In *Xenopus* oocytes the human C3a and C5a receptors elicit a promiscuous response to the anaphylatoxins

Robert S. Ames[a,*], Paru Nuthulaganti[b], Chandrika Kumar[b]

[a]*Department of Molecular Immunology, SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, PA 19406-0939, USA*
[b]*Department of Molecular Genetics , SmithKline Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, PA 19406-0939, USA*

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Abstract The *Xenopus laevis* oocyte has been widely utilized for cloning and functional expression of G-protein coupled receptors (GPCR). This system was used for the functional expression and characterization of the recently identified human C3a receptor. Complementary RNA from the human C3a receptor was transcribed in vitro and microinjected into *Xenopus* oocytes for functional characterization. A positive response to a synthetic C3a peptide agonist and to C3a, but not to platelet activating factor or fMetLeuPhe was detected. In addition, a response of approximately one third the amplitude obtained with C3a was obtained with rC5a. Conversely, oocytes co-injected with the C5a receptor and total RNA isolated from U937 cells responded to C5a as well as to C3a and the C3a synthetic peptide. A functional response with the anaphylatoxin C3a receptor in oocytes was dependent on co-injection of a pertussis toxin sensitive complementary human factor which could be supplied by co-injection of total RNA isolated from U937 cells. Oocytes expressing the anaphylatoxin C3a and C5a receptors responded to both agonists, in each case the response to the cognate ligand was substantially more robust than the response elicited by the other anaphylatoxin.

Key words: C3a receptor; C5a receptor; *Xenopus laevis* oocyte; C3a; C5a; Anaphylatoxin

1. Introduction

The chemotactic receptors for C5a and the fMLF receptor have previously been functionally expressed in *Xenopus laevis* oocytes [1–4] and, unlike all other G-protein coupled receptors (GPCRs) tested in this system, injection of cRNA for the cloned chemotactic receptors alone is insufficient to stimulate signal transduction in response to ligand binding [1,2]. Functional expression of both of these chemotactic receptors, as monitored by induction of Ca^{2+} mediated chloride currents, requires the co-injection of a complementary human factor which can be supplied by total RNA from myeloid or liver cells [1,2]. The co-factor is required for receptor-mediated signal transduction but not for expression of the C5a receptor or fMLF receptor on the surface of the oocytes [4]. The nature of the co-factor is unknown but it does not appear to be an α subunit of G_{i1}, G_{i2} or G_{i3}, since co-injection of cRNA for fMLF receptor along with these α subunits did not induce a functional response [2]. In independent studies, the co-factor has been shown to be encoded by an approximately 3–3.5 kb transcript [2,5]. A recent report demonstrated that Gα-16 complements the signal transduction cascade of both the C5a and fMLF receptors, and it has been proposed to be the complementing co-factor present in HL-60 and U937 [6].

The receptor for the human complement fragment C3a has recently been cloned and shown to be a seven transmembrane spanning GPCR with high sequence homology to the receptors for fMLF and C5a [7,8]. We have compared the functional response elicited by the C3a and C5a receptors in *Xenopus laevis* oocytes and have determined that both of the anaphylatoxins elicit a functional response in oocytes expressing either receptor, however, in each case the cognate ligand elicits a markedly more robust response than the other anaphylatoxin. Functional expression of the C3a receptor in *Xenopus* oocytes is dependent on the presence of a pertussis toxin sensitive co-factor which can be supplied by co-injection of total RNA isolated from U937 or HL-60 cells.

2. Materials and methods

2.1. Materials

The C3a carboxy-terminal analogue synthetic peptide, (WWGKKYRASKLGLAR [9]) was obtained from Bachem Bioscience, Inc., King of Prussia, PA. C3a was purchased from Advanced Research Technologies, San Diego, CA. Human rC5a was expressed in *E. coli* and purified to homogeneity. Other agonists and pertussis toxin were obtained from Sigma, St. Louis, MO. The C3a receptor cDNA (HNFAG09) was isolated from a human neutrophil (lipopolysaccharide activated) cDNA library [7]. The C5a receptor cDNA was isolated via PCR from RNA isolated from dimethyl sulfoxide differentiated U937 cells.

2.2. Receptor expression and functional studies in Xenopus oocytes

Capped cRNA transcripts were generated from linearized C3a receptor and C5a receptor plasmid DNA as previously described [10] and suspended in sterile water at a concentration of 0.2 μg/μl. Ovarian lobes were surgically removed from adult female *Xenopus laevis* frogs and defolliculated. Stage V oocytes were harvested by manual dissection [11]. Oocytes were microinjected with C3a receptor or C5a receptor cRNA transcripts (10 ng/oocyte) with or without U937 total RNA (25 ng/oocyte) in a 50 nl volume using a Drummond microinjection apparatus and maintained in modified Barth's saline [12] at 18°C. Electrophysiological recordings from an average of 10 oocytes were made 48 h later at room temperature using the two electrode voltage clamp (Warner Instruments) technique. Some of the oocytes were treated with 2 μg of pertussis toxin for the last 24 h of the incubation. Membrane potentials were routinely clamped at −60 mV. Results represent average response determined in three separate experiments.

3. Results and discussion

Xenopus oocytes were used to functionally characterize the recently identified human C3a receptor. A C3a carboxy-terminal analogue synthetic peptide (WWGKKYRASKLGLAR [9]), and to a lesser extent rC5a, but not platelet activating factor or the chemotactic peptide fMLF, elicited an electrophysiological response in oocytes co-injected with cRNA for the C3a receptor and total U937 RNA (Fig. 1). Like the C5a and the fMLF receptors, the response elicited by the C3a

*Corresponding author. Fax: (1) (610) 270-5093.

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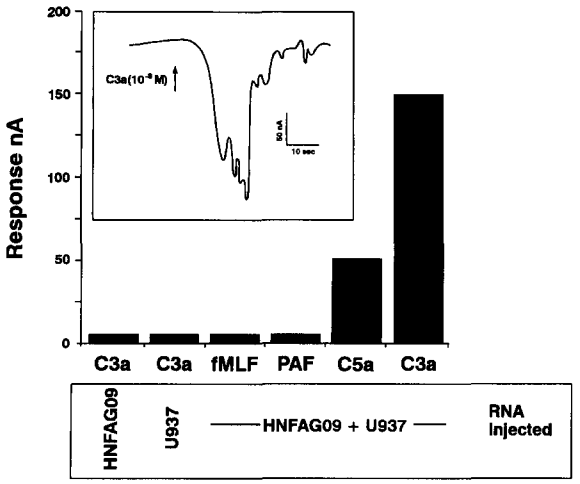

Fig. 1. Oocytes expressing C3a receptor respond to both C3a and C5a. Electrophysiological response of *Xenopus* oocytes injected with cRNA for C3 receptor (HNFAG09, 10 ng), total U937 RNA (25 ng), or a mixture of C3a receptor (HNFAG09, 10 ng) and U937 RNA (25 ng) to 10 nM rC5a, 10 nM C3a analogue peptide, 10 nM platelet activating factor (PAF), 10 nM fMetLeuPhe. Inset presents typical response of oocytes co-injected with C3a receptor cRNA+U937 RNA to 10 nM C3a.

receptor was dependent on the co-injection of a co-factor present in total U937 RNA, as C3a receptor cRNA or U937 RNA when injected alone did not elicit any response to the C3a synthetic peptide or rC5a (Fig. 1). The exact nature of the co-factor is not known, however as the response to the C3a peptide of oocytes co-injected with C3a receptor and U937 RNA was abolished by pertussis toxin (Fig. 2), it does not appear to be Gα-16, which lacks a pertussis toxin ADP-ribosylation site [13]. Gα-16 is encoded by a 2.2 kb [13] transcript, a size significantly smaller than the 3.5 kb RNA fraction which complements the C5a and fMLF receptor expression [2,5]. Although Gα-16 is present in myeloid cells and can complement the functional expression of the C5a and fMLF receptors in *Xenopus* oocytes, there appears to be a complementary factor present in total RNA from HL-60 and U937 which is distinct from Gα-16.

C5a induced rapid activation of chloride currents in C5a receptor cRNA plus U937 RNA co-injected oocytes (Fig. 3). In addition, in oocytes co-injected with the C5a receptor and total U937 RNA, there was also a smaller response to C3a (Fig. 3).

Both the C5a and C3a receptors elicited in oocytes a promiscuous response to the two anaphylatoxins. The responses detected with the C3a synthetic peptide were not an artifact of using the analogue peptide, as the native ligand elicited similar responses in oocytes injected with either receptor (data not shown).

In contrast to the promiscuous functional activity of the anaphylatoxin receptors expressed in *Xenopus* oocytes, RBL-2H3 rat basophils expressing the C3a or C5a receptors respond only to their cognate ligand [7].

It is intriguing that the C3a receptor, in oocytes, also elicits a response to C5a. However, C3a and C5a are very similar in size (77 and 74 amino acids, respectively) and in proported structure [14]. In fact, the crystal structure of C3a has been used to derive a model of C5a [15]. In addition to C3a and C5a receptors, we have observed other instances in which the responses in oocytes are more promiscuous than the response elicited by the same receptor expressed in mammalian cells. For example, oocytes injected with calcitonin gene related peptide (CGRP) receptor respond both to CGRP and calcitonin, while mammalian cells transfected with the CGRP receptor respond only to CGRP (unpublished observation). There are other examples of anomalous behavior of cloned receptors using the oocytes system, most notably the misidentification of the mas oncogene as an angiotensin receptor [16]. It was later demonstrated that rather than encoding an angiotensin receptor, the expression of the mas oncogene in oocytes leads to increased responsiveness of the endogenous AII signalling system [17]. These data exemplify the importance of critical interpretation of the results obtained with cloned GPCRs in *Xenopus* oocytes.

In summary, we have demonstrated that like the C5a and

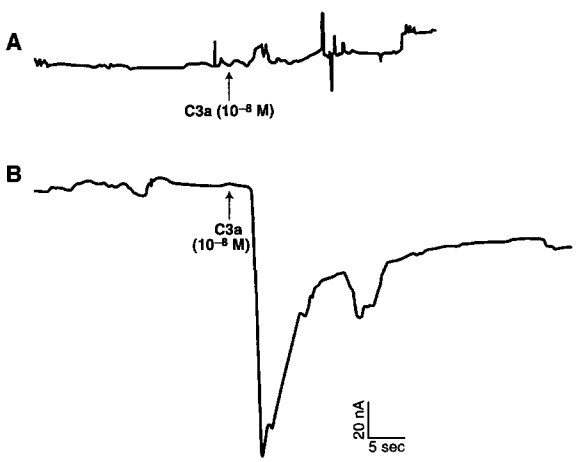

Fig. 2. Pertussis toxin treatement of oocytes co-injected with C3a receptor and total U937 RNA abolishes the response elicited by C3a. Whole cell currents of oocytes exposed to 10 nM C3a analogue peptide. Oocytes were injected with a mixture of cRNA for C3a receptor (HNFAG09, 10 ng) and U937 RNA. Cells were treated with perutssis toxin (2 μg, A), or untreated (B).

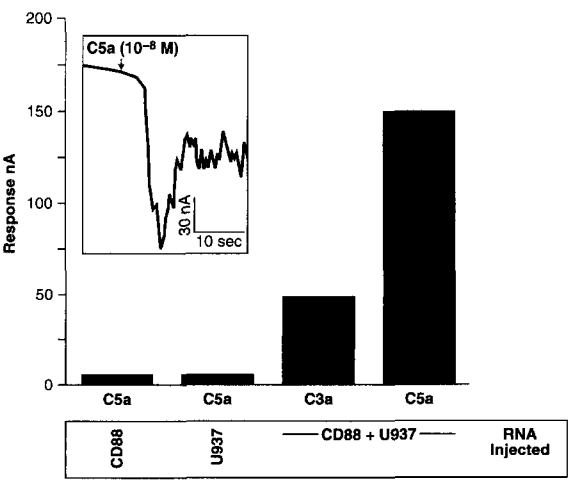

Fig. 3. Electrophysiological response of oocytes injected with C5a receptor cRNA (CD88, 10 ng), U937 RNA (25 ng) or a mixture of C5a receptor and U937 RNA, to 10 nM rC5a or 10 nM C3a analogue peptide. Inset, represents typical response of oocytes co-injected with C5a receptor cRNA+U937 RNA to 10 nM rC5a.

fMLF receptors, the C3a receptor is functionally active in *Xenopus* oocytes, and that activity is dependent on a pertussis toxin sensitive co-factor present in total RNA isolated from myeloid cell lines. In addition, we have demonstrated that both anaphylatoxin receptors, in oocytes, are capable of interacting with both anaphylatoxins however, both receptors elicit a maximal response to their cognate ligand.

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