Molecular Cloning and Characterization of the Human Anaphylatoxin C3a Receptor*

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In a human neutrophil cDNA library, an orphan G-protein-coupled receptor, HNFAG09, with 37% nucleotide identity to the C5a receptor (C5a-R, CD88) was identified. A novel feature of this gene, unlike C5a-R and other G-protein-coupled receptors, is the presence of an extraordinarily large predicted extracellular loop comprised of in excess of 160 amino acid residues between transmembrane domains 4 and 5. Northern blot analysis revealed that expression of mRNA for this receptor in human tissues, while similar, was distinct from C5a-R expression. Although there were differences in expression, transcripts for both receptors were detected in tissues throughout the body and the central nervous system. Mammalian cells stably expressing HNFAG09 specifically bound 125I-C3a and responded to a C3a agonist with a robust calcium mobilization response. HNFAG09 encodes the human anaphylatoxin C3a receptor.

During complement activation the 74–77-amino acid anaphylatoxins C3a, C4a, and C5a are released. They are potent inflammatory mediators, inducing cellular degranulation, smooth muscle contraction, arachidonic acid metabolism, cytokine release, and cellular chemotaxis (reviewed in Refs. 1–3). The C3a carboxyl-terminal analogue synthetic peptide (WWKKKYRASKLGLAR) (9) was obtained from Bachem Bioscience, Rockville, MD. Human rC5a was expressed in Escherichia coli and purified to homogeneity. Other agonists were obtained from Sigma.

cDNA Cloning—cDNA library construction and screening were carried out essentially as described (10), and DNA sequence was determined using a ABI sequencer (11). Expressed sequence tag analysis revealed that expression of mRNA for this receptor in human tissues, while similar, was distinct from C5a-R expression. Although there were differences in expression, transcripts for both receptors were detected in tissues throughout the body and the central nervous system. Mammalian cells stably expressing HNFAG09 specifically bound 125I-C3a and responded to a C3a carboxyl-terminal analogue synthetic peptide and to human C3a but not to rC5a with a robust calcium mobilization response. HNFAG09 encodes the human anaphylatoxin C3a receptor.

The abbreviations used are: PCR, polymerase chain reaction; kb, kilobase; PBL, peripheral blood leukocytes.

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Results and Discussion

Expressed sequence tag analysis (11–13) of cDNA clones derived from a human neutrophil (lipopolysaccharide activated) cDNA library identified a clone demonstrating significant homology (approximately 40% amino acid sequence identity) to the C5a-R. This expressed sequence tag contained an incomplete open reading frame that therefore was used to reprobe the neutrophil cDNA library to obtain a 2040-basepair cDNA encoding a complete orphan G-protein-coupled receptor of 482 amino acids, which shared 37% nucleotide identity throughout the coding regions with the C5a-R (Fig. 1A). Although similar to the C5a-R, this cDNA contains two predicted extracellular N-linked glycosylation sites and an unusually large extracellular domain between transmembrane domains 4 and 5 comprised of over 160 amino acid residues (Fig. 1A). The majority of the identical residues between the C5a-R and HNFAG09 reside in the predicted transmembrane spanning domains and in the second intracellular loop (Fig. 1B).

By Northern blot analysis, expression of HNFAG09 in human tissues and cell lines is distinct from C5a-R expression. An ~2.2-kb C5a-R transcript was abundantly expressed in peripheral blood leukocytes (PBL), lung, spleen, heart, placenta, spinal cord, and throughout the brain. An ~2.1-kb HNFAG09 transcript was predominantly expressed in lung, spleen, ovary, placenta, small intestine, throughout the brain, and to a much lesser extent than C5a-R, in heart and PBL (Fig. 2). Although by Northern blot analysis the specific cells within the various tissues examined, which are expressing C5a-R and HNFAG09, cannot be determined, these data are suggestive that these receptors are abundantly expressed throughout the body. By fluorescent activated cell sorting using polyclonal antibodies generated to fusion proteins composed of glutathione S-transferase or maltose binding protein and the extracellular loop, this receptor has been shown to be expressed on several cell types, including U937, HL-60, PBL, and human neutrophils and monocytes (8).

Preliminary functional characterization in Xenopus laevis oocytes suggested that HNFAG09 encoded a human anaphylatoxin receptor. To confirm these results in mammalian cells, this receptor was expressed in RBL-2H3 cells (19), a rat basophil cell line, which when transfected with an expression plasmid encoding the C5a-R expresses receptors that are functionally active (17). RBL-2H3 cells were stably transfected with mammalian expression plasmids encoding the C5a-R or HNFAG09, and Fura 2-loaded cells were tested for a C5a- or C3a-induced mobilization of intracellular Ca2+. C5a-R but not

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**Fig. 2.** C5a-R and HNFAG09 transcripts are abundantly expressed in the central nervous system and throughout the body. Tissue distribution of C5a-R and HNFAG09 as determined by Northern blot analysis. The tissue source of RNA is indicated above each lane.

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2 R. S. Ames, P. Nuthulaganti, and C. Kumar, unpublished observation.
A robust response to a C3a carboxyl-terminal analogue synthetic peptide (WWGKKYRASKLGLAR) (9) (EC$_{50}$ = 3.9 nM) was detected in cells expressing HNFAG09, but no response was obtained for C5a-R-expressing cells (Fig. 3, D and C, respectively). Similarly, HNFAG09 but not C5a-R expressing RBL-2H3 cells also responded to native human C3a (EC$_{50}$ = 0.3 nM; data not shown).

C3a was radiiodinated and used in whole cell binding assays to further characterize HNFAG09. Binding of $^{125}$I-C3a to HNFAG09 expressing RBL-2H3 cells was competed by increasing concentrations of C3a (IC$_{50}$ = 3.0 nM) and the C3a analogue synthetic peptide (IC$_{50}$ = 155 nM) but not by rC5a (Fig. 3E). By saturation binding and Scatchard analysis a single class of C3a binding sites was identified with an estimated Kd of 0.3 nM and a B$_{max}$ of 32,000 receptors/cell (data not shown). Curiously, a HEK 293 cell line stably expressing HNFAG09 mRNA by Northern blot neither bound nor responded to C3a (data not shown).

RBL-2H3 cells expressing HNFAG09 bind and respond to C3a and a C3a analogue synthetic peptide but not C5a. These data, along with the results of the tissue distribution analysis, are consistent with HNFAG09 (AZ3B) (8) encoding the human C3a receptor.

The demonstration that C5a-R (reviewed in Ref. 20) and C3a-R expression is not limited to myeloid cells but that they both are expressed in a variety of non-myeloid cells throughout the body and that they are abundantly expressed in the central nervous system is consistent with these receptors having a much greater role in the pathogenesis of inflammatory and autoimmune diseases than previously suspected. Now that the receptor for C3a has been identified, further studies to elucidate the role of C3a in immune function and disease will be facilitated.

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Fig. 3. Cells expressing HNFAG09 but not C5a-R bind and respond to C3a. Calcium mobilization by Fura 2-loaded cells expressing C5a-R (A and C) or HNFAG09 (B and D) in response to rC5a (10 nM (A) or 100 nM (B)) or C3a analogue peptide (1 μM, C and D) is shown. E, competition of $^{125}$I-C3a binding to HNFAG09 expressing RBL-2H3 cells by increasing concentrations of C3a analogue synthetic peptide (closed circle), C3a (open square), or rC5a (open triangle).
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