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Gα-16 complements the signal transduction cascade of chemotactic receptors for complement factor C5a (C5a-R) and *N*-formylated peptides (fMLF-R) in *Xenopus laevis* oocytes: Gα-16 couples to chemotactic receptors in *Xenopus* oocytes**

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Abstract The human leukocyte chemoattractant receptors for complement factor C5a (C5a-R) and *N*-formylated peptides (fMLF-R) are important members of the superfamily of G-protein coupled receptors (GPCR). Uniquely among the GPCR, these two receptors cannot be expressed in a functionally active form in the oocytes of the frog *Xenopus laevis*, but require substitution of total RNA of the myelomonocytic U-937 or HL-60 cell lines, respectively. Recently, it was reported that the C5a-R may couple to the α subunit of G-16. We have tested this G-protein for its ability to complement the signal transduction cascade of the C5a-R and fMLF-R in *Xenopus* oocytes. Injection of cRNA for the C5a-R in combination with Gα-16 led to expression of a functional C5a-R as measured by ligand-induced whole cell current. In contrast to a previous report, the fMLF-R exhibited some residual functional activity when transiently expressed in *Xenopus* oocytes the extent of which could, however, substantially be increased by coexpression of Gα-16. Thus, Gα-16 complements the signal transduction cascade of both receptors in *Xenopus laevis* oocytes and is most likely the complementing factor present in the U-937 and HL-60 cell lines.

Key words: C5a-receptor; fMLF-receptor; Gα-16; *Xenopus laevis* oocyte

1. Introduction

The complement factor C5a (C5a) and *N*-formylmethionyl peptides (fMLF) are important mediators of inflammatory reactions acting via two different receptors, the human C5a receptor (C5a-R) and fMLF receptor (fMLF-R). C5a is generated during activation of the complement cascade, *N*-formylated peptides are believed to derive from degraded bacterial proteins [1] or to be generated from mitochondrial proteins upon tissue damage [2]. Both substances are able to trigger the direct locomotion of neutrophils to the sites of bacterial infection and to stimulate a variety of biochemical and cellular responses, like induction of the oxidative burst and release of intracellular enzymes (for review see [3,4]).

The human fMLF-R and C5a-R have both been cloned [5–7]. They belong to the family of G-protein coupled receptors (GPCR) which are characterized by a common motif of seven transmembrane α-helices connected by hydrophilic extra- and intracellular loops, and a signal transduction pathway involving heterotrimeric G-proteins. Much effort has been devoted to the study of the structural and functional characteristics of these receptor-ligand pairs as a prerequisite for the development of rational intervention strategies.

Oocytes of the frog *Xenopus laevis* are commonly used to express GPCR in a functionally active form. This expression system has been successfully used to clone and characterize several GPCR, like the receptors for serotonin [8] and platelet-activating factor [9], and to characterize receptor mutants [10,11]. Several groups, including ourselves, have employed this system in an attempt to establish a functional expression system for the human fMLF-R and C5a-R. Uniquely among the GPCR tested so far, it was found that these two receptors require a complementing factor present in the RNA of myelomonocytic cell lines, like HL-60 and U-937, for functional activity [12–15]. Recently, it was reported that a G-protein α subunit, Gα-16, may selectively couple to the human C5a-R in 293 and COS cell lines [16,17]. Here, we report that Gα-16, a G-protein that is expressed in HL-60 and U-937 cell lines, is able to restore the signal transduction cascade of the C5a-R and fMLF-R in *Xenopus* oocytes and therefore most probably represents the complementing factor previously described in these cell lines.

2. Materials and methods

2.1. Materials

The U-937 cell line was obtained from ATCC. The fMLF-R cDNA in plasmid vector pCDM8 (Invitrogen) coding for the fMLF-R98 variant [5] was donated by F. Boulay (Grenoble, France). The coding sequence for Gα-16 in vector pCMV5 was kindly given by G.L. Johnson (Denver, USA). Construction of pCC1 containing the C5a-R coding sequence in plasmid pCDM8 was reported earlier [14]. Recombinant human C5a was purified from our own recombinant *E. coli* strain as described elsewhere [18]. Adult *Xenopus laevis* frogs were purchased from Nasco (Alabama, USA). Composition of ND96: 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.4. Bovine serum albumin (0.2% (w/v); Boehringer) was added to ND96 in all electrophysiological measurements.

2.2. Preparation of cRNA

For all experiments with Gα-16, a subclone in the *Hin*dIII site of plasmid vector pCDM8 was used. cRNA for the human fMLF-R,

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Abbreviations: C5a-R, human C5a-receptor; fMLF, *N*-formyl-methionylleucylphenylalaline; fMLF-R, human fMLF-receptor; GPCR, G-protein-coupled receptor; rhC5a, recombinant human C5a.

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C5a-R and Gα-16 was prepared from the T7 promotor of the recombinant pCDM8 constructs after linearization with *Hpa*I (fMLF-R and Gα-16) or *Xba*I (C5a-R) using the mCAP mRNA capping kit (Stratagene). cRNA was quantitated by densitometric evaluation from ethidium bromide stained agarose gels against a known RNA standard using the CS-1 photographic system (Cybertech).

U-937 cells were grown at 37°C in a humidified atmosphere with 5% CO_2 in RPMI 1640 medium (Gibco) supplemented with 10% (w/v) heat-inactivated fetal calf serum, 50 U/ml penicillin and 50 μg/ml streptomycin. Total RNA was prepared by the guanidinium isothiocyanate method [19] and stored at −70°C in H_2O.

2.3. Oocyte expression experiments

Preparation of defolliculated *Xenopus laevis* oocytes and electrophysiological recordings were performed essentially as described previously [11,14,20]. RNA (46 nl/oocyte) was injected by a motor-driven microinjector (Drummond) using glass capillaries broken to an outer diameter of 12–25 μm. For co-injection experiments equal amounts of each RNA were used. Two days later, the oocytes were voltage-clamped at −70 mV and functional receptor activity was measured by whole cell current recordings after exposure to ND96 containing 20 nM rhC5a or 1 μM fMLF, respectively, for 60 s. A response ≥5 nA was considered positive.

3. Results and discussion

In previous experiments we have demonstrated the absolute dependence of functional C5a-R expression in *Xenopus laevis* oocytes from one or several factors present in the total RNA fraction of the myelomonocytic U-937 cell line [14]. Similar results have been independently obtained by other researchers [15].

The recent description of Gα-16 as signalling pathway complementing factor for functional C5a-R expression in eukaryotic cell lines [16,17] prompted us to test whether this G-protein was able to complement the signal transducing pathway in *Xenopus* oocytes, as well. As shown in Table 1, co-injection of cRNA for Gα-16 together with cRNA for the human C5a-R resulted in functional C5a-R expression. In comparison to complementation with total RNA of the U-937 cell line the signal intensity was considerably increased. Current amplitudes >1000 nA were easily detected in the electrophysiological assay while the signal intensity with complementing RNA from U-937 cells rarely exceeded 100 nA (and was much smaller in most cases). The typical shape of the current response (I_{fast} followed by an extended I_{slow} with a lower amplitude) was conserved.

A similar dependence of functional receptor expression in *Xenopus* oocytes on co-injected complementing factor(s) present in the RNA of the myelomonocytic HL-60 cell line had been reported for the human fMLF-R [12,13]. We thus checked whether Gα-16 would also complement fMLF-R activity in *Xenopus* oocytes. As shown in Table 2 and Fig. 1, expression of the fMLF-R alone led – in contrast to the above-mentioned reports – to some functional activity which in our hands could only marginally increased by co-injection of U-937 RNA. However, co-injection of cRNA for Gα-16 together with cRNA for the human fMLF-R significantly increased the electrophysiological response. Thus, the human fMLF-R couples to Gα-16 in *Xenopus laevis*, too.

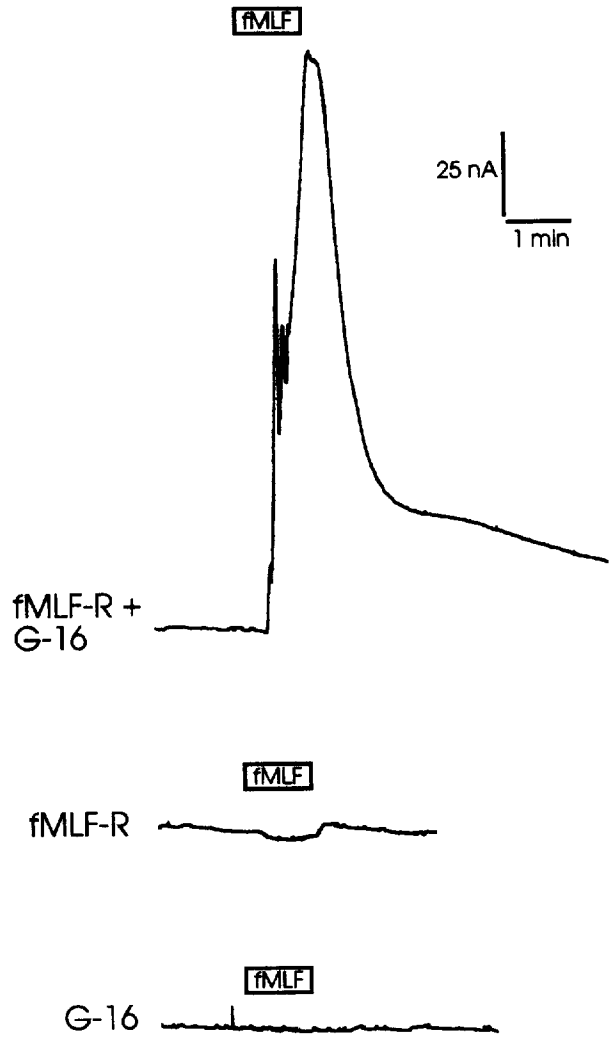

Fig. 1. Whole cell currents of oocytes exposed to 1 μM fMLF for 60 s (horizontal bar). Oocytes were injected with 23 ng cRNA for the fMLF-R and 23 ng of cRNA for Gα-16 (top), 46 ng of cRNA for the fMLF-R (middle) and 46 ng cRNA for Gα-16 (bottom).

Very recently, it was reported that Gα-16 may couple to a

Table 1
Summarized data of the C5a-R expression experiments in *Xenopus* oocytes (with and without Gα-16)

	n (series no.)	Current response		Mean amplitude ± S.D. (positive eggs)
		yes	no	
C5a-R cRNA plus U-937-RNA	10 (1)	6 (60%)	4 (40%)	38 ± 55
	7 (2)	4 (57%)	3 (43%)	16 ± 14
C5a-R cRNA plus Gα-16 cRNA	9 (1)	9 (100%)	0 (0%)	685 ± 483
	11 (2)	10 (91%)	1 (9%)	345 ± 377
Gα-16 cRNA	13 (1)	0 (0%)	13 (100%)	
	10 (2)	0 (0%)	10 (100%)	

Injected oocytes were voltage-clamped at −70 mV and the ligand-induced current response was measured at day 3 post injectionem after superfusion with 20 nM rhC5a for 60 s.

Table 2
Summarized data of the fMLF-R expression experiments in *Xenopus* oocytes (with and without Gα-16)

	n (series no.)	Current response		Mean amplitude ± S.D. (positive eggs)
		yes	no	
fMLF-R cRNA	9 (1)	6 (67%)	3	7.5 ± 6.1
	4 (3)	1 (25%)	3	30
fMLF-R cRNA plus U-937-RNA	9 (1)	6 (67%)	3 (33%)	25 ± 13
	8 (2)	3 (38%)	5 (63%)	8 ± 8.5
	3 (3)	3 (100%)	0 (0%)	11.5 ± 7.5
fMLF-R cRNA plus Gα-16 cRNA	12 (1)	11 (92%)	1 (8%)	117 ± 88
	8 (2)	8 (100%)	0 (0%)	116 ± 73
	6 (3)	6 (100%)	0 (0%)	59 ± 22
	4 (4)	4 (100%)	0 (0%)	295 ± 164
Gα-16 cRNA	5 (1)	0 (0%)	5 (100%)	
	5 (2)	0 (0%)	5 (100%)	
	5 (3)	0 (0%)	5 (100%)	

Injected oocytes were voltage-clamped at −70 mV and the ligand-induced current response was measured at day 3 post-injectionem after superfusion with 1 μM fMLF for 60 s.

wide variety of GPCR in eukaryotic cell lines [21]. This observation does also apply to the *Xenopus* expression system, as shown by the data presented herein, and should allow for the establishment of a functional test system for virtually any GPCR in *Xenopus laevis* oocytes. The physiological significance of this rather unselective coupling of Gα-16 to every GPCR tested so far remains unknown, as expression of this G-protein seems to be restricted to cells of hematopoietic origin [22]. But it is certainly expressed in the HL-60 [22] and U-937 cell lines [23] as shown by Northern hybridizations. These data clearly suggest that Gα-16 is the complementing factor previously described in these cell lines. The failure to detect this G-protein in U-937 cells by Vanek et al. [24] is probably simply due to differences in cell treatment or may indicate variant cell clones.

In summary, we have shown that Gα-16 complements the signal transduction cascade of the human fMLF-R and C5a-R in *Xenopus laevis* oocytes and is most probably the complementing factor previously described in HL-60 and U-937 cells [12–15]. The signal intensity could be considerably increased as compared to our previous results obtained with co-injection of total RNA from these cell lines. Thus, injection of cRNA for Gα-16 considerably improves the sensitivity of the *Xenopus laevis* oocyte test system which now recommends itself for the functional characterization of GPCR or appropriate receptor mutants, respectively, as we have recently demonstrated using some C5a-R mutants [25].

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