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*J Immunol* 2001; 166:2317-2322; doi: 10.4049/jimmunol.166.4.2317

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Lysophosphatidic Acid Receptor-Selective Effects on Jurkat T Cell Migration Through a Matrigel Model Basement Membrane

Yuhua Zheng, Yvonne Kong, and Edward J. Goetzl

Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (SIP) are growth factors secreted by stimulated platelets, macrophages, and epithelial cells, as well as some types of tumor cells, which bind specifically to G protein-coupled receptors of a recently characterized subfamily encoded by endothelial differentiation genes (Edg Rs) (1–8). Of the Edg Rs, Edg-2 and Edg-4 Rs, as assessed by semiquantification of mRNA with PCR and of protein using a new set of mAbs for Western blots (14), have more sharply focused patterns of constitutive expression of Edg Rs than have other Edg Rs, which, respectively, express predominantly Edg-2 Rs or Edg-4 Rs. The generation of IL-2 by CD4+ T cells, but not CD8+ T cells, stimulated through binding of adherent anti-CD3 plus anti-CD28 mAbs was inhibited by 10^{-6} M LPA and by 30 to 300 ng/ml of anti-Edg-2 R Ab, but not anti-Edg-4 R Ab. LPA and anti-Edg-4 R Ab also enhanced by up to 4-fold the expression of matrix metalloproteinase by Jurkat-T-4 cells, but not Jurkat-T-2 cells, as assessed by cleavage of [3H] type IV human collagen in the Matrigel. Enhancement of matrix metalloproteinase-dependent trans-Matrigel migration of Jurkat-T cells by the chemokine RANTES was suppressed by anti-Edg-2 R Abs, but was stimulated by anti-Edg-4 R Abs. The opposite effects of Edg-2 and Edg-4 LPA receptors on trans-Matrigel migration and some other T cell functions provide receptor-selective mechanisms for regulation of T cell recruitment and immune contributions. The Journal of Immunology, 2001, 166: 2317–2322.

Received for publication July 25, 2000. Accepted for publication November 27, 2000.

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1 This work was supported by National Institutes of Health Grant HL-31809 and Grant 02702-28-RG from American Foundation for AIDS Research.

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

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

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100 U/ml penicillin, 100 μg/ml streptomycin, and 10% heat-treated FBS at 37°C in 5% CO₂. Jurkat-T-2 cells (expressing predominantly Edg-2 Rs), Jurkat-T-4 cells (expressing predominantly Edg-4 Rs), and Jurkat-T-3 cells (expressing predominantly Edg-3 Rs) and serving as occasional controls for Jurkat-T-2 and -4 cells) were generated as described for Jurkat and other human T cell lines (17, 18). A stimulus of 10⁻² M LPA, S1P, or the control lyso- 

Western blot analyses of Edg Rs 

Proteins were extracted from replicate suspensions of 3–5 × 10⁶ Jurkat-T cells, Jurkat-T-Edg R cells, and HTCC4 cell Edg-2, -3, -4, and -5 R stable transfectants, and resolved by electrophoresis along with a rainbow-

Assessment of migration of Jurkat-T cells and blood CD4⁺ T cells through a model basement membrane 

CD4⁺ T cells were isolated from heparinized venous blood of healthy volunteers using immunomagnetic retention chromatography after incubation of whole blood with biotinylated mouse monoclonal anti-human CD4⁺ Abs and streptavidin metallic microbeads (Miltenyi Biotec, Auburn, CA), as described (14). Jurkat-T cells and CD4⁺ T cells were washed twice and resuspended at 5 × 10⁶/ml in Iscove’s medium with 100 U/ml penicillin, 100 μg/ml streptomycin (University of California at San Francisco Cell Culture Facility), and 50 μg/ml fatty acid-free human serum albumin (Sigma, St. Louis, MO). Replicate 0.2-ml suspensions of Jurkat-T cells and CD4⁺ T cells were added to the 6.5-μm diameter inserts of Transwell inserts with MMP-neutral 1% Nutridoma-SP (Boehringer Mannheim, Indianapolis, IN). Duplicate aliquots of 30 μl were removed from the lower compartment of each chamber, as well as control chambers without stimuli and without cells, after 4 and 24 h for assessment of fluid phase radioactivity (21). The activity inhibited by 10⁻³ M GM6001 was attributed to specific in situ MMP. 

Results 

Jurkat-T cells transfected with expression plasmids encoding one Edg R and antisense mRNA specific for the other Edg Rs were enriched by selection in hygromycin for 10–16 days. The level of expression of each Edg R then was determined by semiquantitative RT-PCR and Western blots. The untransfected control Jurkat-T cell contains mRNA for Edg-2, -3, -4, and -5 R, but not Edg-5 R (Fig. 1), and not Edg-1 or -7 Rs (data not shown). Radio-PCR semiquantification of mRNA encoding Edg Rs in untransfected Jurkat-T cells revealed an approximate rank-order of expression of Edg-3>Edg-2>Edg-4 (Table I). Transfection and selection led to stabilization of three distinct sets of Jurkat-T cells, which express almost exclusively mRNA encoding the respective Edg-2 and Edg-4 mRNA, and the Edg-3 S1P R (Fig. 1). The Edg-2 R (Jurkat-T-2)- and Edg-4 R (Jurkat-T-4)-predominant sets were selected for further studies to permit analyses of Edg R-dependent determinants of T cell responses to LPA. The Jurkat-T-3 cells were used as controls expressing no detectable LPA receptor mRNA. Radio-PCR semiquantification of Edg R mRNA profiles in Jurkat-T-2 and Jurkat-T-4 cells demonstrated predominance of the expected major Edg R in each set (Table I). Jurkat-T-2 cells had no Edg-4 R, and Jurkat-T-4 cells had no Edg-2 R. However, there was slight retention of mRNA for S1P Edg Rs, especially Edg-3R. Western blot studies of Edg R protein expression in the Jurkat-T cell transfectants confirmed the results of analyses of mRNA. Each set of transfectants expressed predominantly the protein Ag of the major R without a gradient for chemokinesis. In some studies of intrinsic mobility alone, filters were not coated with Matrigel. Chambers were incubated at 37°C in 5% CO₂ in air for the known optimal times of 4 h without Matrigel and 42 h with Matrigel. Inserts then were shaken in the medium of the lower chamber for 5 min to detach cells adherent to the lower surface of filters, and cells in this medium were counted microscopically. Migration responses are expressed as a percentage of the total number of Jurkat-T cells or CD4⁺ T cells added to the chamber or as a percentage of the control value (100%) in the absence of an inhibitor or enhancer. The hydroxamic acid dipipeptide analog inhibitor of MMPs, HONHCO-CH₂-ButCO₂-trp-methylamide (GM6001) and the inactive control GM2454 were added to the cells and lower compartment medium at 10⁻⁶ to 10⁻⁸ M.
Edg R, and none expressed Edg-5 R (Fig. 2). Jurkat-T-2 cells also contain a low level of Edg-3 R but, importantly, had no Edg-4 R, and Jurkat-T-4 cells have low levels of Edg-3 R but only a trace of Edg-2 R Ag.

Jurkat-T-2 and Jurkat-T-4 cells responded differently to LPA stimulation in a model basement membrane transmigration assay. This system measures the integrated effects of enhanced motility and increased T cell MMP activity required to create channels through the Matrigel barrier (22) (Fig. 3). LPA stimulated trans-Matrigel migration of Jurkat-T-4 cells significantly at 10^{-9} M and maximally at 10^{-7} M to a level 4-fold higher than the baseline of unstimulated migration, whereas S1P elicited significant, but lesser, migration only at 10^{-6} M. As equal concentrations of LPA on both sides of the micropore filter evoked Jurkat-T-4 cell migration of the same magnitude as an identical concentration of LPA only in the stimulus compartment, chemokinesis appears to be the major mechanism. Jurkat-T-2 and Jurkat-T-3 cells responded only marginally to the highest concentration of LPA. The specificity controls dioleoylphosphatidic acid and phosphatidylethanolamine had no effects on migration of Jurkat-T-2 or Jurkat-T-4 cells.

A mouse mAb to a substituent peptide in the extracellular amino terminus of Edg-4 R evoked predominantly chemotactic trans-Matrigel responses of Jurkat-T-4 cells, but not Jurkat-T-2 cells (Tables II and III). The level of chemotaxis was dependent on anti-Edg-4 R mAb concentration and attained a maximal increment similar to that for LPA. Anti-Edg-2 R mAb specific for a substituent peptide in the extracellular amino terminus of Edg-2 R failed to induce significant trans-Matrigel migration of Jurkat-T-2 or Jurkat-T-4 cells. Neither LPA nor mAbs affected migration of Jurkat-T-3 cells. Mitogen-activated human blood CD4^+ T cells express both Edg-2 and Edg-4 Rs (15). The anti-Edg-4 R mAb, but not the anti-Edg-2 R mAb, elicited trans-Matrigel chemotactic responses of activated CD4^+ T cells (Table IV). The chemotactic responses

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**Table I.** Predominance of expression of one Edg R by Jurkat-T-2 and -4 cells

<table>
<thead>
<tr>
<th>Type of Cell</th>
<th>Edg-1</th>
<th>Edg-2</th>
<th>Edg-3</th>
<th>Edg-4</th>
<th>Edg-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jurkat-T cell, control</td>
<td>&lt;0.02</td>
<td>0.49</td>
<td>0.72</td>
<td>0.23</td>
<td>0.05</td>
</tr>
<tr>
<td>Jurkat-T-2 cell</td>
<td>&lt;0.02</td>
<td>0.97</td>
<td>0.17</td>
<td>0.03</td>
<td>0.07</td>
</tr>
<tr>
<td>Jurkat-T-4 cell</td>
<td>&lt;0.02</td>
<td>0.07</td>
<td>0.20</td>
<td>1.10</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*All values are the mean of results of two RT-PCR determinations of mRNA encoding Edg Rs. Each number is the ratio of ^32P in the Edg-R cDNA band to that in the standard G3PDH band.*

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**FIGURE 2.** Western blot of Edg Rs extracted from Jurkat-T cell transfectants. Two micrograms of cellular proteins from HTC4 cell standard transfectants (HS) and 10 μg each from Jurkat-T cells transfected with human Edg-2 R (J2), Edg-3 R (J3), or Edg-4 R (J4) were electrophoresed and transferred for staining with the respective anti-Edg R mAbs noted at the top of each frame. The horizontal line at the left of all frames depicts the position of a 51-kDa prestained protein marker.

**FIGURE 3.** Edg R-dependent stimulation of trans-Matrigel migration of Jurkat-T cells by LPA. Each column and bracket represents the mean ± SD of the results of three different studies. Symbols depicting levels of statistical significance calculated by a paired Student’s t test are: +, p < 0.05 and *, p < 0.01.
of activated CD4+ T cells attained a maximum level similar to that evoked by RANTES (Table IV). The lack of migratory response of Jurkat-T-2 cells to concentrations of LPA that optimally stimulated trans-Matrigel migration of Jurkat-T-4 cells and to homologous anti-Edg-2 R Abs suggested that Edg-2 R might transduce inhibitory signals capable of suppressing migration elicited by factors of other classes. At 1 nM, the chemokine RANTES evoked a level of trans-Matrigel chemotactic migration for both sets of Jurkat-T cell transfectants equal to that of Jurkat-T-4 cells to LPA (Fig. 4). LPA suppressed significantly the trans-Matrigel migration of Jurkat-T-2 cells elicited by RANTES with LPA concentration dependence. In contrast, LPA increased slightly RANTES-induced migration of Jurkat-T-4 cells with less than additive total responses (Fig. 4). For activated CD4+ T cells expressing Edg-2 and Edg-4 Rs, anti-Edg-2 R mAb suppressed the chemotactic response to RANTES with mAb concentration dependence, whereas anti-Edg-4 R mAb increased significantly, but less than additively, the net chemotactic response to RANTES (Table V).

The trans-Matrigel migration of many types of T cells in this basement membrane model is dependent on the proteolytic activity of endogenous MMP-9 (20–22). In situ functional MMP activity, expressed by Jurkat-T-2 and Jurkat-T-4 cells in the course of trans-Matrigel migration, may be quantified by measuring the release of radioactivity from [3H]type IV human collagen suspended in the Matrigel (21). At concentrations that elicit significant trans-Matrigel migration of Jurkat-T-4 cells, MMP activity was increased similarly of endogenous MMP-9 (20 –22). In situ functional MMP activity, expressed by Jurkat-T-2 and Jurkat-T-4 cells in the course of trans-Matrigel migration across a simulated basement membrane. LPA, but not S1P, enhanced trans-Matrigel migration of Jurkat-T-4 cells, but not Jurkat-T-2 cells (Fig. 3). Transduction of stimulation of T cell migration by Edg-4 Rs, but not Edg-2 Rs, was confirmed by applying anti-Edg R-selective mAbs in the same assay system. Anti-Edg-4 R mAb evoked trans-Matrigel migration of Jurkat-T-4 cells, but not Jurkat-T-2 cells, whereas anti-Edg-2 R Ab had no stimulatory effect on migration of either type of Jurkat transfectant (Tables II and III). The differential effects of anti-Edg R Abs also was observed with human blood CD4+ T cells after mitogen activation, which results in codominant expression of both Edg-2 and Edg-4 Rs (14, 15). Anti-Edg-4 R mAb, but not anti-Edg-2 R mAb, elicited chemotaxis of mitogen-activated CD4+ T cells (Table IV). That LPA-elicited migration appears to be principally chemokinesis and that elicited by anti-Edg-4 R Ab largely chemotaxis, may reflect only differences in the physicochemical properties of the two stimuli (Fig. 3, Tables II and III). Peptides and proteins readily establish

Table II. Anti-Edg-4 R mAb stimulation of trans-Matrigel migration of Jurkat-T-4 cells*  

<table>
<thead>
<tr>
<th>Anti-Edg-4 R mAb (ng/ml)</th>
<th>Anti-Edg-2 R mAb (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 10/CT 30/CT 30/CK 100/CT 100/CK 300/CT 30/CT 100/CT</td>
<td></td>
</tr>
<tr>
<td>3.7 3.5 10.3* 4.4 13.6* 3.1 16.9** 4.6 4.2</td>
<td></td>
</tr>
<tr>
<td>0.8 0.5 2.7 1.9 3.7 0.6 2.6 1.5 1.8</td>
<td></td>
</tr>
</tbody>
</table>

* Each upper number is the mean and each lower number the SD of the results of three different studies, expressed as the percentage of initial Jurkat cells to cross the filter. CT, Chemotaxis; CK, chemokinesis. Symbols depicting the level of statistical significance are the same as in Fig. 5.

Table III. Lack of anti-Edg-4 R mAb stimulation of trans-Matrigel migration of Jurkat-T-2 cells*  

<table>
<thead>
<tr>
<th>Anti-Edg-2 R mAb (ng/ml)</th>
<th>Anti-Edg-4 R mAb (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 10/CT 30/CT 30/CK 100/CT 100/CK 300/CT 30/CT 100/CT</td>
<td></td>
</tr>
<tr>
<td>2.8 3.2 4.6 2.5 4.8 3.9 6.7 6.1 7.0</td>
<td></td>
</tr>
<tr>
<td>1.2 0.7 1.8 0.6 1.5 1.1 2.4 2.0 2.8</td>
<td></td>
</tr>
</tbody>
</table>

* Each upper number is the mean and each lower number the SD of the results of three different studies, expressed as the percentage of initial Jurkat cells to cross the filter. CT, Chemotaxis; CK, chemokinesis. Symbols depicting the level of statistical significance are the same as in Fig. 5.

Table IV. Trans-Matrigel chemotactic effects of anti-Edg R mAbs for mitogen-activated human blood CD4+ T cells*  

<table>
<thead>
<tr>
<th>Anti-Edg-4 R mAb (ng/ml)</th>
<th>Anti-Edg-2 R mAb (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 10/CT 30/CT 30/CK 100/CT 100/CK 300/CT 30/CT 100/CT</td>
<td></td>
</tr>
<tr>
<td>3.3 5.4* 12.1* 3.9 17.3* 3.2 22.1* 3.8 4.3</td>
<td></td>
</tr>
<tr>
<td>0.7 0.6 1.8 0.8 2.9 0.6 3.1 0.6 1.2</td>
<td></td>
</tr>
</tbody>
</table>

* Each upper number is the mean and each lower number the SD of the results of three studies of CD4+ T cells from different subjects, expressed as the percentage of initial T cells to cross the filter. CT, Chemotaxis; CK, chemokinesis. Symbols depicting the statistical significance are the same as in Fig. 5.

Discussion

Transfection of Jurkat-T cells with plasmids encoding one sense and combined other antisense messages for human Edg Rs, followed by hygromycin selection, induced vastly predominant expression of the Edg-2, -3, or -4 R (Figs. 1 and 2, and Table I). Analyses of migration and related responses of the Jurkat-T-2 and Jurkat-T-4 transfectants to LPA and agonist anti-Edg R mAbs permitted definition of some functional differences between Edg-2 and -4 Rs. This had not been possible in human blood T cells because there is no known developmental stage or functional state in which Edg-2 Rs are expressed alone or with sufficient predominance (14, 15).

The distinctive role of each LPA-specific Edg R in T cells was demonstrated in the trans-Matrigel model of migration across a simulated basement membrane. LPA, but not S1P, enhanced trans-Matrigel migration of Jurkat-T-4 cells, but not Jurkat-T-2 cells (Fig. 3). Transduction of stimulation of T cell migration by Edg-4 Rs, but not Edg-2 Rs, was confirmed by applying anti-Edg R-selective mAbs in the same assay system. Anti-Edg-4 R mAb evoked trans-Matrigel migration of Jurkat-T-4 cells, but not Jurkat-T-2 cells, whereas anti-Edg-2 R Ab had no stimulatory effect on migration of either type of Jurkat transfectant (Tables II and III). The differential effects of anti-Edg R Abs also was observed with human blood CD4+ T cells after mitogen activation, which results in codominant expression of both Edg-2 and Edg-4 Rs (14, 15). Anti-Edg-4 R mAb, but not anti-Edg-2 R mAb, elicited chemotaxis of mitogen-activated CD4+ T cells (Table IV). That LPA-elicited migration appears to be principally chemokinesis and that elicited by anti-Edg-4 R Ab largely chemotaxis, may reflect only differences in the physicochemical properties of the two stimuli (Fig. 3, Tables II and III). Peptides and proteins readily establish
a concentration gradient across a micropore filter, but the amphiphatic phospholipids adhere to numerous surfaces including micropore filters, which may diminish effective concentrations in the cellular compartment.

Instead of transducing an LPA signal stimulatory to T cell migration, Edg-2 Rs inhibit responses to the chemokine RANTES in settings where the Edg-4 R has an enhancing effect (Fig. 4). The same effect was observed with mitogen-activated CD4+ T cells, where anti-Edg-2 R mAb, but not anti-Edg-4 R mAb, suppressed RANTES-induced chemotaxis (Table V). As Edg-2 Rs did not suppress spontaneous migration of Jurkat-T cell transfectants or mitogen-activated CD4+ T cells, it seemed possible that the observed inhibition of stimulated trans-Matrigel migration might be attributable to a negative effect on a critical component of the response other than intrinsic motility. One striking effect of many factors that enhance T cell migration is augmentation of MMP activity, which is predominantly due to increases in secretion of activated MMP-9 (21, 22). A functional assay for enhanced activity of the gelatinase-type MMPs on or near migrating T cells is quantification of release of degraded fragments from human type IV [3H]collagen suspended in the Matrigel above filters. In this assay, LPA and anti-Edg-4 R mAb, but not anti-Edg-2 R mAb, stimulated MMP functional activity of Jurkat-T-4 cells during trans-Matrigel migration, without affecting Jurkat-T-2 cell MMP activity (Fig. 5A). Migration-enhancing concentrations of RANTES also stimulated MMP in situ activity of Jurkat T-2 and Jurkat-T-4 cells (Fig. 5B). MMP activity of Jurkat-T-2 cells was inhibited significantly by LPA and anti-Edg-2 R mAb, but not anti-Edg-4 R mAb. Under the same conditions, LPA and anti-Edg-4 R mAb, but not anti-Edg-2 mAb, increased more than additively RANTES-evoked migration of Jurkat-T-4 cells (Fig. 5B).

Transduction of opposite signals from LPA by Edg-2 and -4 Rs thus has been documented with respect to two different functions of T cells. TCR-dependent stimulation of generation and secretion of IL-2 by Jurkat T cells and human blood-derived CD4+ T cells is suppressed by Edg-4 R-transduced signals from LPA and by anti-Edg-4 R mAb (14, 15). In the same setting, Edg-2 R-transduced signals from LPA and anti-Edg-2 R mAb enhance IL-2 release (15). Regulation of trans-Matrigel migration of T cells by Edg-2 Rs and -4 Rs now is observed to be the inverse of that documented for IL-2 secretion (14, 15). Edg-4 R signals enhance...
and Edg-2 R signals inhibit trans-Matrigel migration and the related function of release of endogenous MMP. Such results strengthen the hypothesis that LPA-evoked responses of activated native T cells bearing both LPA Edg Rs are the summation of separate signals from Edg-2 and Edg-4 Rs. Integration of these findings suggests that contributions of LPA in recruitment and immunostimulation of T cells differ with their state of activation. The Edg-4 Rs that predominate on unactivated blood CD4+ T cells signal amplification of MMP-dependent migration across basement membranes and into sites of immune responses, while suppressing secretion of IL-2 and possibly other cytokines. After T cells are activated by Ag, Edg-2 R expression is up-regulated, and signaling would shift partially to inhibition of responses of MMP-dependent migration to chemokines and other chemotactic factors, localizing the T cells at the site of the immune response, while enhancing generation of IL-2 and possibly other cytokines. Thus in both the recruitment and effector phases of the immune responses of helper T cells, LPA has complex facilitatory functional roles. Much additional data will be required to support or modify this tentative formulation of the contributions of LPA in immunity.

References