent small molecule agonists also displayed comparable profiles of agonist function and potency to human C3a in other assays such as chemotaxis (migration), ERK phosphorylation, inflammatory gene expression (*TNF, IL1β, IL8, CCL3, PTGS2, IL6, FOSB, EGR1*) in human macrophages; mast cell activation and degranulation; and neutrophil migration and activation. These and other activities gave us confidence to examine the agonists in many other *in vitro* and *in vivo* assays to stimulate the C3a receptor and anticipate that the responses would be similar to those of C3a, which is only maintained intact at or near the cell surface where it is formed during complement activation.

However, when a thiazole was incorporated into these peptidomimetics there was an interesting finding of either agonist or antagonist activity, depending upon the location of the sulfur and nitrogen atoms relative to the adjacent amide carbonyl group (Figure 4). When the thiazole nitrogen was adjacent to the carbonyl, agonist activity was observed. When the thiazole sulfur was adjacent to the carbonyl the compound was instead an antagonist. The latter is attributed [16] to orbital interaction between the sulfur and oxygen, indicated by the distance between sulfur and oxygen being less than the sum of the van der Waals radii, and different δ+…δ- dipole alignments (Figure 4).

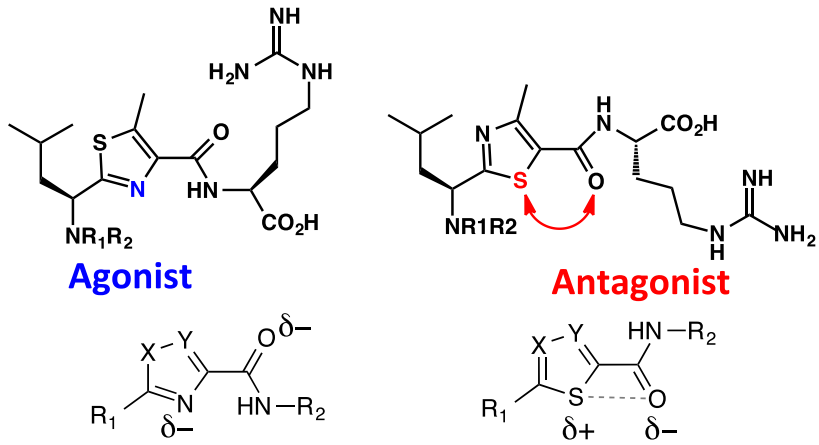

Fig. 4. Heterocycle can control conformation of adjacent amide and hence projection of arginine, dictating agonist versus antagonist action.

As a consequence of these findings, we have been able to gain access to both potent agonists (e.g. Figure 3) and antagonists (e.g. Figure 5) which can be used to probe the effects of modulating the C3a receptor in a range of human and rodent cells and in animal models of C3aR-mediated inflammation and disease. Indications alluded to in the seminar are that antagonists are far more potent than the known feeble antagonist SB290157 [17], in macrophages, mast cells and neutrophils and in animal models such as the rat paw oedema induced by small stable C3aR agonists. Oral delivery of C3aR antagonists (5-20 mg/kg doses) to rats prior to intraplantar administration of C3aR agonist (350 µg/paw) was able to inhibit the resulting acute inflammation manifested by paw swelling, mast cell activation and degranulation at 30 mins, neutrophil infiltration and activation at 6h, and inflammatory gene and protein expression induced by C3aR agonists *in vivo*.

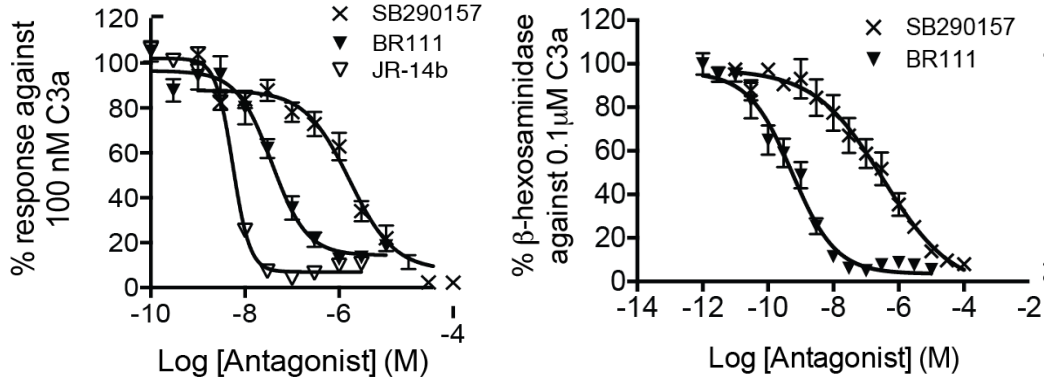

Fig. 5. Antagonism by heterocyclic peptidomimetics of C3a-induced (left): Ca^{2+}release in human monocyte-derived macrophages, (right): β-hexosaminidase release in LAD2 human mast cells.

Acknowledgments

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