Available online at www.sciencedirect.com
SCIENCE DIRECT
Immunology Letters 100 (2005) 139–145
Immunology Letters
www.elsevier.com/locate/

The C3a receptor antagonist SB 290157 has agonist activity

Marie-Claude Mathieu [a], Nicole Sawyer [a], Gillian M. Greig [a], Martine Hamel [a], Stacia Kargman [a], Yves Ducharme [b], Cheuk K. Lau [b], Richard W. Friesen [b], Gary P. O'Neill [a], Francois G. Gervais [a], Alex G. Therien [a,*]

[a] *Department of Biochemistry and Molecular Biology, Merck Frosst Center for Therapeutic Research, Kirkland, Que., Canada H9H 3L1*
[b] *Department of Medicinal Chemistry, Merck Frosst Center for Therapeutic Research, Kirkland, Que., Canada H9H 3L1*

Received 21 January 2005; received in revised form 2 March 2005; accepted 10 March 2005
Available online 25 March 2005

Abstract

The anaphylatoxin C3a is an important immune regulator with a number of distinct functions in both innate and adaptive immunity. Many of these roles have been ascribed to C3a based on studies in mice genetically modified to lack its precursor, C3, or its receptor, C3aR. However, other presumed functions of C3a are based on results obtained with a recently described small molecule ligand of C3aR, SB 290157. Although this compound was originally described as an antagonist and appears to act as such in some systems, it has recently been shown to have effects that cannot be explained by simple antagonism of C3aR. In the current study, SB 290157 is shown to have full agonist activity on C3aR in a variety of cell systems, including a calcium mobilization assay in transfected RBL cells, a β-lactamase assay in CHO-NFAT-*bla*-Gα_{16} cells and an enzyme-release assay in differentiated U-937 cells. On the other hand, the compound lacks agonist activity in guinea pig platelets, cells known to express C3aR at very low levels. SB 290157 agonism of C3aR is consistent with recent discrepant data obtained using this molecule. These results caution against attributing novel roles to C3a based on data obtained with SB 290157 and highlight a continuing need for the identification of true small molecule C3aR antagonists.

Keywords: Anaphylatoxin; GPCR; Asthma

1. Introduction

Complement factor 3a (C3a) is a 77-amino acid peptide produced following activation of the complement cascade. Specifically, it is derived from C3 upon cleavage of this protein by C3 convertase, the central mediator of the complement cascade [1]. Some of the cellular and physiological events associated with C3a include contraction of smooth muscle, chemotaxis and activation of leukocytes, primarily mast cells and eosinophils, and an increase in vascular permeability [1,2]. The functional receptor for C3a, C3aR, is a predominantly G_i-coupled GPCR, the binding kinetics and downstream mechanisms of which have been studied in a number of cellular systems [3–7].

Although it has been described as having broad pro-inflammatory effects, the main role of C3a appears to be in T_h2-type inflammatory reactions [2,8–11]. Consistent with this, a large body of evidence has emerged over the last few years implicating C3a in allergic asthma. These data include: (i) a complete abrogation of airway hyper-reactivity upon allergen-challenge in mice lacking C3 or C3aR [8–10,12], (ii) an upregulation of C3 and C3aR mRNA [13], as well as C3aR protein [14] in ovalbumin-sensitized and -challenged mice, (iii) increased C3a levels in bronchoalveolar lavage fluid and serum of asthmatics upon allergen challenge [10,15] or during exacerbations [16], (iv) elevated C3aR levels in patients

Abbreviations: AHR, airway hyper-reactivity; C3a, complement factor 3a; C3aR, complement factor 3a receptor; C5a, complement factor 5a; cC3aR, cynomolgus monkey complement factor 3a receptor; dU-937, differentiated U-937; GPCR, G-protein-coupled receptor; HBSS, Hank's balanced salt solution; hC3aR, human complement factor 3a receptor; mC3aR, mouse complement factor 3a receptor; NAG, *N*-acetyl-β-D-glucosaminidase

* Corresponding author. Tel.: +1 514 428 3937; fax: +1 514 428 8615.
E-mail address: alex_therien@merck.com (A.G. Therien).

0165-2478/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.imlet.2005.03.003

with fatal asthma (Fregonese et al., 99th American Thoracic Society International Conference, 2003) and (v) genetic linkage of the C3 and C3aR genes with asthma [17,18].

One of the difficulties in evaluating the role of C3a in animal models of asthma has been the lack of a potent, orally available small molecule C3aR antagonist. Recently, Ames et al. [19] described just such an antagonist, SB 290157. The arginine-like molecule was shown to compete with C3a binding to C3aR, expressed in RBL cells, as well as inhibit C3a-induced calcium mobilization in these cells and in human neutrophils (with IC_{50} values ranging from 28 to 200 nM for the human enzyme). The molecule also inhibits chemotaxis of HMC-1 cells and ATP-release from guinea pig platelets. In addition, SB 290157 was found to be active in two animal models of inflammation, a guinea pig LPS-induced lung neutrophilia model, and a rat adjuvant-induced arthritis model. SB 290157 has since been used in a number of in vitro and in vivo studies [20–24] with sometimes unexpected results [20,23]. We wished to further characterize SB 290157 with the goal of evaluating its effects in T_h2-driven in vivo models. Surprisingly, we found that SB 290157 has full agonist activity in a variety of cell systems, particularly those with a relatively high receptor density. These results reconcile the discordant results obtained in vivo with SB 290157 and serve to caution against use of this compound as a C3aR antagonist.

2. Materials and methods

2.1. DNA constructs and cell lines

The genes for human C3aR (hC3aR; GenBank Accession Number: NM_004054) and mouse C3aR (mC3aR; GenBank Accession Number: NM_009779) were amplified by polymerase chain reaction from Marathon-ready cDNA libraries (BD Biosciences, San Jose, CA, USA) or full-length cDNA clones (Invitrogen, Carlsbad, CA, USA). The gene for the cynomolgus monkey C3aR (cC3aR; GenBank Accession Number: AY426336) was amplified using primers corresponding to the human sequence flanking the coding region, and from a leucocyte cDNA library synthesized using the SMART PCR cDNA synthesis kit (BD Biosciences, San Jose, CA, USA). All genes were subcloned into the pCDEF3 vector [25] and transfected into RBL or CHO-NFAT-*bla*-$G\alpha_{16}$ cells using the Lipofectamine Plus reagent (Invitrogen, Carlsbad, CA, USA). Stably expressing cells were generated through selection in G418 (Gibco; 400 μg/ml for RBL cells, 700 μg/ml for CHO cells) and high-expressing clones were isolated based either on increased binding to [^{125}I]-C3a (for RBL cells; see below) or on high receptor expression (for CHO cells) as assessed by fluorescence-assisted cell sorting using anti-hC3aR (BD Biosciences, San Jose, CA, USA). The CHO-NFAT-*bla*-$G\alpha_{16}$ cells used above were generated through transfection of CHO-NFAT-*bla* cells (Invitrogen, Carlsbad, CA, USA) with the $G\alpha_{16}$ gene (GenBank Accession Number: NM_002068) and selection in hygromycin B (Roche, Indianapolis, IN, USA; 500 μg/ml) for generation of stably expressing cells.

2.2. Synthesis of SB 290157

The C3aR ligand SB 290157 was synthesized as described previously [19] and isolated in >95% purity (^{1}H NMR) as the TFA salt. MS (CI, −ve ion mode) $m/e = 411.3$ $(M-1)^-$. ^{1}H NMR (400 MHz, CD_3OD) δ 1.45–1.70 (m, 3H), 1.90 (m, 1H), 3.18 (m, 2H), 4.05 (s, 2H), 4.11 (dd, 1H), 4.19 (dd, 1H), 4.40 (t, 1H), 4.45 (m, 1H), 7.20 (m, 2H) and 7.30 (m, 8H). As an alternative source of SB 290157, a commercial version was purchased from EMD Biosciences.

2.3. Radioligand binding assay

Binding of [^{125}I]-C3a to RBL-hC3aR membranes was assessed by scintillation proximity assay (SPA). Briefly, RBL-hC3aR membranes (0.3 μg/well) were incubated to equilibrium in 96-well plates with 0.05 mg/well beads (polyethyleneimine-treated wheat germ agglutinin-coated polyvinyltoluene SPA beads, type A; Amersham Biosciences, Piscataway, NJ, USA), 70 pM [^{125}I]-C3a (2200 Ci/mmol; Perkin Elmer, Torrance, CA, USA) and in the absence or presence of various concentrations of unlabeled C3a (Advanced Research Technologies, San Diego, CA, USA), or SB 290157 for 60 min at RT with shaking in 20 mM HEPES, 3 mM $MgCl_2$, 0.1 mM EDTA, 0.0003% (v/v) Tween-20, pH 7.4. Bound [^{125}I]-C3a was then assessed by scintillation counting on a 1450 Microbeta β-counter (Perkin Elmer, Torrance, CA, USA). Specific [^{125}I]-C3a binding is defined as the difference between total binding and non-specific binding as determined in the presence of 70 nM unlabeled C3a. K_i values were determined from IC_{50} values using the relationship $K_i = IC_{50}/(1+[C3a]/K_d)$. IC_{50} and K_i values are reported as means ± S.D. of multiple determinations.

2.4. Calcium-mobilization assay

C3a-induced calcium mobilization in RBL-hC3aR or RBL-mC3aR cells was assessed as follows. Cells were plated into 96-well, black-walled, clear-bottom plates (Corning Life Sciences, Acton, MA, USA) at 45,000 cells/well and incubated at 37 °C (in 5% CO_2) for 18–24 h. Cells were then washed once in Hank's balanced salt solution (HBSS) containing 20 mM HEPES, followed by incubation in the same buffer containing "no wash" calcium dye (Molecular Devices, Sunnyvale, CA, USA) for 45 min at 37 °C. Plates were then transferred to a FlexStation II instrument (Molecular Devices, Sunnyvale, CA, USA) for processing. Using the built-in sample-addition function of the instrument, C3a or SB 290157 was added to the wells, at the concentrations specified in the figure legends. Fluorescence emission (λ_{exc} = 485 nm; λ_{emm} = 525 nm) was measured at 3-s intervals prior to, during, and following ligand addition. For dose–response curves, individual points represent the differences between baseline