An Anti-Inflammatory Function for the Complement Anaphylatoxin C5a Binding Protein, C5L2*

Norma P. Gerard†§, Bao Lu†, Pixu Liu†, Stewart Craig‡, Yuko Fujiwara¶, Shoji Okinaga‡, and Craig Gerard†§

†Pulmonary Division, Department of Pediatrics, ¶Division of Hematology and Oncology, Children’s Hospital, §Department of Medicine, Harvard Medical School, Boston, Massachusetts
‡ Department of Geriatric and Respiratory Medicine, Tohoku University School of Medicine, Sendai, Japan

Running title: An anti-Inflammatory Role for the C5a Binding Protein, C5L2

Address correspondence to: Craig Gerard, Ina Sue Perlmutter Laboratory, Children’s Hospital, Harvard Medical School, 320 Longwood Ave., Boston, MA, Phone: 617 355-6174; Fax: 617 730-0240; Email: Craig.Gerard@childrens.harvard.edu

Summary

C5L2 is an enigmatic serpentine receptor that is co-expressed with the C5a receptor on many cells including PMN neutrophils. The apparent absence of coupling of C5L2 with G proteins suggests that this receptor may modulate the biological activity of C5a, perhaps by acting as a decoy receptor. Alternatively, C5L2 may affect C5a function through formation of a heteromeric complex with the C5aR, or it may utilize a G protein-independent signaling pathway. Here we show that in mice bearing a targeted deletion of C5L2, the biological activity of C5a/C5a desArg is enhanced both in vivo and in vitro. The biological role of C5L2 thus appears to be limiting to the pro-inflammatory response to the anaphylatoxin. Accordingly, up-regulation of C5L2 may be of benefit in inflammatory states driven by C5a, including sepsis, asthma, cystic fibrosis and chronic obstructive lung disease.

Introduction

During the proteolytic cascade of complement activation, small (~10kDa) cationic fragments of C3, C4 and C5 are released, known as the anaphylatoxins C3a, C4a and C5a. The anaphylatoxins participate in host defense through initiating chemotaxis and activation of myeloid cells, enhancement of vascular permeability, contraction of smooth muscle and as yet poorly understood functions on endothelial and epithelial cells (1, 2). Conversely, inappropriate activation of complement in vivo may be involved in a number of autoimmune diseases, asthma, rheumatoid arthritis, and cardiac disease (3).

Biological insights into the function of the anaphylatoxins and their receptors have been obtained through gene deletion in mice. Mice deficient in the C5a receptor show slower clearance and hence higher mortality in a model of Pseudomonas pneumonia (4). In contrast, these animals are completely protected from pulmonary injury in a model of immune complex injury in the lung (5). C3a receptor deficient mice do not develop airway hyperresponsiveness compared with wild type animals upon allergen sensitization and challenge (6, 7). These mice have also shown a role for
C3a/C3a receptor interactions in the clearance of bacteria (8).

Given the potential for the anaphylatoxins to cause injury, mechanisms exist to limit the duration of their biologic activity. Serum carboxypeptidase N is also recognized as an anaphylatoxin inactivator (9). All three anaphylatoxins contain carboxy-terminal arginine residues. In the case of C3a and C4a, the desArginine derivatives are virtually incapable of binding to the C3a receptor. In the case of C5a, the biologic activity decreases by more than an order of magnitude, and the binding affinity of the desArginine derivative changes from sub-nanomolar to micromolar. In whole serum or plasma, removal of the arginine by carboxypeptidase is close to instantaneous. Control of the residual activity of C5adesArg is presumed to be through clearance and degradation of the ligand following receptor internalization.

In 2000, Ohno and colleagues described an orphan receptor with significant homology to the C5a receptor that is expressed on immature (but not mature) dendritic cells (10). Chromosome analysis reveals that C5L2 is adjacent to the C5a receptor gene on human chromosome 19. Cain and Monk (11) and Okinaga et al (12) demonstrated that C5L2 is a high affinity binding protein for C5a and C5adesArg. Some evidence was provided that C5L2 may be weakly coupled to G proteins in stably transfected RBL cells and potentiates IgE mediated degranulation (11). Kalant and colleagues (13) further demonstrated that C5L2 binds C3a, C3adesArg, and C4a, and suggested that C5L2 mediates the action of acylation-stimulating protein (14). We have consistently been unable to detect binding of ligands other than C5a and C5adesArg, using both transiently transfected 293T cells and stably transfected L1.2 mouse lymphoblasts (12, Gerard, unpublished). We have also been unable to detect an interaction of native C5L2 with G proteins, although when leucine 132 is mutated to arginine, a weak calcium response is observed upon stimulation with C5a (12). In a recent study by Gavrilyuk, et al (15), noradrenaline (NA) was shown to upregulate C5L2 message and protein in rat astrocytes; this correlated with an anti-inflammatory response induced by NA. Additionally, antisense oligonucleotides against C5L2 reversed some of the anti-inflammatory properties of NA.

In the current report, we describe a murine line deficient in C5L2, which retains the classic C5a receptor. Using a well-characterized model of C5a-dependent immune complex pulmonary injury, we show that C5L2 deficient mice exhibit an exaggerated inflammatory response. The data support a function for C5L2 in limiting the effects of C5a/C5adesArg.

Materials and Methods

Targeting the C5L2 gene through homologous recombination: A genomic clone of ~11kb containing the C5L2 gene was cloned from a mouse SV129 DNA library. A 6kb genomic fragment including the C5L2 coding sequence was used to construct the targeting vector. Approximately 1kb of coding sequence was excised and replaced with genes encoding green fluorescent protein (GFP) and neomycin resistance under control of the phosphoglucokinase promoter. The mutant construct was subcloned into pPNT, linearized and electroporated into CJ7 embryonic stem cells derived from mouse strain 129. Transfectants were selected with G418 and gancyclovir, and
the correctly targeted event was screened by Southern blot. Six targeted lines out of ~300 doubly resistant colonies were isolated. Three of these were injected into blastocysts derived from C57/BL6 females. Chimeric males were bred to C57/BL6 females to yield germline transmission of the targeted allele. Animals were backcrossed to C57/BL6 through at least 5 generations prior to initiating the studies presented.

Preparation of recombinant murine C5a: A cDNA encoding murine C5a was prepared by RT-PCR from mouse liver based on previously published sequence data (accession number M35525; 16), and cloned into p3XFLAG-CMV-9 (Sigma). The plasmid was transfected into HEK293T cells using calcium phosphate (17). After 6 hours, cells were transferred to serum-free media containing 5 mM sodium butyrate and protease inhibitor cocktail (Sigma) and cultured for an additional 48 hours. Culture supernatants were harvested and the Flag-murine C5a was purified by affinity chromatography on M2 anti-Flag resin as described by the supplier (Sigma).

In vitro chemotaxis: Bone marrow cells were isolated from the femurs and tibias of C5L2-/- mice and wild type littermates by perfusion with sterile PBS. Cells were fluorescently labeled with calcein-AM (Molecular Probes), suspended in 20mM HEPES, pH 7.5, 125mM NaCl, 5mM KCl, 1mM MgCl2, 1mM CaCl2, 0.5mM glucose, 0.2% BSA, at 1x10^7/ml and tested for chemotactic activity in modified Boyden chambers. Cells (0.1 ml) were placed in the upper wells of 3 micron, 6.4 mm FluoroBlok filter inserts (Falcon) with 0.6 ml buffer containing 0 of 10nM C5a of 8µM fMLP in the lower wells. Chambers were incubated at 37°C for 45 min and chemotaxis determined by measuring the fluorescence intensity (excitation 485nm, emission 535nm) passing to the underside of the filter. Measurements were determined in duplicate for three independent experiments.

In vivo chemotaxis: Recombinant mouse C5a, 5 µg in 500 µl sterile PBS, was injected ip into C5L2-/-, C5aR-/-, or wild type mice. Four hours later, animals were sacrificed and the peritoneal cavity lavaged with 10 ml PBS and the cellular infiltrate quantified by hemocytometer.

Immune complex mediated lung injury: All mouse protocols were approved by the Animal Care and Use Committee of Children’s Hospital. Mice matched for sex and age (female, 10-12 weeks) were used for this model as previously described (5). Mice were anesthetized with ketamine and xylazine. A single incision was made at the neck, and salivary glands were parted by blunt dissection to expose the trachea. A single 30-µl injection containing rabbit antibody to chicken egg albumin (300 µg) diluted in normal saline was injected using a syringe with 25 gauge needle, followed by injection of chicken egg albumin (20 mg/kg) into the tail vein. Four hours later, the mice were sacrificed and bronchoalveolar lavage (BAL) was performed. The BAL fluid was analyzed for protein concentration, cytokine measurement, total cell count and differential. The lung was perfused via the right ventricle with 5 ml of PBS, and individual lobes were removed for histology or homogenized and assessed for myeloperoxidase and cytokines (ELISA).

Statistical analysis: Arithmetic means and standard deviations were calculated using Prism 4.0a (Graphpad) software. Data were analyzed using Student’s t-test for unpaired samples and
statistical significance was defined as $P<0.05$.

**Results and Discussion**

The murine C5L2 gene was targeted using the construct shown in Figure 1A. As shown in Figure 1B (upper panel), HindIII digested DNA revealed the expected 5.7kb restriction fragments for the targeted gene hybridizing with probe B, confirming homologous recombination. Three independent ES cell clones transmitted through the germ line and produced viable offspring in the expected Mendelian ratios. The mice are fecund, grow normally, and have a normal weight and lifespan.

Because the C5L2 locus is only ~15kb away from the C5aR locus, it was important to demonstrate normal expression of the C5aR message and functional receptor protein. As shown in Figure 1B (lower panel), Bam H1 digested genomic DNA revealed identical restriction fragments hybridizing with the C5aR gene for both wild type and C5L2 deficient animals. Similarly, northern blots demonstrated normal C5aR mRNA (Figure 1C) in wild type and C5L2-/- mice, but no C5L2 mRNA in C5L2 deficient animals. Further, as indicated by the data shown in Figure 2, the C5a receptor protein exhibits apparently normal function, as intraperitoneal instillation of a single dose of recombinant murine C5a ($5\mu g$) into C5L2 deficient and wild-type mice yielded indistinguishable neutrophilic infiltrates that were increased approximately two-fold over C5aR-/- animals ($P<0.05$, between wild type or C5L2-/- and C5aR-/- mice, n=3-5 animals per group). C5a receptor deficient animals yielded cell numbers comparable to injection of PBS alone (Figure 2).

Immune complex lung injury, an intrapulmonary version of a reverse passive Arthus reaction, has been demonstrated to be dependent on C5a acting on the C5a receptor (5, 18). A recent report elegantly demonstrated the mechanism for this phenotype (19). Fcγ receptor activation by immune complexes results in non-complement mediated production of C5a by alveolar macrophages. C5a signaling through the classical C5aR and $G_{\alpha i}$ results in a decrease in expression of the anti-inflammatory FcγRIIB without affecting the inflammatory FcγRIII. As a result, signaling through the latter receptor stimulates production of TNFα and CXCR2 ligands, which are central to the generation of hemorrhagic pneumonitis.

Since this phenotype is virtually “black and white” in comparing wild type and C5a receptor deficient mice, we elected to ascertain the functional role of C5L2 in this model. Mice were given ovalbumin via tail vein injection, and anti-ovalbumin IgG was instilled into the trachea. After 4 hours, animals were sacrificed and analyzed for inflammatory parameters. The bronchoalveolar lavage fluid was quantified for both total cells (Figure 3A) and for neutrophils and monocytes (Figure 3B). A dramatic increase in both total cells ($8.3\pm1.1\times10^5$ for C5L2-/-, vs. $3.2\pm1.0\times10^5$ for wild type mice, $P=0.008$, n=5 per group) and neutrophils ($2.70\pm0.56\times10^5$ for C5L2-/- vs. $1.07\pm0.56\times10^5$ for wild type, $P=0.0005$, n=5 per group) was observed in C5L2 deficient mice compared to wild type animals. The cellular influx in the lungs of control mice of either strain treated with antibody alone or antigen alone was significantly reduced, as previously observed (5). No difference in influx of monocytes was apparent. This phenotype
for C5L2-/- mice is opposite the result obtained with mice deficient in the classical C5a receptor (5).

As indicated by the data of Figure 4, the increased cellular influx correlated with an increase in the concentration of TNFα and IL6 in lung homogenates and in TNFα levels in BAL (not shown). Both cytokines were increased approximately 4-fold in immune complex treated C5L2-/- mice compared with wild type animals (TNFα 166±15 pg/ml for C5L2-/- vs. 38±7 pg/ml for wild type mice, P=0.002, n=3 per group; IL6 1117±251 pg/ml for C5L2-/- vs. 263±63 pg/ml for wild type mice, P=0.03, n=3 per group). These findings were confirmed by histologic examination of the lavage fluid and lung parenchyma as shown in Figure 5.

C5L2 deficient mouse cells are also more responsive to C5a than wild type cells expressing both receptors in in vitro chemotaxis assays. Bone marrow cells isolated from C5L2-/- mice or wild type littermates were examined for their ability to migrate to 10nM C5a relative to 8µM fMLP. As shown in Figure 6 chemotaxis of C5L2-/- cells to C5a was ~40% greater relative to the response to 8µM fMLP than cells from wild type mice (C5L2-/- cells responded to 10nM C5a with 7.9±0.4 fold increase in chemotactic cells over 8µM fMLP compared to 4.8±0.3 fold more cells for wild type, P=0.008, n=3 independent experiments performed in duplicate). This difference is substantially less than the 250-300% increase in cellular influx in the lungs following immune complex injury (Figure 3), likely reflecting absence of the amplification resulting from secondary release of additional chemotactic factors observed in this model. The observation of similar neutrophil influx into the peritoneal cavity of wild type vs C5L2-/- mice following injection of 5µg C5a (Figure 2) is likely the result of inaccuracies inherent in this assay and reflective of the relatively small number of animals tested.

The overall expression level of mRNA for C5L2 is significantly lower than that for the C5a receptor, and generally does not have a 1:1 correspondence among tissues (20). The median expression values for C5L2 are approximately one third that of the C5aR. In some murine tissues, for example, pancreas and spleen, high levels of C5aR expression are associated with below median expression of C5L2, while in lactating mammary gland and skeletal muscle, the mRNA levels of for the two receptors are nearly equal. Clearly, the significance of these differences is subject to evaluation of the protein levels, but it suggests that C5L2 acts independently of the C5a receptor to counteract C5a/C5a receptor mediated inflammation. Whether it does so by reducing the effective C5a levels available to act at the classical C5a receptor as suggested by Gao, et al (21), by triggering an anti-inflammatory signaling pathway, or both, awaits further study.
References
1. Guo RF, Ward PA. Role of C5a in inflammatory responses. 2005. Annu. Rev. Immunol. 23:821-852.
2. Gerard C, Gerard NP. 1994. C5a anaphylatoxin and its seven transmembrane-segment receptor. Annu. Rev. Immunol. 12:775-808.
3. Ward PA. 2004. The dark side of C5a in sepsis. Nat. Rev. Immunol. 4:133-142.
4. Hopken UE, Lu B, Gerard NP, Gerard C. 1996. The C5a chemoattractant receptor mediates mucosal defense to infection. Nature; 383:86-89.
5. Hopken UE, Lu B, Gerard NP, Gerard C. 1997. Impaired inflammatory responses in the reverse Arthus reaction through genetic deletion of the C5a receptor. J. Exp. Med. 186:749-756.
6. Humbles AA, Lu B, Nilsson CA, Lilly C, Israel E, Fujiwara Y, Gerard NP, Gerard C. 2000. A role for the C3a anaphylatoxin receptor in the effector phase of asthma. Nature 406:998-1001.
7. Drouin SM, Corry DB, Holman TJ, Kildsgaard J, Wetsel RA. 2002. Absence of the complement anaphylatoxin C3a receptor suppresses Th2 effector functions in a murine model of pulmonary allergy. J. Immunol. 169:5926-5933.
8. Kildsgaard J, Hollmann TJ, Matthews KW, Bian K, Murad F, Wetsel RA. 2000. Cutting edge: targeted disruption of the C3a receptor gene demonstrates a novel protective anti-inflammatory role for C3a in endotoxin-shock. J. Immunol. 165:5406-5409.
9. Matthews KW, Mueller-Ortiz SL, Wetsel RA. 2004. Carboxypeptidase N: a pleiotropic regulator of inflammation. Mol. Immunol. 40:785-793.
10. Ohno M, Hirata T, Enomoto M, Araki T, Ishimaru H, Takahashi TA. 2000. A putative chemoattractant receptor, C5L2, is expressed in granulocyte and immature dendritic cells, but not in mature dendritic cells. Mol. Immunol. 37:407-412.
11. Cain SA, Monk PN. 2002. The orphan receptor C5L2 has high affinity binding sites for complement fragments C5a and C5a des-Arg(74). J. Biol. Chem. 277:7165-7169.
12. Okinaga S, Slattery D, Humbles A, Zsengeller Z, Morteau O, Kinrade MB, Brodebeck RM Krause JE, Choe HR, Gerard NP, Gerard C. 2003. C5L2, a nonsignaling C5A binding protein. Biochem. 42:9406-9415.
13. Kalant D, Cain SA, Maslowska M, Sniderman AD, Cianflone K, Monk PN. 2003. The chemoattractant receptor-like protein C5L2 binds the C3a des-Arg77/ acylation-stimulating protein. J. Biol. Chem. 278:11123-11129.
14. Kalant D, Maclaren R, Cui W, Samanta R, Monk PN, Laporte SA, Cianflone K. 2005. C5L2 is a functional receptor for acylation stimulating protein. J. Biol. Chem. (in press).
15. Gavriluk V, Kalinin S, Hilbush BS, Middlecamp A, McGuire S, Pelligrino D, Weinberg G, Feinstein DL. 2005. Identification of complement 5a-like receptor (C5L2) from astrocytes: characterization of anti-inflammatory properties. J. Neurochem. 92:1140-1149.
16. Wetsel, R.A., Ogata, R.T. Tack, B.F. 1987. Primary structure of the fifth component of murine complement. Biochem. 26:737-743.
17. Gao, J. Choe, H. Bota, D. Wright, P. L. Gerard, C. Gerard, N. P. 2003. Sulfation
of tyrosine 174 in the human C3a receptor is essential for binding of C3a anaphylatoxin. J. Biol. Chem. 278:37902-37908

18. Godau J, Heller T, Hawlisch H, Trappe M, Howells E, Best J, Zwirner J, Verbeek JS, Hogarth PM, Gerard C, Van Rooijen N, Klos A, Gessner JE, Kohl J. 2004. C5a initiates the inflammatory cascade in immune complex peritonitis. J. Immunol. 173:3437-3445.

19. Skokowa J, Ali SR, Felda O, Kumar V, Konrad S, Shushakova N, Schmidt RE, Piekorz RP, Nurnberg B, Spicher K, Birnbaumer L, Zwirner J, Claassens JW, Verbeek JS, van Rooijen N, KohlJ, Gessner JE. 2005. Macrophages induce the inflammatory response in the pulmonary Arthus reaction through G alpha i2 activation that controls C5aR and Fc receptor cooperation. J. Immunol. 174:3041-3050.

20. Su AI, Cooke MP, Ching KA, Hakak Y, Walker JR, Wiltshire T, Orth AP, Vega RG, Sapinoso LM, Mogrich A, Patapoutian A, Hampton GM, Schultz PG, Hogenesch JB. (2002) Large-scale analysis of the human and mouse transcriptosomes. Proc. Natl. Acad. Sci. USA. 99:4465-4470.

21. Gao H, Neff TA, Guo RF, Speyer CL, Sarma JV, Tomlins S, Man Y, Riedemann NC, Hoesel LM, Younkin E, Zetoune FS, Ward PA. 2005. Evidence for a functional role of the second C5a receptor C5L2. FASEB J. 19:1003-1005.

Acknowledgments: This work was supported in part by NIH grants HL36162 (NPG), HL69511 (CG), and the Perlmutter Foundation at Children’s Hospital.

Key Words: G protein coupled receptors, complement, inflammation, C5a, knockout mice, chemoattractant receptors.

Figure Legends

Figure 1. Molecular characterization of C5L2 deficient mice. A. Targeting construct for the murine C5L2 gene. The majority of the coding sequence for C5L2 was excised and replaced with genes encoding GFP and PGK-neomycin resistance. B. Restriction digests of tail snip DNA from wild type, C5L2+/- and C5L2-/- mice hybridized with probe B indicated in panel A (upper blot) or a C5a receptor probe (lower blot). C. RT-PCR of bone marrow mRNA from wild type and C5L2 mice demonstrating absence of C5L2 in the targeted animals (upper blot) and presence of the classical C5aR in all animals (middle blot). Comparable loading of mRNA is shown with GAPDH (lower blot).

Figure 2. Functional expression of the classical C5a receptor in C5L2 deficient mice. Normal function of the classical C5aR in C5L2-/- mice is shown by the magnitude of neutrophil influx following intraperitoneal injection of PBS containing 0 (open bars) or 5 µg recombinant murine C5a (solid bars). Four hours after injection the peritoneal cavity was lavaged and infiltrating cells quantified. Cells recovered in C5L2-/- animals were comparable to wild type, and significantly elevated compared to C5aR-/- mice. * P<0.05 for C5L2-/- or wild type vs. C5aR-/-, n=3-5 animals per group.

Figure 3. Pulmonary immune complex injury in C5L2-/- mice results in greater influx of inflammatory cells compared to wild type animals. Four hours after tail vein injection of ovalbumin and intratracheal instillation of anti-ovalbumin IgG, mice were sacrificed, lungs lavaged and cells were quantified. A. C5L2-/- mice exhibited
significantly greater total cells compared with wild type animals. ** P=0.008, n=5-7 mice per group. B. Lavaged neutrophil levels were also significantly elevated in C5L2 deficient animals. *** P=0.0005 for neutrophils, n=5-7 mice per group. No difference was observed in monocytes. Control animals received either antigen alone (Ag) or antibody alone (Ab).

**Figure 4.** C5L2 deficient mice release significantly more IL6 and TNFα than wild type animals following immune complex injury. Lung homogenates were assessed for IL6 and TNFα levels by ELISA as described in Materials and Methods. The cytokine levels assessed were each ~4-fold elevated in C5L2-/- mice compared to wild type animals. * P=0.03, ** P=0.002, n=3 animals per group.

**Figure 5.** Histologic analysis of lung lavage and tissue sections exhibit significantly enhanced inflammation in C5L2-/- mice compared to wild type animals following immune complex injury. A. Cyto centrifuge preparations of lung lavage fluid from wild type or C5L2-/- mice following immune complex formation in the lungs. Lungs were lavaged four hours after tail vein injection of ovalbumin and intratracheal instillation of anti-ovalbumin IgG. Lavage fluids from antigen alone (Ag) or antibody alone (Ab) controls are shown for comparison. B. Frozen sections of lungs from wild type or C5L2-/- mice following immune complex formation in the lungs, stained with hematoxylin and eosin. Antigen alone (Ag) and antibody alone (Ab) tissues are shown as controls.

**Figure 6.** C5a elicits elevated chemotactic responses relative to fMLP in C5L2 deficient mouse bone marrow cells compared with cells from wild type littermates. Fluorescently labeled bone marrow cells from C5L2-/- or wild type mice were tested for chemotactic activity in modified Boyden chambers as described in Materials and Methods. Chemotactic agents, 10nM C5a or 8µM fMLP, were placed in the lower chambers, with 1x10⁶ cells in the upper chambers. Fluorescence intensity passing to the underside of the filter was determined after a 45 min incubation, and the response to C5a normalized to fMLP. The relative chemotaxis to C5a for wild type cells was assigned as 100 (±0.04%) to facilitate comparison among experiments. The response of C5L2-/- cells was 141 ± 8.46% of wild type (** P=0.008, n=3 independent experiments performed in duplicate).
Figure 1
Figure 2
Figure 3

A

Cell Numbers x10^4

Ag
Ab
WT-IC
C5L2-/-IC

B

Cell Number x10^4

Neutrophils
Monocytes

Ag(n=4)
Ab(n=3)
WT-IC(n=7)
C5L2-/-IC(n=5)
Figure 4

**Panel A:**

C5L2+/+IC vs. C5L2-/-IC

- TNFα concentration pg/ml
- 0 to 200

**Panel B:**

C5L2+/+IC vs. C5L2-/-IC

- IL6 concentration pg/ml
- 0 to 1500

*Statistical significance:
- *: p < 0.05
- **: p < 0.01
Figure 5

BAL RBC is increased in C5L2-/- mice.

Figure 5
Figure 6

![Graph showing relative chemotaxis between WT and C5L2-/-. The graph indicates a significant difference with a ** symbol.]
An anti-inflammatory function for the complement anaphylatoxin C5a binding protein, C5L2
Norma P. Gerard, Bao Lu, Pixu Liu, Stewart Craig, Yuko Fujiwara, Shoji Okinaga and Craig Gerard

J. Biol. Chem. published online October 4, 2005

Access the most updated version of this article at doi: 10.1074/jbc.C500287200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts