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<td>Published Version</td>
<td>doi:10.3389/fimmu.2015.00445</td>
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Cloning of the human C5a anaphylatoxin receptor, and more

Norma P. Gerard1,2 and Craig Gerard1

1 Ina Sue Perlmutter Laboratory, Division of Respiratory Diseases, Department of Medicine, Children’s Hospital, Harvard Medical School, Boston, MA, USA, 2 Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA, USA

Keywords: C5aR/C5R1, 7-TMS receptors, complement anaphylatoxin, C5a, G protein coupled receptor

The initial observation in 1973 that the complement C5 activation product, C5a, has the ability to stimulate human neutrophils led to the concept of a specific C5a receptor (1). In the mid-1980s, a groundbreaking paper published in Nature announced the cloning of the beta-adrenergic receptor, and for the first time established seven transmembrane (7TM) G protein-coupled receptors as members of the rhodopsin superfamily (2). Almost simultaneously, Feltner and colleagues demonstrated that the FMLP, C5a, and LTE4 activities on rabbit neutrophils could be inhibited by pertussis toxin, indicating coupling to GTP binding proteins (3). In 1987, Masu and colleagues used an oocyte expression cloning system to isolate a cDNA encoding the neuropeptide substance K receptor; and later, the same group identified the related substance P receptor (4, 5).

In 1989, we realized that the pertussis sensitivity of the FMLP, C5a, and LTE4 receptors suggested that they would also be members of the rhodopsin superfamily. When we aligned the handful of structures for the then known 7TM receptors (adrenergic, serotonin, dopamine, FSH/LH, and substance P and K receptors), we recognized homologies in both the transmembrane segments and intracellular loops, which presumably facilitated interactions with G proteins. This observation actually presented us with an opportunity to attempt to clone receptors by homology to the superfamily as “orphan receptors.”

We constructed an antisense oligonucleotide with minimal degeneracy that encompassed a highly conserved NPXXY motif in the seventh transmembrane segment of the known rhodopsin family members. In order to enrich in C5a receptors, we took advantage of the fact that the receptors were induced by cyclic-AMP in U937 cells, and in retinoic acid differentiated human HL60 cells. By summer of 1990, we had isolated ~20 cDNAs using this approach from the cAMP induced U937 cell library. About half of these clones were an identical cDNA that we named NPIIY-18. Using this as a probe, we demonstrated that NPIIY-18 recognized a ~2.2 kb mRNA only in cAMP differentiated U937 cells. “Orphan receptors.”

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In November 1990, within months of the identification of the human FPR1, Thomas et al. reported the cloning of the rabbit receptor for FMLP, F3R (9), which had almost no significant homology to the human receptor. This was troubling, because we knew that when the ligand...
was identical from species to species, the receptors were generally highly conserved. Thus, the adrenergic, dopamine, serotonin, and histamine receptors are >90% identical across species. Curiously, the cDNA was reported to bind the FPR1 radioligand and transduce calcium transients. Because the expression of the claimed rabbit F3R formyl peptide receptor was restricted to neutrophils, we wondered if, in fact, the Navarro lab had misidentified an interleukin-8 receptor. One of us (Craig Gerard) actually traveled to the Navarro lab to obtain the F3R cDNA to establish a collaboration and test its identity as a receptor for IL-8. At that time, there was no radioligand IL-8 commercially available. Henry Showell, of Pfizer Central Research, was able to provide us with a custom iodinated IL-8, which we demonstrated to bind F3R. Unfortunately, we did not have sufficient quantities of the reagent to perform comprehensive studies to publish our findings. We disclosed our result to Javier Navarro, but were left in silence. Unbeknownst to us, Dan Witt, at Repligen, had reportedly approached the Navarro lab with a similar idea. Thomas et al. went on to publish F3R as an IL8 receptor, without retracting the previous paper (10). During this time, Tom Schall and I met at a FASEB meeting with Phil Murphy, and suggested to him that he use F3R to clone a human homolog from HL60 cells and test it against IL8. The

References