The orphan receptor C5L2 has recently been described as a high affinity binding protein for complement fragments C5a and C3a that, unlike the previously described C5a receptor (CD88), couples only weakly to G-like G proteins (Cain, S. A., and Monk, P. N. (2002) J. Biol. Chem. 277, 7165–7169). Here we demonstrate that C5L2 binds the metabolites of C4a and C3a, C4a des-Arg77, and C3a des-Arg77 (also known as the acylation-stimulating protein or ASP) at a site distinct from the C5a binding site. The binding of these metabolites to C5L2 does not stimulate the degranulation of transfected rat basophilic leukemia cells either through endogenous rat G proteins or when co-transfected with human Gαs. C3a des-Arg77/ASP and C3a can potentially stimulate triglyceride synthesis in human skin fibroblasts and 3T3-L1 preadipocytes. Here we show that both cell types and human adipose tissue express C5L2 mRNA and that the human fibroblasts express C5L2 protein at the cell surface. This is the first demonstration of the expression of C5L2 in cells that bind and respond to C3a des-Arg77/ASP and C3a. Thus C5L2, a promiscuous complement fragment-binding protein with a high affinity site that binds C3a des-Arg77/ASP, may mediate the acylation-stimulating properties of this peptide.

C5a and C3a have wide ranging effects in humans. Although initially described as leukocyte chemoattractants and anaphytoxins, it is now clear that C5a and C3a are involved in microbial host defense, immune regulation (1), and protection against toxic insult (2–5). C5a and C3a are also reported to stimulate prostanoid production by guinea pig peritoneal macrophages and rat Kupffer cells (10), or human monocyte-like U937 cells (10), and unlike C3a, C3a des-Arg77 does not stimulate eosinophil chemotaxis (9), prostanoid production by guinea pig peritoneal macrophages and rat Kupffer cells (10), or human monocyte-like U937 cell degranulation (11). However, the following responses to C3a des-Arg77 have been reported. (i) The cytotoxicity of NK cells is inhibited by both C3a and C3a des-Arg77 (12). (ii) Cytokine production by human monocyte/macrophages and PBMC is enhanced by these ligands but inhibited in human tonsil-derived B cells (13, 14). (iii) Histamine release from rat peritoneal mast cells is stimulated (15). In addition, C3a des-Arg77 has well documented acylation-stimulating properties and increases triacylglycerol synthesis in human adipocytes, preadipocytes, and human skin fibroblasts (HSF). Here we demonstrate that the expression of C3a des-Arg77 form to the previously cloned and characterized C3a receptor (C3aR) is observed in transfected RBL cells or mouse macrophage/monocytes (8) and, unlike C3a, C3a des-Arg77 does not stimulate eosinophil chemotaxis (9), prostanoid production by guinea pig peritoneal macrophages and rat Kupffer cells (10), or human monocyte-like U937 cell degranulation (11). However, the following responses to C3a des-Arg77 have been reported. (i) The cytotoxicity of NK cells is inhibited by both C3a and C3a des-Arg77 (12). (ii) Cytokine production by human monocyte/macrophages and PBMC is enhanced by these ligands but inhibited in human tonsil-derived B cells (13, 14). (iii) Histamine release from rat peritoneal mast cells is stimulated (15). In addition, C3a des-Arg77 has well documented acylation-stimulating properties and increases triacylglycerol synthesis in human adipocytes, preadipocytes, and human skin fibroblasts (HSF). Here we show that both cell types and human adipose tissue express C5L2 mRNA and that the human fibroblasts express C5L2 protein at the cell surface. This is the first demonstration of the expression of C5L2 in cells that bind and respond to C3a des-Arg77/ASP and C3a. Thus C5L2, a promiscuous complement fragment-binding protein with a high affinity site that binds C3a des-Arg77/ASP, may mediate the acylation-stimulating properties of this peptide.

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The Chemoattractant Receptor-like Protein C5L2 Binds the C3a des-Arg77/Acylation-stimulating Protein*

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¶ The abbreviations used are: CD88, human C5a receptor; C3aR, human C3a receptor; HSF, human skin fibroblasts; ASP, acylation-stimulating protein; RBL, rat basophilic leukemia cell line; HEK 293, human embryonic kidney 293 cell; FACS, fluorescence-activated cell scanning; FLUOS, 5(6)-carboxyfluorescein-N-hydroxysuccinimide ester; PBS, phosphate-buffered saline; RT, reverse transcription; PT, pertussis toxin; PMA, phorbol 12-myristate 13-acetate; TGS, triglyceride synthesis.
FACS using FLUOS-C3a des-Arg77/ASP binding, selecting the top 50% of the population of positive cells each time.

**Transient Transfection of HEK 293 Cells**—HEK 293 cells were seeded into 6-well plates at 1 x 10^6 cells/well the day before transfection. C5L2 in vector pEE6hCMV.neo (Celltech) or C3aR in vector pCDNA1/AMP (Invitrogen) at 2 μg of DNA/well was transfected with LipofectAMINE 2000 (5 μl/well) (Invitrogen) according to the manufacturer’s protocol. Cells were assayed for binding/uptake 3 days post-transfection.

**Production of Anaphylatoxins**—Expression and purification of the recombinant His6-tagged C5a, C5a des-Arg74, and C3a were performed under denaturing conditions as described (21). Recombinant C4a, C4a des-Arg77, and C3a des-Arg77 were expressed and purified under non-denaturing conditions by sonication in the presence of BugBuster Protein Extraction Reagent (Novagen) using manufacturer’s conditions. Plasma C3a des-Arg77/ASP and plasma C3a were purified as described previously (17).

**Fluorescent Labeling of C3a des-Arg77/ASP and C3a—C3a des-Arg77/ASP and C3a were labeled with FLUOS (Roche Molecular Biochemicals) at a molar ratio of 1:10 (ligand to FLUOS) for 2 h according to the manufacturer’s recommendations. Labeled ligand was separated from free FLUOS on a Sephadex G25 M column and stored in aliquots at −80 °C.

**Radiolabeled Ligand Competition Receptor Binding Assays**—Competition binding assays were performed using 50 μM 125I-C5a or 125I-C3a (PerkinElmer Life Sciences) on adherent C3aR-, C5L2- or C3aR-, C5L2-transfected RBL cells in 96-well microtiter plates (55,000 cells/well) at 4 °C as described previously (22). Competition assays for HSF, 3T3-L1, U937, and HEK 293 were performed using 1 nM 125I-C3a or 125I-C3a des-Arg77/ASP on adherent cells in 96-well microtiter plates. Competition curves were generated by preincubating adherent cells with increasing concentrations of unlabeled complement fragments. The IC50, standard error values and linear regression analyses were obtained by using GraphPad Prism 2.0 or Sigma Plot.

**Production of Antiserum against C5L2**—Antiserum was raised in rabbits using the extracellular N-terminal sequence of human C5L2 (MGDNSVSYEYDYSDLSDRPVFC) coupled to keyhole limpet hemocyanin, as described previously (23). The serum recognized RBL cells transfected with human C5L2 (but not untransfected control cells) at dilutions as low as 1/10,000, and binding to C5L2 was totally inhibited by preincubation of serum with 100 μg/ml immunizing peptide.

**Fluorescence-activated Cell Scanning for Ligand Binding/Uptake Assays**—Cells were incubated with the indicated concentrations of FLUOS-labeled C3a des-Arg77/ASP or C3a for 30 min at 37 °C in binding buffer (24) and washed three times with cold binding buffer. Cells were then detached with 0.25% trypsin/0.02% EDTA in phosphate-buffered saline (PBS), fixed with 1% paraformaldehyde, washed with 0.3% PBS, and assayed by FACS. For anti-human C5L2 binding, cells were released from the culture dishes with non-enzymatic cell dissociation solution (Sigma), pelleted (600 g, 5 min), resuspended with anti-C5L2 antiserum (1:2000 in 3% bovine serum albumin in PBS), and incubated at 4 °C for 60 min. Again, cells were pelleted, washed twice with PBS, and resuspended in fluorescein isothiocyanate-labeled anti-rabbit IgG, (Sigma) at 1:1000 dilution in PBS and incubated at 4 °C for 60 min. Finally, cells were pelleted, washed twice, and resuspended in 0.3% paraformaldehyde in PBS for FACS analysis.

**Cellular Activation Assays**—Cellular activation was measured as the release of β-hexosaminidase from RBL intracellular granules (25) or as the stimulation of triglyceride synthesis in HSF and 3T3-L1 cells (17). For β-hexosaminidase assays, EC50 and standard error values were obtained by iterative curve fitting using GraphPad Prism 2.0. For triglyceride synthesis, cells were incubated with 100 μM [3H]oleate complexed to albumin (molar ratio 5:1) for 4 h. Triglyceride synthesis was calculated as [3H]oleate incorporation into triglyceride.

**Analysis of Receptor Expression by RT-PCR**—Total RNA was isolated by Trizol extraction from freshly isolated samples of human C5L2 (MGDNSVSYEYDYSDLSDRPVFC) coupled to keyhole limpet hemocyanin, as described previously (23). For β-hexosaminidase assays, EC50 and standard error values were obtained by iterative curve fitting using GraphPad Prism 2.0. For triglyceride synthesis, cells were incubated with 100 μM [3H]oleate complexed to albumin (molar ratio 5:1) for 4 h. Triglyceride synthesis was calculated as [3H]oleate incorporation into triglyceride.

**Summary of competition binding data for human chemoattractant receptors expressed in RBL cells**

| Unlabeled ligand | CD88 (125I-C3a) | C3aR (125I-C3a) | C5L2 (125I-C5a) | C5L2 (125I-C3a) |
|------------------|-----------------|-----------------|-----------------|-----------------|
|                  | IC50 (nM)       | IC50 (nM)       | IC50 (nM)       | IC50 (nM)       |
| C5a              |                 |                 |                 |                 |
| C5a des-Arg74    | 74.6            | 15              | 2900            | 3               |
| C4a              | 250             | 4               | 9670            | 3               |
| C4a des-Arg77    | 3               | 10,000          | 2               | 19,200          |
| C3a              | 12              | 155             | 2               | 19,200          |
| C3a des-Arg77/ASP| 3               | >100,000        | 3               | 18,500          |

* IC50 concentration (nM) of unlabeled ligand resulting in 50% competition of maximal radioligand binding.
* n, number of separate experiments performed in triplicate.
* ND, assay not done.

Fig. 1. C3a des-Arg77/ASP and C4a des-Arg77 bind to RBL cells expressing C5L2. RBL cells stably transfected with C5L2 were incubated with the stated concentrations of complement fragments for 10 min prior to the addition of 50 pM 125I-C5a (A) or 125I-C3a (B). Results are the means of n (n shown in Table I) separate experiments performed in triplicate ± S.E.
RESULTS AND DISCUSSION

C5L2 Is a Promiscuous Complement Fragment-binding Protein—We have shown previously that C5L2 has binding sites for C5a, C5a des-Arg^74, C4a, and C3a (18). Here we show that the des-Arg^77 forms of C4a and C3a are also ligands for this receptor when expressed in the RBL-2H3 cell line (Fig. 1, A and B, and Table I) and can compete strongly with 125I-C3a for C5L2 binding (Fig. 1A). In contrast, C4a des-Arg^77 and C3a des-Arg^77/ASP cannot compete effectively with 125I-C5a for C5L2 or CD88 binding (Fig. 1B, and Table I). Although C3aR and C5L2 bind C3a with similar affinities, C3aR has no detectable affinity for C3a des-Arg^77/ASP (Table I). Similarly, although C4a can compete with 125I-C3a for binding to both C3aR and C5L2, suggesting a similar affinity for both receptors, C4a des-Arg^77 is >50-fold more effective at competing with 125I-C3a binding at C5L2 than at C3aR (Table I). The data suggest either that C5L2 has two conformations with different ligand binding profiles or that the receptor has two binding sites. As we have shown previously that the B-max values for 125I-C3a and 125I-C5a binding to C5L2-transfected RBL cells are identical (18), the most likely explanation is that a single form of C5L2 has separate binding sites. We propose that one site binds 125I-C3a and C3a des-Arg^77/ASP, at which all of the complement fragments except C5a des-Arg^74 can compete with similar affinities, and that the second high affinity site, which preferentially binds 125I-C5a, can only be competed by C5a des-Arg^74 and, to a lesser extent, C4a.

C3a des-Arg^77/ASP Binds Directly to C5L2 but Not to C3aR or CD88—Because recombinant C3a des-Arg^77/ASP can clearly compete with 125I-C3a (but not C5a) for binding to C5L2, we then directly measured the affinity of C3a des-Arg^77/ASP for C5L2 using protein purified from human plasma as C3a des-Arg^77/ASP and tested for acylation-stimulating bioactivity. Plasma-purified human C3a des-Arg^77/ASP and C3a were both labeled with FLUOS. Increasing concentrations of C3a des-Arg^77/ASP were incubated with HEK 293 cells transiently transfected with C5L2, and binding and uptake were assessed by flow cytometry (Fig. 2A). FLUOS-C3a des-Arg^77/ASP clearly binds to C5L2 with half-maximal fluorescence intensity at ~3 nM, whereas mock-transfected cells (Fig. 2A, inset) show no binding of C3a des-Arg^77/ASP, even at a high concentration of 10 nM. For comparison purposes, the binding of FLUOS-C3a to HEK 293 cells transiently transfected with C3aR is shown (Fig. 2B) with half-maximal binding of FLUOS-C3a at 2.5 nM. In

| Unlabeled ligand | Human skin fibroblast | HEK C3aR |
|------------------|-----------------------|----------|
|                  | 125I-C3a des-Arg^77/ASP | 125I-C3a |
|                  | IC_50 (nM) | n | IC_50 (nM) | n |
| C3a des-Arg^77/ASP | 71.5 ± 17.0 | 5 | 178 ± 10.8 | 6 |
| C3a | 50.7 ± 4.2 | 5 | 56.6 ± 4.2 | 6 |
| No binding | 3 | No competition | 3 |
| ND ^d | 14.0 ± 3.7 | 3 |

^a IC_50 concentration (nM) of unlabeled ligand resulting in 50% competition of maximal radio ligand binding, mean ± S.E.

^n number of separate experiments, each performed using 15 concentrations of competing ligand each in triplicate.

^c Significantly different from IC_50 for C3a competition of 125I-C3a by paired t-test, p < 0.025.

^d ND, assay not done.

^e p < 0.001, IC_50 for HEK C3aR versus HSF for 125I-C3a.
C5L2 binds C3a des-Arg^{77/ASP}

TABLE III
Summary of receptor activation data for human chemoattractant receptors expressed in RBL cells

| Ligand        | CD88 EC_{50} | CD88 n | C3aR EC_{50} | C3aR n |
|---------------|--------------|--------|--------------|--------|
| C5a           | 5.85         | 16     | >10,000      | 2      |
| C5a des-Arg^{74} | 21.2       | 5      | >10,000      | 2      |
| C4a           | >10,000      | 2      | >10,000      | 2      |
| C4a des-Arg^{77} | ND         |       | >10,000      | 2      |
| C3a           | >10,000      | 2      | 50.2         | 8      |
| rC3a des-Arg^{77/ASP} | ND       |       | >10,000      | 2      |
| Plasma C3a des-Arg^{77/ASP} | >10,000 | 1      | >10,000      | 2      |

\* EC_{50}, concentration of ligand (nm) resulting in 50% of maximal degranulation.
\* n, number of separate experiments performed in triplicate.
\* ND, assay not done.

separate experiments, FLUOS-C3a des-Arg^{77/ASP} binding to C3aR transfected cells was found to be not significantly different from basal (basal fluorescence = 100%; FLUOS-C3a des-Arg^{77/ASP} = 103% ± 8%, mean ± S.E., n = 3), and neither FLUOS-C3a des-Arg^{77/ASP} nor FLUOS-C3a showed binding to cells transiently transfected with CD88, the C5a receptor (Fig. 2C).

C3a des-Arg^{77/ASP} binding was further examined in cells that are responsive to the acylation-stimulating properties of C3a des-Arg^{77/ASP} and compared with that in HEK cells transfected with C3aR and CD88. 125I-C3a des-Arg^{77/ASP} does not bind to C3aR-transfected HEK cells and does not compete with 125I-C3a (Table II), as found previously (26). Similarly, B_{L}cAMP-differentiated U937 macrophages (which are reported to express the C3a receptor and respond to C3a) demonstrated no specific C3a des-Arg^{77/ASP} binding (data not shown). The result was also negative for undifferentiated U937 cells (data not shown). Also, C3a des-Arg^{77/ASP} does not bind to HEK 293 cells transfected with CD88 (binding of 125I-C3a des-Arg^{77/ASP}, mock transfection 100% ± 4%, n = 6; irrelevant receptor transfection, 102% ± 11%, n = 6; CD88 transfection, 110% ± 22%, n = 6). Similar results were obtained for 125I-C3a binding to CD88 (irrelevant receptor transfection, 100% ± 6%, n = 6; CD88 transfection, 99% ± 17, n = 6). By contrast, human skin fibroblasts, which respond to C3a des-Arg^{77/ASP} by increasing triglyceride synthesis (27), bind both 125I-C3a des-Arg^{77/ASP} and 125I-C3a with high affinity (Table II). As observed in C5L2-transfected RBL cells, unlabeled C3a des-Arg^{77/ASP} is slightly less effective at competing for 125I-C3a binding than unlabeled C3a in both HSF- and C5L2-transfected RBL cells (Tables II and I, respectively), whereas C3a was an effective competitor for 125I-C3a des-Arg^{77/ASP} binding (Table II). Thus, C5L2 has binding characteristics that overlap with both CD88 and C3aR but also has the unique ability to bind C3a des-Arg^{77/ASP}, which parallels the binding characteristics of HSF cells.

C3a des-Arg^{77} Binding to C5L2 Does Not Stimulate Degranulation in C5L2-Transfected RBL Cells—We have shown previously that C5a, C5a des-Arg^{74}, C4a, and C3a binding to C5L2 does not stimulate either an increase in intracellular C{A}^{2+} or the degranulation of transfected RBL cells due to weak coupling to endogenous G_{i} like G proteins (18). We also examined the effects of C3a des-Arg^{77/ASP} and C4a des-Arg^{77/ASP} and found that these ligands did not stimulate degranulation in transfected RBL cells at concentrations of up to 10 μM (data not shown). In addition, there was no effect of these two ligands on either CD88 or C3aR activation of degranulation (Table III) although the expected responses to C5a, C5a des-Arg^{77}, and C3a, respectively, are robust. Neither recombinant nor plasma-purified C3a des-Arg^{77/ASP} (nor any other ligand) is able to activate endogenous G proteins in C5L2-transfected RBL cells.
represents 100%.

We reasoned that the moderate response of Cell lines that are negative for C3a des-Arg77/ASP binding and response to C3a des-Arg77/ASP and C3a.

Co-transfected cells (Fig. 3) with human G/H9251 do not respond with an increase in TGS to either C3a or C3a des-Arg/ASP (Table IV). However, these cell types may express in RBL cells and HSF with comparable affinity, because the presence of the carboxypeptidase inhibitor (Plummer’s inhibitor) has no effect on C3a bioactivity (Table IV). Increased TGS is not simply a response to C3a binding, however, as C3aR-transfected HEK cells and Bt2-cAMP-differentiated U937 monocytes cells (which express the C3aR and bind C3a) do not respond with an increase in TGS to either C3a or C3a des-Arg/ASP (Table IV). However, these cell types may lack all or part of the machinery to mount an increase in TGS, as there is no significant response to treatment with PMMA or insulin (Table IV).

Both C3a and C3a des-Arg/ASP bind to the C5L2 receptor expressed in RBL cells and HEK with comparable affinity, suggesting that C5L2 may be the C3a des-Arg/ASP receptor on HEK. As C5L2 has already been shown to bind several complement fragments, we examined the degranulation response of other C5L2 ligands in cells that respond to C3a des-Arg/ASP. Even at higher concentrations than those usu-

### Table IV

| Stimulus                  | Conc | Cell type  |
|---------------------------|------|------------|
|                           |      | HSF        |
|                           |      | U937       |
|                           |      | Bt2cAMP-U937 |
|                           |      | HEK        |
|                           |      | HEK C3aR   |
|------------------------------------------|------|------------|
| C3a des-Arg77/ASP                    | 5 µM | 175.9 ± 10.9  |
| C3a                                    | 5 µM | 212.6 ± 27.7  |
| C3a + CBPI inhibitor                  | 5 µM | 197.0 ± 6.5   |
| Insulin                               | 10 nm | 206.4 ± 14.3  |
| PMA                                   | 1 nm  | 184.6 ± 19.3  |

**a n, number of values.**
**b Percentage of stimulation of triglyceride synthesis.** The results are expressed as mean ± S.E. relative to basal triglyceride synthesis, which represents 100%.
**c Significantly different from basal, p < 0.025.**
**d CBP, carboxypeptidase inhibitor.**
**e ND, assay not done.**

### Table V

| Concentration | Peptide | C3a des-Arg77/ASP (n = 3) | C5a (n = 6) | C5a des-Arg74 (n = 6) | C4a (n = 4) | C4a des-Arg77 (n = 4) |
|---------------|---------|---------------------------|------------|----------------------|------------|----------------------|
| 1 µM          | ND      | 91.1 ± 6.6               | 102.0 ± 4.1| 106.4 ± 2.5          | 105.2 ± 3.0|
| 2 µM          | ND      | 101.7 ± 13.3             | 100.7 ± 3.6| 90.8 ± 5.1           | 104.6 ± 7.4|
| 5 µM          | 213.0 ± 9.1 | 102.6 ± 10.1            | 84.9 ± 8.5 | 98.4 ± 3.5           | 104.6 ± 8.0|
| 10 µM         | ND      | 111.2 ± 12.6             | 101.2 ± 5.7| 92.6 ± 4.6           | 104.3 ± 3.7|

**a n, number of values.**
**b Percentage of stimulation of triglyceride synthesis.** Results are expressed as mean ± S.E. relative to basal triglyceride synthesis, which represents 100%.
**c ND, assay not done.**
**d Significantly different from basal; p < 0.0001.**
different roles, one involved solely in ligand binding and one involved in both binding and activation of TGS. Thus, C5a, which binds to the first site on C5L2, may be able to sterically hinder the binding of ligands that interact primarily with the second site (C3a and C3a des-Arg77/ASP) without activation of receptor. The ability of C5a to influence binding to the second site is presumably dependent on the C-terminal Arg residue, as C5a des-Arg74 cannot compete for 125I-C3a binding to C5L2.

C5L2 mRNA and Cell Surface Protein Are Expressed in Adipose Tissue, Skin Fibroblasts, and 3T3-L1 Preadipocytes—Although C3a des-Arg77/ASP is regarded as biologically inactive in most myeloid systems, the acylation-stimulating properties of this complement fragment are well documented in adipocytes and related cells (33). We therefore investigated the expression of C5L2 in human adipose tissue, HSF, and 3T3-L1 preadipocytes, because fibroblasts, preadipocytes, and adipocytes are all known to respond directly to C3a and C3a des-Arg77/ASP by an increase in triglyceride synthesis (Table IV) and glucose transport (17). We performed RT-PCR using species-specific sets of primers to detect expression in human adipocytes, HSF, and mouse 3T3-L1 preadipocyte mRNA. Both primer sets (human and murine) produced a band as seen on polyacrylamide electrophoresis gels at sizes similar to those expected for a C5L2 transcript (Fig. 4). As the DNA markers are standardized for agarose gels and not polyacrylamide gels, the human adipose tissue PCR product was extracted from an agarose gel and sequenced. We confirmed the authenticity of the transcript as that of C5L2. By contrast, RT-PCR of RNA from the human monocytic cell line U937 and non-transfected HSF 293 cells did not result in any PCR product using C5L2 primers despite equal levels of glyceraldehyde-3-phosphate dehydrogenase (Fig. 4).

These results were further confirmed using an antisera specific to the N-terminal region of human C5L2. FACS analysis clearly demonstrates that HSFs (Fig. 5A) express endogenous C5L2 on their cell surface, although the fluorescent intensity was lower than that of HEK 293 cells overexpressing C5L2 (Fig. 5B). In contrast, untransfected HSF 293 cells did not bind the anti-serum (Fig. 5C). As the antisera does not appear to recognize murine C5L2, cells transfected with mouse C5L2 were negative (data not shown), and we were unable to test for the expression of C5L2 on the surface of the murine 3T3-L1 cells.

In summary, we have shown that adipocytes, HSF, and 3T3-L1 preadipocytes, cell types that have been shown to bind both C3a and C3a des-Arg77/ASP and to respond to these ligands with increased triglyceride synthesis, also express C5L2. C5L2 binds both ligands with high affinity, suggesting that it may be a functional C3a des-Arg77/ASP and C3a receptor when expressed in appropriate cell types. In contrast, C5a and C5a des-Arg74, which bind preferentially to a different site on C5L2, do not stimulate triglyceride synthesis. The role of C5L2 in cellular responses to complement fragments is clearly complex and remains to be elucidated.

REFERENCES

1. Kohl, J. (2001) Mol. Immunol. 38, 175–187
2. Bhattacharya, S., Littman, J., Schupf, N., and Williams, C. A. (1983) J. Immunol. 126, 277–287
3. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
4. O’Halloran, P. J., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
5. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
6. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
7. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
8. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
9. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
10. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
11. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
12. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
13. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
14. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
15. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
16. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
17. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
18. Mukherjee, P., and Pasinetti, G. M. (2001) J. Neurochem. 77, 43–49
19. Daffarn, P. J., Pfeifer, P. H., and Hugli, T. E. (1995) J. Exp. Med. 181, 2177–2184
C5L2 binds C3a des-Arg77/ASP

181, 2119–2127

10. Puschel, G. P., Nolte, A., Schieferdecker, H. L., Rothermel, E., Gotze, O., and Jungermann, K. (1996) Hepatology 24, 685–690
11. Klos, A., Bank, S., Gietz, C., Bautsch, W., Kohl, J., Burg, M., and Kretzschmar, T. (1992) Biochemistry 31, 11274–11282
12. Charriaut, C., Senik, A., Kolb, J. P., Barel, M., and Frade, R. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 6083–6087
13. Fischer, W. H., Jagels, M. A., and Hugli, T. E. (1999) J. Immunol. 162, 453–459
14. Fischer, W. H., and Hugli, T. E. (1997) J. Immunol. 159, 4279–4286
15. Mousli, M., Hugli, T. E., Landry, Y., and Bronner, C. (1992) J. Immunol. 148, 2456–2461
16. Cianflone, K., and Maslowska, M. (1995) Eur J. Clin. Invest. 25, 817–825
17. Murray, L., Parker, R. A., Kirchgessner, T. G., Tran, J., Zhang, Z. J., Westerlund, J., and Cianflone, K. (1997) J. Lipid Res. 38, 2492–2501
18. Cain, S. A., and Monk, P. N. (2000) J. Biol. Chem. 275, 7165–7169
19. Cianflone, K., Maslowska, M. H., and Sniderman, A. D. (1999) J. Clin. Invest. 85, 722–730
20. Monk, P. N., Barker, M. D., Partridge, L. J., and Pease, J. E. (1995) J. Biol. Chem. 270, 16625–16629
21. Paczkowski, N. J., Finch, A. M., Whitmore, J. B., Short, A. J., Wong, A. K., Monk, P. N., Cain, S. A., Fairlie, D. P., and Taylor, S. M. (1999) Br. J. Pharmacol. 128, 1461–1466
22. Monk, P. N., Barker, M. D., and Partridge, L. J. (1994) Biochem. Biophys. Acta 1221, 323–329
23. Ohno, M., Hirata, T., Enomoto, M., Araki, T., Ishimaru, H., and Takahashi, T. A. (2000) Mol. Immunol. 37, 407–412
24. Crass, T., Raffetseder, U., Martin, U., Grove, M., Klos, A., Kohl, J., and Bautsch, W. (1996) Eur. J. Immunol. 26, 1844–1850
25. Cain, S. A., Coughlan, T., and Monk, P. N. (2001) Biochemistry 40, 14047–14052
26. Wilken, H. C., Rogge, S., Gotze, O., Werfel, T., and Zvirner, J. (1999) J. Immunol. Methods 226, 139–145
27. Cianflone, K., Vu, H., Walsh, M., Baldo, A., and Sniderman, A. (1989) J. Lipid Res. 30, 1727–1733
28. Sheth, B., Banks, P., Burton, D. R., and Monk, P. N. (1991) Biochem. J. 275, 809–811
29. Shum, J. K., Allen, R. A., and Wong, Y. H. (1995) Biochem. Biophys. Res. Commun. 208, 223–229
30. Buhl, A. M., Eisfelder, B. J., Worthen, G. S., Johnson, G. L., and Russell, M. (1993) FEBS Lett. 323, 132–134
31. Baldo, A., Sniderman, A. D., St. Luce, S., Zhang, X. J., and Cianflone, K. (1995) J. Lipid Res. 36, 1415–1423
32. Cianflone, K., Maslowska, M., and Sniderman, A. D. (1999) Semin. Cell Dev. Biol. 10, 31–41