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Functional expression of a human C5a receptor clone in *Xenopus* oocytes requires additional RNA

Beate Kroll[1], Monica Emde[1], Andreas Jeromin[2], Lothar Penner[1], Gerhard Rechkemmer[2], Titus Kretzschmar[1], Andreas Klos[1], Jörg Köhl[1] and Wilfried Bautsch[1]

[1]*Institut für Medizinische Mikrobiologie, Medizinische Hochschule Hannover, Konstanty-Gutschow-Str 8, 3000 Hannover 61, Germany* and [2]*Physiologisches Institut, Tierärztliche Hochschule Hannover, Bischofsholer Damm 15, 3000 Hannover 1, Germany*

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cRNA from a PCR-generated C5aR clone was prepared by in vitro transcription and microinjected into *Xenopus laevis* oocytes Ligand-induced whole cell current could be detected after co-injection of cRNA for the C5aR with total RNA of the unstimulated U937 cell line, but not with either of the components injected alone These data clearly demonstrate an absolute requirement of the C5aR for an additional human factor to become functionally expressed in *Xenopus* oocytes

Reconstitution C5a receptor, U937 *Xenopus* oocyte

1. INTRODUCTION

The human C5a anaphylatoxin is a potent mediator of inflammatory reactions including chemotaxis and activation of neutrophils, smooth muscle contraction and increase in vascular permeability. It is generated by proteolytic cleavage of the complement component C5 during activation of the complement system (see [1] for review) and exerts its various functions by binding to a specific C5a receptor found in the membrane of several cells, like neutrophils, eosinophils and the U937 and HL60 cell line [2–5] These latter two cell lines express high levels of C5aR if stimulated with cAMP while no C5aR expression can be demonstrated in unstimulated cells Cloning of the cDNA for the human C5aR from the U937 and HL60 cell lines has recently been reported [4,5]. The C5aR identity of the cDNA clones was demonstrated by their ability to confer a high-affinity C5a binding property to transfected COS cells

Functional expression of the C5aR in *Xenopus* oocytes by microinjection of mRNA from cAMP-stimulated HL60 cells has been reported by Murphy et al [6]. The oocyte expression system would therefore seem well suited to supplement the preliminary characterization of the recombinant C5aR by functional data We now report the cloning and functional expression of the recombinant human C5aR in *Xenopus* oocytes and demonstrate its absolute dependence on an additional human factor for functional expression

Abbreviations cRNA, complementary RNA, C5aR, C5a receptor, rhC5a, recombinant human C5a, PCR, polymerase chain reaction, fMLP, *N*-formyl methionyl-leucyl-phenylalanine, G-protein, GTP binding protein, G_i, subclass of G-proteins originally described to inhibit adenyl cyclase, HEPES, *N* [2-hydroxyethyl]piperazine-*N*'-[2-ethanesulfonic acid]

Correspondence address W. Bautsch, Institut für Medizinische Mikrobiologie, Medizinische Hochschule Hannover, Konstanty Gutschow-Str 8, 3000 Hannover 61, Germany.

2 MATERIALS AND METHODS

2 1 *Materials*

Oligonucleotides P1(5'-CGGAATTCATGAACTCCTTCAATTATAC-3'), P2 (5'-CCAAGCTTGACGTGTCGCCTACACTGCCT-3'), P3 (5'-ATAGAAGCTTAGCCCAGGAGACCAGAACATGAACTCCTTCAATTAT-3') and all sequencing primers were synthesized on a GENE Assembler Plus (Pharmacia) and purified according to the manufacturer's instructions Restriction endonucleases were obtained from Pharmacia, deoxynucleotide-triphosphate solutions and T4-DNA ligase from Gibco RhC5a was obtained from Sigma or purified from our own recombinant *E coli* strain (Bautsch et al, submitted) Identical results were obtained irrespective of the source of the rhC5a Adult *Xenopus laevis* were purchased from Kähler (Hamburg, Germany) Composition of oocyte Ringer solution (OR, in mM) NaCl 96, KCl 2, $CaCl_2$ 1 8, $MgCl_2$ 1, HEPES 5, pH 7 4 Bovine serum albumin (0 2%, Boehringer) was added to OR in all electrophysiological measurements

2 2 *Cloning of the C5aR*

Total human DNA was prepared from peripheral blood leukocytes [8] The DNA sequence for the C5aR was amplified from 10 ng total human DNA using P1, P2 and VENT DNA polymerase (Biolabs) according to the manufacturer's instructions 36 cycles at an annealing temperature of 50°C were run The DNA fragment was digested by *Eco*RI/*Hin*dIII and cloned into pGEM-9Zf(−) (Promega) Positive clones were completely sequenced using the T7 sequencing kit (Pharmacia) One out of 5 sequenced clones contained the complete mutation-free DNA sequence corresponding to position 1–1063 of the C5aR sequence To introduce part of the native leader sequence of the C5aR 1 ng of the recombinant DNA clone was reamplified by 25 cycles PCR using P3 and P2 as amplification primers and cloned into

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the HindIII site of pGEM-9Zf(−) Positive clones were completely sequenced as described above The final recombinant plasmid, called pCG1, contained the complete DNA sequence of the C5aR from position −18 to 1063 downstream of the T7 promotor [5] This clone (or a subclone in pCDM8 (Invitrogen) called pCC1) served for the production of cRNA [7]

2 3 *Preparation of RNA*

U937 cells were grown in RPMI 1640 medium (Gibco) For induction of C5aR expression cells were treated with 1 mM dibutyryl-cAMP (Boehringer) for 40 h Total RNA was prepared by the guanidinium isothiocyanate method [7] and stored at −70°C in water cRNA for the C5aR was prepared from the T7 promotor of the *NsiI*-digested pCG1 (or *XbaI*-digested pCC1, respectively) using the mCAP mRNA capping kit (Stratagene) and quantitated by densitometric evaluation from ethidium bromide-stained agarose gels against a known RNA standard using the CS-1 system (Cybertech)

2 4 *Oocyte expression experiments*

Preparation of defolliculated oocytes and electrophysiological recordings were performed essentially as described previously [8] RNA (46 nl/oocyte) was injected by a motor-driven microinjector (Drummond) using glass capillaries broken to an outer diameter of 12 25 μm C5aR activity was tested 1–6 days after RNA injection by current recording of voltage-clamped oocytes (−70 mV) exposed to OR containing 20 nM rhC5a for 100 s

3 RESULTS

Xenopus oocytes express functional C5a receptors after injection of total RNA prepared from cAMP-stimulated cells of the human U937 cell line Fig 1a displays a rhC5a induced current response of a voltage-clamped oocyte injected two days before with total RNA of cAMP-stimulated U937 cells Functional C5a receptor activity appeared 1–2 days after RNA injection and reached a maximum at the third day. The response desensitized strongly upon repeated application of the ligand (data not shown) Oocytes injected with RNA from unstimulated U937 cells or with C5aR-cRNA alone showed no C5a response as demonstrated in Fig 1b,c. However, co-injection of a 1·1 mixture of both receptor cRNA and RNA of unstimulated U937 cells led to expression of functional C5a receptors (Fig 1d) These C5a responses showed the same electrophysiological characteristics as those of oocytes injected with cAMP-stimulated U937 RNA. Table I summarizes the data of our expression experiments with oocytes of 4 different frogs 44% of the oocytes injected with RNA of cAMP-stimulated U937 cells and 43% of the oocytes injected with the cRNA/RNA mixture responded to C5a The shape and amplitude (ranging from 5 to 320 nA) of the acquired responses varied between different oocytes as described for other expressed receptors too [9] In contrast, none of the oocytes injected with C5aR clone cRNA or RNA from unstimulated U937 cells alone displayed functional C5a receptor activity

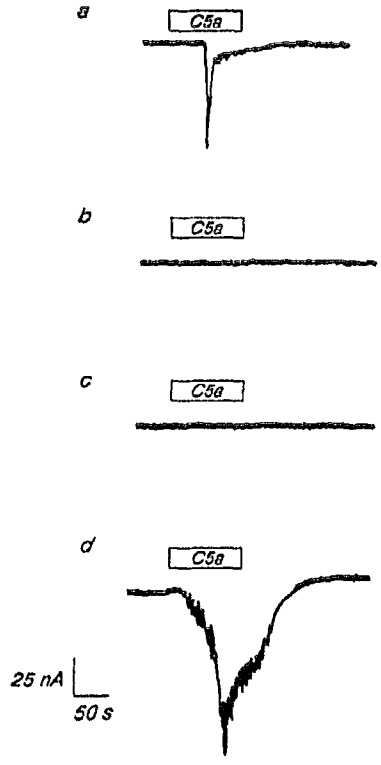

Fig 1 Whole cell currents of oocytes exposed to 20 nM rhC5a for 100 s (horizontal bar) Oocytes were injected with (a) 46 ng total RNA of cAMP-stimulated U937 cells, (b) 46 ng total RNA of unstimulated U937 cells, (c) 0 2 ng of C5aR-cRNA and (d) 0 1 ng of C5aR-cRNA plus 23 ng total RNA of unstimulated U937 cells Oocytes derived from the same frog were voltage clamped to −70 mV

4 DISCUSSION

The C5aR belongs to the rhodopsin-like receptor superfamily, a subgroup of G-protein coupled receptors [10] Several of these receptors such as the serotonin 5-HT_{1C} and substance K receptor [11,12] have been functionally expressed in *Xenopus* oocytes from their specific cRNA Ligand-induced current responses were mediated by the endogenous signal transduction pathway of the oocyte via activation of phosphatidylinositol breakdown and opening of chloride channels In the

Table I

Summarized data of the RNA expression experiments with oocytes of 4 different frogs examined 1–6 days after injection of the indicated RNA for functional C5aR activity

		Current response	
	n	yes	no
total RNA of cAMP-stimulated U937 cells	71	31 (44%)	40 (56%)
total RNA of unstimulated U937 cells	39	0 (0%)	39 (100%)
C5aR-cRNA	34	0 (0%)	34 (100%)
C5aR cRNA plus total RNA of unstimulated U937 cells	88	38 (43%)	50 (57%)

Oocytes were voltage-clamped at −70 mV and superfused for 100 s with 20 nM rhC5a

present paper we clearly demonstrate that functional expression of a C5aR clone requires a complementary human factor. Similar results have quite recently been obtained by Murphy and McDermott for functional fMLP receptor expression in oocytes [13]. The identity of this complementary factor is still unknown. A special feature of the C5a as well as the fMLP receptor is their tight association with a G_i-like protein [14,15] These observations suggest a G_i-protein to function as this complementary factor in the oocyte system However, coinjection of the fMLP receptor cRNA with cRNA coding for the α-subunits G_{i1}, G_{i2} and G_{i3} failed to induce functional fMLP receptors [13]

In summary, we describe a functional expression system for the human C5a receptor in *Xenopus* oocytes and conclusively demonstrate the requirement of an additional human factor derived from coinjected RNA This system provides a functional assay for the study of the C5a–C5aR interactions by site-directed mutagenesis and may serve as a tool for expression cloning of the complementary human factor

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REFERENCES

[1] Bitter-Suermann, D (1988) in The Complement System, The Anaphylatoxins (Rother, K and Till, G O eds) pp 367–395, Springer, Berlin

[2] Chenoweth, D E and Hugli, T E (1978) Proc Natl Acad Sci USA 75, 3943–3947

[3] Gerard, N P, Hodges, M K, Drazen, J M, Weller, P F and Gerard, C (1989) J Biol Chem 264, 1760–1766

[4] Gerard, N P and Gerard, C (1991) Nature 349, 614–617

[5] Boulay, F, Mery, L, Tardif, M, Brouchon, L and Vignais, P (1991) Biochemistry 30, 2993–2999

[6] Murphy, P M, Gallin, E K and Tiffany, H L (1990) J Immunol 145, 2227–2234

[7] Sambrook, J, Fritsch, E F and Maniatis, T (1989) Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

[8] Kroll, B, Bautsch, W, Bremer, S, Wilke, M, Tümmler, B and Fromter, E (1989) Am J Physiol 257, L284–L288

[9] Singer, D, Boton, R, Moran, O and Dascal, N (1990) Pflügers Arch 416, 7–16

[10] Dixon, R A F, Kobilka, B K, Strader, D J, Benovic, J L, Dohlmann, H G, Frielle, T, Bolanowski, M A, Bennett, C D, Rands, E, Diehl, R E, Mumford, R A, Sleter, E E, Sigal, I S, Caron, M G, Lefkowitz, R J and Strader, C D (1986) Nature 321, 75–79

[11] Lubbert, H, Hoffmann, B J, Snutch, T P, van-Dyke, T, Levine, A J, Hartig, P R, Lester, H A and Davidson, N (1987) Proc Natl Acad Sci USA 84, 4332–4336

[12] Masu, Y, Nakayama, K, Tamaki, H, Harada, Y, Kuno, M and Nakanishi, S (1987) Nature 329, 836–838

[13] Murphy, P M and McDermott, D (1991) J Biol Chem 266, 12560–12567

[14] Rollins, T E, Siciliano, S, Kobayashi, S, Cianciarulo, D N, Bonilla-Argudo, V, Collier, K and Springer, M S (1991) Proc Natl Acad Sci USA 88, 971–975

[15] Williamson, K, Dickey, B F, Pyun, H Y and Navarro, J (1988) Biochemistry 27, 5371–5377