SARS-CoV-2
Biology and Reagents
Request a Complimentary Wall Poster

Cutting Edge: Differential Constitutive Expression of Functional Receptors for Lysophosphatidic Acid by Human Blood Lymphocytes

This information is current as of May 9, 2021.

Edward J. Goetzl, Yvonne Kong and Julia K. Voice

J Immunol 2000; 164:4996-4999; ;
doi: 10.4049/jimmunol.164.10.4996
http://www.jimmunol.org/content/164/10/4996

References This article **cites 20 articles**, 14 of which you can access for free at:
http://www.jimmunol.org/content/164/10/4996.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

**average*

Subscription Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

Downloaded from http://www.jimmunol.org/ by guest on May 9, 2021

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2000 by The American Association of
Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.

CUTTING EDGE

Cutting Edge: Differential Constitutive Expression of Functional Receptors for Lysophosphatidic Acid by Human Blood Lymphocytes[1]

Edward J. Goetzl,[2] Yvonne Kong, and Julia K. Voice

Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) from platelets and macrophages mediate T cell functions. Endothelial differentiation gene-encoded G protein-coupled receptors (Edg Rs) are specific for S1P (Edg-1, -3, -5, and -8 Rs) and LPA (Edg-2, -4, and -7 Rs). Human T cell tumors express many Edg Rs for both LPA and S1P. In contrast, human blood CD4$^+$ T cells express predominantly Edg-4, and CD8$^+$ T cells show only traces of Edg-2 and -5, by quantification of mRNA and Edg R Ags. LPA at 10^{-10}–10^{-6} M suppressed significantly the secretion of IL-2 from anti-CD3 plus anti-CD28 Ab-challenged CD4$^+$ T cells, but not CD8$^+$ T cells. Monoclonal anti-Edg-4 R Ab, like LPA, suppressed stimulated IL-2 secretion from CD4$^+$ T cells, but not CD8$^+$ T cells. Constitutive expression of Edg-4 by CD4$^+$, but not CD8$^+$, human T cells accounts for differential functional responsiveness of the T cell subsets to LPA. ***The Journal of Immunology,*** **2000, 164: 4996–4999.**

The lysophospholipid growth factors lysophosphatidic acid (LPA)[3] and sphingosine 1-phosphate (S1P) are generated and released predominantly by platelets, macrophages, epithelial cells, and some tumor cells. LPS and S1P are bound extensively by albumin and other plasma proteins, and attain concentrations as high as micromolar in serum and tissues (1–4). LPA and S1P both evoke proliferation and diverse functional responses of many types of cells by binding to one or more members of a subfamily of G protein-coupled receptors encoded by endothelial differentiation genes (Edg Rs) (5–8). Edg Rs differ in lysophospholipid ligand specificity and relative levels of expression by each type of cell. Of the Edg Rs for which ligands have been defined, Edg-1, -3, -5, and -8 bind S1P most avidly, whereas Edg-2, -4, and -7 vastly prefer LPA.

LPA and S1P both stimulate and inhibit proliferation, enhance survival, suppress apoptosis, and elicit migration of T cell lymphoma or lymphoblastoma cells (9–13). In the few analyses of Edg Rs in such T cell lines, there was broad expression of Edg-1, -2, -3, -4, and -5 with quantitative predominance of Edg-2, -3, and -4 (11, 12, 14). Systematic evaluation of Edg Rs in human native immune cells and of the effects of LPA and S1P on immune functions of the major sets of normal T cells have not been undertaken previously, in part because of the unavailability of Abs highly specific for each Edg R. The present analyses of Edg Rs in human normal immune cells use recently generated mouse mAbs to peptide substituents of Edg Rs for Western blots in parallel with quantification of mRNA.

Materials and Methods

Isolation of human blood immune cells

Mixed mononuclear leukocytes were recovered from heparinized venous blood of healthy volunteers by centrifugation at 400 × *g* for 25 min at room temperature on Ficoll-Paque (Pharmacia, Piscataway, NJ), washed twice, and resuspended at 1×10^6/ml in RPMI 1640 containing 100 μg/ml fatty acid-free BSA (Calbiochem, La Jolla, CA) (RPMI 1640-faf-BSA). Monocytes and B cells, respectively, were isolated directly with metallic microbeads bearing anti-CD14 and anti-CD19 Abs before two cycles of magnetic retention chromatography (Miltenyi Biotec, Auburn, CA). CD4$^+$ and CD8$^+$ subsets of T cells were isolated from nonadherent mononuclear leukocytes by sequential reaction with biotinylated mouse monoclonal anti-human CD4 and anti-human CD8 Abs (PharMingen, San Diego, CA), respectively, washing, binding of streptavidin metallic microbeads (Miltenyi Biotec), and two cycles of magnetic retention chromatography. The purity of each set of mononuclear leukocytes was >96%, as assessed by flow cytometry.

RT-PCR semiquantification of mRNA encoding Edg receptors

Total cellular RNA was extracted by the TRIzol method (Life Technologies, Grand Island, NY) from suspensions of CD4$^+$, CD8$^+$, CD19$^+$, and CD14$^+$ sets of human blood cells, as well as four lines of rat HTC4 hepatoma cells, that have very low endogenous expression of native Edg-Rs and stably overexpress recombinant human Edg-2, -3, -4, or -5 Rs (15, 16). RT-PCR was performed as described (11, 15). [α-^{32}P]dCTP, 2 μCi, was added to some sets of reaction mixtures to allow radioactive quantification of mRNA encoding each Edg R relative to that of the constitutive standard G3PDH (17). The sequences of oligonucleotide primer pairs for G3PDH and human Edg-1 to -5 have been provided (11, 12). The primers for Edg-7 were: 5′-CCATAGCAACCTGACCAAAAAGAG (450–473) and 5′-TCCTTGTAGGAGTAGATGATGGGG (909–932) (18). Ethidium bromide-stained G3PDH and Edg R cDNA bands were cut from 2 g/100 ml agarose gels and solubilized in 0.5 ml sodium perchlorate solution at 55°C for 1 h before β-scintillation counting (Elu-Quick, Schleicher and Schuell, Keene, NH). Relative quantities of cDNA encoding each Edg R were expressed in terms of the ratio of radioactivity to that in the corresponding G3PDH band (11, 15).

Departments of Medicine and Microbiology-Immunology, University of California, San Francisco, CA 94143

Received for publication February 3, 2000. Accepted for publication March 14, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Institutes of Health Grant HL-31809.

[2] Address correspondence and reprint requests to Dr. Edward J. Goetzl, University of California, UB8B, Box 0711, 533 Parnassus at 4th San Francisco, CA 94143-0711. E-mail address: egoetzl@itsa.ucsf.edu

[3] Abbreviations used in this paper: LPA, lysophosphatidic acid; S1P, sphingosine 1-phosphate; Edg R, G protein-coupled receptor encoded by an endothelial differentiation gene; faf-BSA, fatty acid-free BSA.

Copyright © 2000 by The American Association of Immunologists

0022-1767/00/$02.00

Downloaded from http://www.jimmunol.org/ by guest on May 9, 2021

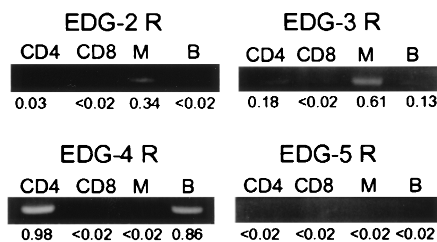

FIGURE 1. Semiquantitative PCR analyses of cDNAs encoding Edg-2 Rs, Edg-3 Rs, Edg-4 Rs, and Edg-5 Rs in human immune cells. Immune cells were from venous blood of 20 healthy female and male volunteers of different races and ages 27–58 years: CD4, helper/inducer T cells; CD8, suppressor/cytotoxic T cells; CD14, monocytes (M); and CD19, B cells (B). The ratio of ^{32}P radioactivity in each Edg R PCR cDNA to that in the G3PDH cDNA is shown below the corresponding lane.

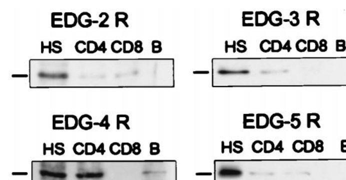

FIGURE 2. Western blot of Edg Rs extracted from immune cells. The immune cells were isolated from a single healthy volunteer whose CD4$^+$ cells had detectable mRNA for Edg-2, -3, and -5, as well as Edg-4, and CD8$^+$ cells had mRNA for Edg-2 and -5, but B cells had mRNA only for Edg-4 (Table I). Two micrograms of cellular proteins from HTC4 cell standard transfectants (HS) and 10 μg from T helper cells (CD4), T suppressor cells (CD8), and B cells (B) were electrophoresed, transferred, and labeled with the anti-Edg R Abs noted for each frame. The horizontal line at the margin of each frame depicts the position of a 45-kDa prestained marker. This pattern is representative of those from five donors.

Western blot analyses of Edg Rs

Proteins were extracted from replicate suspensions of 3–5 $\times$ 10^6 human purified immune cells and HTC4 cell Edg-2, -3, -4, and -5 R stable transfectants and resolved by electrophoresis with a rainbow-prestained set of m.w. markers (New England Nuclear, Boston, MA or Amersham, Arlington Heights, IL) as described (12, 15). Blots were developed with 0.5–1 μg/ml mouse monoclonal anti-Edg-2, -3, -4, or -5 R Ab and then HRP-labeled goat anti-mouse IgG, before development with an ECL kit (Amersham) (11, 12). The amino-terminal peptide epitopes for Edg-2, -3, -4, and -5 Rs consisted of amino acids 6–25, 1–21, 9–27, and 1–23, respectively.

Stimulation and quantification of IL-2 secretion by CD4$^+$ and CD8$^+$ T cells

Replicate suspensions of 3–4 $\times$ 10^5 CD4$^+$ and CD8$^+$ T cells in 0.5 ml RPMI 1640-faf-BSA with 100 U/ml penicillin and 100 μg/ml streptomycin were added to 24-well polystyrene plates without and with precoating by 0.5 μg each of mouse monoclonal anti-CD3 and anti-CD28 for 2 h at 37°C and 16 h at 4°C (PharMingen). T cells were incubated for 24 h at 37°C in 5% CO_2 in air after addition of 10^{-11}–10^{-6} M LPA, S1P, control phospholipids (Biomol, Plymouth Meeting, PA), an optimally effective amount of mouse monoclonal anti-Edg-4 R peptide Ab capable of signaling through the amino terminus of Edg-4 specifically (19), or mouse isotype-matched monoclonal anti-Edg-3 R amino-terminal peptide Ab in RPMI 1640-faf-BSA. Plates then were centrifuged at 1200 $\times$ g for 10 min at 4°C, and 150 μl of supernatant were removed from each well. The concentration of IL-2 in each well was determined by ELISA (Endogen, Woburn, MA), which is highly specific for human IL-2 and has a coefficient of variation <10% and a sensitivity of 6 pg/ml. As concentrations of IL-2 attained by CD4$^+$ and CD8$^+$ T cells stimulated with anti-CD3 plus anti-CD28 varied among sets from healthy volunteers by up to 10-fold, each control positive value was set at 100%, and the decreases achieved by lipids or anti-Edg R Ab were expressed as lower percentages of the control level. The significance of each mean decrease relative to each mean positive control value was calculated by a paired t test comparing every absolute suppressed value with the absolute value of its respective positive control.

Results

Edg Rs expressed by the functionally distinct sets of immune cells isolated immunomagnetically from blood of healthy volunteers were mapped first by RT-PCR, using pools of first-strand cDNAs from 20 subjects of different ages, sexes, and races (Fig. 1). The CD4$^+$ T cells and B cells had mRNA encoding predominantly Edg-4. Levels of Edg-2 and -3 mRNA much lower than that encoding Edg-4 were detected in CD4$^+$ T cells and B cells of some subjects, and traces of Edg-5 rarely were found in CD4$^+$ T cells (Table I). In sharp contrast, isolated CD8$^+$ T cells had no detectable mRNA encoding Edg-4 R and only occasionally trace levels of Edg-2 and Edg-5 (Table I). Monocytes had similarly high levels of mRNA for Edg-2 and -3 Rs, but none for Edg-4 or -5. None of the immune cells had Edg-1 or -7 (Table I). The same sets of immune cells isolated immunomagnetically from venous blood of three additional normal subjects showed patterns with considerable individual variation in the minor bands, but with universal prominence of Edg-4 in CD4$^+$ T cells and absence of Edg-4 from CD8$^+$ T cells (Table I).

Western blot analyses of the principal Edg-2 to -5 Rs detected in T cells and B cells by RT-PCR confirmed the striking differences in expression for the CD4$^+$ and CD8$^+$ subsets of T cells (Fig. 2). CD4$^+$ T cells showed predominantly Edg-4 protein and only very low levels of Edg-2, -3, or -5 proteins. In contrast, CD8$^+$ T cells showed no Edg-4 protein and either only very low levels of Edg-2 and/or -5 proteins or no Edg proteins. B cells had the expected predominance of Edg-4 with occasional traces of Edg-3. These constitutive patterns of expression of Edg Rs detected in human blood T cells predicted functional responses of the CD4$^+$ subset to LPA and possibly high concentrations of S1P, but no responses of the CD8$^+$ subset to either LPA or S1P.

LPA and S1P both have many stimulatory and inhibitory effects on diverse functions of cultured lines of human neoplastic T cells. In such T cell lines, LPA and S1P evoke very similar patterns of functional responses (9–14). However, LPA and S1P had very different effects in human blood-derived sets of T cells. LPA, but not S1P, inhibited IL-2 secretion by human blood CD4$^+$ T cells

Table I. *Relative levels of mRNA encoding Edg Rs in human blood immune cells*[a]

Cell	Edg-1	Edg-2	Edg-3	Edg-4	Edg-5	Edg-7
CD4$^+$	<0.02	0.02–0.14	0.14–0.23	0.79–1.16	<0.02–0.07	<0.02
CD8$^+$	<0.02	<0.02–0.19	<0.02	<0.02	<0.02–0.08	<0.02
B	<0.02	<0.02–0.06	<0.02–0.15	0.67–0.94	<0.02	<0.02
M	<0.02	0.21–0.44	0.47–0.70	<0.02	<0.02	<0.02
HS	1.14	1.18	1.22	1.26	0.98	1.06

[a] Each number is the mean for the extracts of HTC4 cell transfectants (HS) overexpressing one Edg R or the range (n = 3) for blood immune cells. Values represent the ratio of ^{32}P in the respective Edg R cDNA to that in the G3PDH cDNA amplified from the same sample.

Downloaded from http://www.jimmunol.org/ by guest on May 9, 2021

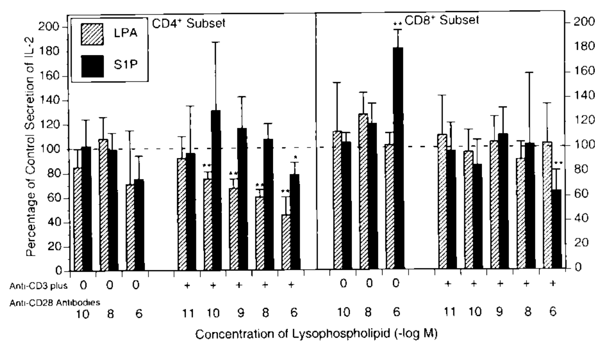

FIGURE 3. LPA inhibition of IL-2 generation by CD4$^+$ T cells but not by CD8$^+$ T cells. Each bar and bracket depicts the mean ± SD of the results of three separate studies of T cells from three healthy volunteers. The respective control values (100%) in the absence of phospholipids showed ranges of: CD4$^+$ in medium alone, 28–46 pg/ml; CD4$^+$ with anti-CD3 plus anti-CD28 Abs, 214-1086 pg/ml; CD8$^+$ in medium alone, 19–22 pg/ml; and CD8$^+$ with anti-CD3 plus anti-CD28 Abs, 116–224 pg/ml. Exposure of CD4$^+$ and CD8$^+$ sets of T cells to anti-CD3 plus anti-CD28 mAbs for 24 h, under the conditions used to generate IL-2, did not change the patterns of relative expression of Edg Rs. The range of maximal inhibition by 10^{-6} M LPA of IL-2 secretion by stimulated CD4$^+$ T cells was 32–61%. The symbols denoting statistical significance calculated by the paired *t* test were: *, $p < 0.025$; and **, $p < 0.01$.

stimulated with anti-CD3 plus anti-CD28 Abs (Fig. 3). Suppression of IL-2 secretion by stimulated CD4$^+$ T cells was significant and increased in extent from 10^{-10}–10^{-6} M LPA, attained mean maximum inhibition of 55% at 10^{-6} M LPA after 24 h, and was not accompanied by effects of LPA on IL-2 secretion by unstimulated CD4$^+$ T cells or stimulated or unstimulated CD8$^+$ T cells. S1P inhibited significantly, but modestly, stimulated secretion of IL-2 by CD4$^+$ T cells only at 10^{-6} M (Fig. 3). The control lysophospholipids phosphatidic acid and sphingosine had no effect on stimulated IL-2 secretion by CD4$^+$ T cells. The prominent suppressive activity of LPA for stimulated secretion of IL-2 by CD4$^+$ T cells presumably is mediated by Edg-4 Rs, whereas the marginal activity of S1P for the same T cells is dependent on the lesser expression of Edg-3 Rs (Figs. 1 and 2). The capacity of 10^{-6} M S1P to enhance secretion of IL-2 by unstimulated CD8$^+$ T cells and to inhibit IL-2 secretion by stimulated CD8$^+$ T cells is not explained by Edg R mediation but may reflect an intracellular messenger role of S1P (20).

A monoclonal anti-Edg-4 Ab directed to the amino terminus of Edg-4 Rs elicits cellular signals similar to those evoked by LPA in other types of human cells (19). This anti-Edg-4 R Ab, but not an isotype-matched monoclonal anti-Edg-3 R Ab, suppressed IL-2 secretion by stimulated CD4$^+$ T cells, but not unstimulated CD4$^+$ T cells or unstimulated or stimulated CD8$^+$ T cells after 24 h (Fig. 4). The pattern of anti-Edg-4 R Ab inhibition of IL-2 secretion by sets of human blood T cells was identical with that observed with LPA.

Discussion

Most types of cells of adult humans and other mammals, which have been studied to date, express two or more different Edg Rs in nearly equivalent abundance (5–8). For example, endothelial cells have high levels of Edg-1, -2, and -3 Rs, whereas epithelial cells from several sources have similarly prominent levels of Edg-2, -3, and -5 Rs. The first analyses of Edg Rs and responses to LPA and S1P in cultured lines of T cell lymphomas and lymphoblastomas provided profiles of a breadth equivalent to or greater than nonlymphoid cells with predominant expression of Edg-2, -3, and -4, and lower levels of Edg-1 and -5 (11, 12, 14). It thus was surprising to find that freshly isolated human blood CD4$^+$ T cells constitutively expressed predominantly Edg-4 Rs and only very low levels of Edg-2, -3, and/or -5. In contrast, CD8$^+$ T cells isolated from the same donors had no Edg-4 Rs and only marginally detectable Edg-2 and/or Edg-5 or no detectable Edg Rs (Figs. 1 and 2 and Table I).

Inhibition of stimulated generation of IL-2 by LPA was observed only with CD4$^+$ T cells and not CD8$^+$ T cells (Fig. 3). This inhibition of IL-2 secretion by LPA, as for LPA suppression of proliferation of some T cell clones (9), was not blocked by prior treatment with pertussis toxin under conditions known to prevent Gi-mediated LPA enhancement of proliferation of many types of cells (7). Pertussis toxin-resistant regulation of cellular proliferation and functions by LPA in some cultured lines is mediated by $\beta\gamma$ dimers of G proteins capable of sequentially altering phosphatidylinositol 3-kinase and extracellular signal-regulated kinases by *ras*-dependent or -independent mechanisms (21). A second pathway capable of regulating extracellular signal-regulated kinases

Downloaded from http://www.jimmunol.org/ by guest on May 9, 2021

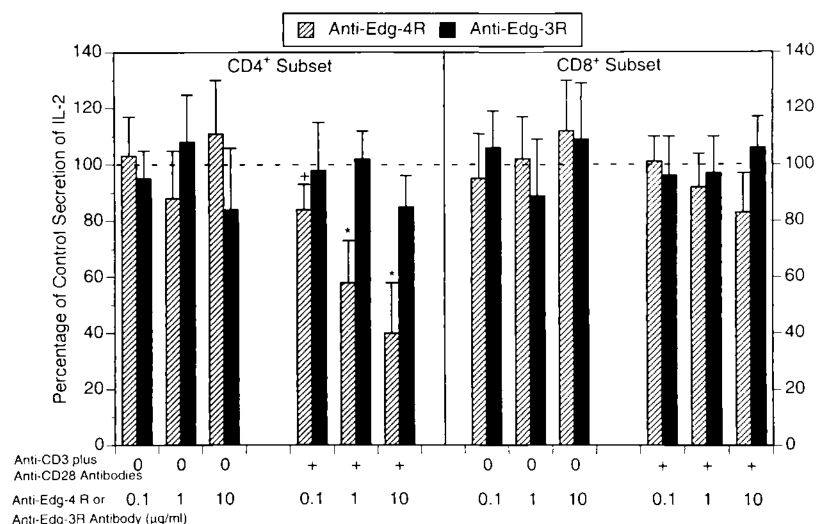

FIGURE 4. Mouse monoclonal anti-Edg-4 R Ab inhibition of IL-2 generation by CD4$^+$ T cells but not by CD8$^+$ T cells. Each bar and bracket depicts the mean ± SD of the results of three separate studies of T cells from three healthy volunteers. The respective control values (100%) in the absence of Abs showed ranges of: CD4$^+$ in medium alone, 26–35 pg/ml; CD4$^+$ with anti-CD3 plus anti-CD28 Abs, 331-3587 pg/ml; CD8$^+$ in medium alone, 29–68 pg/ml; and CD8$^+$ with anti-CD3 plus anti-CD28 Abs, 164–710 pg/ml. The range of maximal inhibition by 10 μg/ml anti-Edg-4 R Ab of IL-2 secretion by stimulated CD4$^+$ T cells was 33–56%. The symbols denoting statistical significance calculated by the paired *t* test were: +, $p < 0.05$; and *, $p < 0.025$.

involves suppression of oncogene-encoded and constitutively activated αi2, without involvement of *ras* (22). These alternative mechanisms must be examined in detail in different types of T cells to determine whether they participate in LPA suppression of IL-2 generation. Even less is known of the mechanisms that couple anti-Edg R Ab binding to Edg Rs with signals relevant to IL-2 generation. The current demonstration that $CD4^+$ T cell Edg-4 R binding of anti-Edg-4 R Ab, but not Edg-3 R binding of anti-Edg-3 R Ab, suppresses stimulated secretion of IL-2 simply confirms a role for the Edg-4 R as the principal transducer of the suppressive effect of LPA (Figs. 3 and 4).

Although the present data suggest the possibility of a predominant role for Edg-4 Rs in LPA effects on many $CD4^+$ T cell activities, additional studies are required to establish such actions and to delineate any functionally relevant changes in expression of Edg Rs elicited by T cell stimuli. The combination of anti-CD3 plus anti-CD28 Abs did not change the profile of Edg Rs on $CD4^+$ T cells or $CD8^+$ T cells. The present results suggest that LPA occupies and activates Edg-4 Rs of Th1 cells, which are a major source of IL-2. This critical point must be examined in Th1 and Th2 cell clones and other defined populations, using a range of functional assays. If the differential activity of LPA for $CD4^+$ T cells, as contrasted with $CD8^+$ T cells, transcends a single function or if qualitatively distinctive effects are observed in Th1 and Th2 subsets, then LPA may be considered a significant specific immunoregulatory mediator.

Acknowledgments

We thank Bethann Easterly for expert completion of all illustrations.

References

1. Spiegel, S., and S. Milstein. 1995. Sphingolipid metabolites: members of a new class of lipid second messengers. *J. Membr. Biol. 146:225.*
2. Moolenaar, W. H., O. Kranenburg, F. R. Postma, and G. Zondag. 1997. Lysophosphatidic acid: G protein signaling and cellular responses. *Curr. Opin. Cell Biol. 9:168.*
3. Goetzl, E. J., and S. An. 1998. Diversity of cellular receptors and functions for the lysophospholipid growth factors lysophosphatidic acid and sphingosine 1-phosphate. *FASEB J. 12:1589.*
4. An, S., E. J. Goetzl, and H. Lee. 1998. Signaling mechanisms and molecular characteristics of G protein-coupled receptors for lysophosphatidic acid and sphingosine 1-phosphate. *J. Cell. Biochem. Suppl. 30/31:147.*
5. Hecht, J. H., J. A. Weiner, S. R. Post, and J. Chun. 1996. Ventricular zone gene-1 (*vzg-1*) encodes a lysophosphatidic acid receptor expressed in neurogenic regions of the developing cerebral cortex. *J. Cell Biol. 135:1071.*
6. Lee, M.- J., J. R. Van Brocklyn, S. Thangada, C. H. Liu, A. R. Hand, R. Menzeleev, S. Spiegel, and T. Hla. 1998. Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. *Science 279:1552.*
7. Lynch, K. R., and D.- S. Im. 1999. Life on the Edg. *Trends Pharmacol. Sci. 20:473.*
8. Goetzl, E. J., and K. R. Lynch, eds. 2000. Lysophospholipid and eicosanoid mediators in physiology and diseases. *Ann. NY Acad. Sci. In press.*
9. Tigyi, G., D. L. Dyer, and R. Miledi. 1994. Lysophosphatidic acid possesses dual action in cell proliferation. *Proc. Natl. Acad. Sci. USA 91:1908.*
10. Cuvillier, O., D. S. Rosenthal, M. E. Smulson, and S. Spiegel. 1998. Sphingosine 1-phosphate inhibits activation of caspases that cleave poly(ADP-ribose) polymerase and lamins during Fas- and ceramide-mediated apoptosis in Jurkat T lymphocytes. *J. Biol. Chem. 273:2910.*
11. Goetzl, E. J., Y. Kong, and B. Mei. 1999. Lysophosphatidic acid and sphingosine 1-phosphate protection of T cells from apoptosis in association with suppression of Bax. *J. Immunol. 162:2049.*
12. Goetzl, E. J., Y. Kong, and J. S. Kenney. 1999. Lysophospholipid enhancement of human T cell sensitivity to diphtheria toxin by increased expression of heparin-binding epidermal growth factor. *Proc. Assoc. Am. Physicians 111:259.*
13. Stam, J. C., F. Michiels, R. A. van der Kammen, W. H. Moolenaar, and J. G. Collard. 1998. Invasion of T lymphoma cells: cooperation between Rho family GTPases and lysophospholipid receptor signaling. *EMBO J. 17:4066.*
14. Goetzl, E. J., Y. Kong, and J. K. Voice. 2000. Lysophosphatidic acid and sphingosine 1-phosphate stimulation of Jurkat T cell transmigration of a model basement membrane. *FASEB J. In press (Abstr.).*
15. An, S., T. Bleu, O. G. Hallmark, and E. J. Goetzl. 1998. Characterization of a novel subtype of human G protein-coupled receptor for lysophosphatidic acid. *J. Biol. Chem. 273:7906.*
16. An, S., T. Bleu, Y. Zheng, and E. J. Goetzl. 1998. Recombinant human G protein-coupled lysophosphatidic acid receptors mediate intracellular calcium mobilization. *Mol. Pharmacol. 54:881.*
17. Goetzl, E. J., H. Dolezalova, Y. Kong, and L. Zeng. 1999. Dual mechanisms for lysophospholipid induction of proliferation of human breast carcinoma cells. *Cancer Res. 59:4732.*
18. Bandoh, K., J. Aoki, H. Hosono, S. Kobayashi, T. Kobayashi, K. Murakami-Murofushi, M. Tsugimoto, H. Arai, and K. Inoue. 1999. Molecular cloning and characterization of a novel G protein-coupled receptor, EDG-7, for lysophosphatidic acid. *J. Biol. Chem. 274:27776.*
19. Goetzl, E. J., H. Dolezalova, Y. Kong, Y.-L. Hu, R. B. Jaffe, K. R. Kalli, and C. A. Conover. 1999. Distinctive expression and functions of the type 4 endothelial differentiation gene-encoded G protein-coupled receptor for lysophosphatidic acid in ovarian cancer. *Cancer Res. 59:5370.*
20. Olivera, A., T. Kohama, L. Edsall, V. Nava, O. Cuvillier, S. Poulton, and S. Spiegel. 1999. Sphingosine kinase expression increases intracellular sphingosine 1-phosphate and promotes cell growth and survival. *J. Cell Biol. 147:545.*
21. Hedin, K. E., M. P. Bell, C. J. Huntoon, L. M. Karnitz, and D. J. McKean. 1999. Gi proteins use a novel β/γ- and *ras*-independent pathway to activate extracellular signal-regulated kinase and mobilize AP-1 transcription factors in Jurkat T lymphocytes. *J. Biol. Chem. 274:19992.*
22. Gupta, S. K., C. Gallego, G. L. Johnson, and L. E. Heasley. 1992. MAP kinase (p42) is constitutively activated in gip2 and *src* transformed rat 1a fibroblasts. *J. Biol. Chem. 267:7987.*

Downloaded from http://www.jimmunol.org/ by guest on May 9, 2021