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Regulation of CD8 T cell signaling, metabolism and cytotoxic activity by extracellular lysophosphatidic acid

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Summary

Lysophosphatidic acid (LPA) is an endogenous bioactive lipid that is produced extracellularly and signals to cells via cognate LPA receptors, which are G-protein coupled receptors (GPCRs). Mature lymphocytes in mice and humans express three LPA receptors, LPA_2, LPA_5 and LPA_6, and work from our group has determined that LPA_5 signaling by T lymphocytes inhibits specific antigen-receptor signaling pathways that ultimately impair lymphocyte activation, proliferation and function. In this review, we discuss previous and ongoing work characterizing the ability of an LPA-LPA_5 axis to serve as a peripheral immunological tolerance mechanism that restrains adaptive immunity but is subverted during settings of chronic inflammation. Specifically, LPA-LPA_5 signaling is found to regulate effector cytotoxic CD8 T cells by (at least) two mechanisms: i) regulating the actin-microtubule cytoskeleton in a manner that impairs immunological synapse formation between an effector CD8 T cell and antigen-specific target cell, thus directly impairing cytotoxic activity, and ii) shifting T cell metabolism to depend on fatty-acid oxidation for mitochondrial respiration and reducing metabolic efficiency. The *in vivo* outcome of LPA_5 inhibitory activity impairs CD8 T cell killing and tumor immunity in mouse models providing impetus to consider LPA_5 antagonism for the treatment of malignancies and chronic infections.

Keywords

T cells; cytotoxic; lipid mediators; cytotoxicity; signal transduction cancer

Introduction

Adaptive immunity depends on the ability of naïve T and B lymphocytes to recognize foreign antigen by their antigen receptors that subsequently leads to the neutralization or elimination of the foreign antigen or infected, damaged or malignant cell. Critical

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Conflict of Interest Statement

The authors declare no competing interests.

to initiating this response are the signals transmitted by the T cell and B cell antigen receptors (TCR and BCR, respectively). These antigen receptor signaling events can be further positively or negatively regulated by cell intrinsic or extrinsic mechanisms, including the expression of cell-surface co-receptors (co-stimulatory or inhibitory) as well as paracrine signals from the microenvironment in which the cell activation occurs. Cancers and viruses that establish chronic infections often co-opt T cell inhibitory receptor signaling to evade T cell immunity by suppressing T cell functional activity. This review highlights work from our lab that has revealed the ability of the lysophosphatidic acid, or LPA, bioactive lysophospholipid to signal via the LPA_5 G-protein-coupled receptor to antagonize T and B cell antigen receptor signaling, activation, proliferation and function while simultaneously regulating T cell metabolism. Through biochemical, molecular, cell biological, and metabolic analyses coupled with *in vitro* and *in vivo* models of lymphocyte function, this review summarizes our major findings on how lysophosphatidic acid, or LPA, regulates adaptive cytotoxic CD8 T cell immunity.

Lysophosphatidic acid (LPA) is a lysophospholipid which are a family of simple phospholipids comprised of a polar phosphate head group, glycerol backbone and a long-chain fatty acid that exists in distinct species based on carbon chain length and degree of saturation. Lysophospholipids are signaling lipids that bind surface and intracellular receptors to regulate a broad range of processes. LPA can be found both inside and outside cells where it is generated through different enzymatic pathways and subsequently signals in different manners.[1–3] The ability of LPA to regulate lymphocyte activation, function, and adaptive immunity discussed in this review is restricted to extracellularly produced bioactive lipid (for references and review of LPA intracellular signaling see.[2,4,5]) Originally identified in mammalian and plant cells over 60 years ago, LPA was considered to be a metabolite involved in membrane phospholipid production.[6] Approximately 20 years later a number of groups documented that LPA displayed bioactivity as an extracellular signaling molecule capable of acting on diverse cell types, including platelets and neutrophils. Examples of LPA bioactivity implicated LPA in inducing hypertension in rodents, promoting intracellular signaling, inducing morphological changes, promoting cell aggregation, and enhancing chemotaxis.[7–13] Moolenaar and colleagues provided early and compelling evidence that LPA signaled in fibroblasts via a G-protein coupled receptor(s) (GPCRs) and by the mid-90s LPA was found to be the ligand for specific and distinct (orphan) G-protein coupled receptors (GPCRs) expressed by a variety of cell types.[14,15] In the past 20 years considerable attention has been devoted towards investigating cell-type specific signaling including how LPA receptor (LPAR) signaling impacts the nervous, vascular and immune systems.[3,16–20]

Determining that LPA was a cognate GPCR ligand placed it in a class of other inflammatory lipids that signal through GPCRs including eicosanoids and sphingosine-1-phosphate (S1P).[3,21] These signaling lipids regulate cell-type specific functions ranging from innate inflammation, hemostasis and angiogenesis and lymphocyte recirculation. Similar to LPA, S1P is also an endogenously-produced lysophospholipid and, as extracellular bioactive lipids, both lipids signal to cells via low nM affinity association with cognate GPCRs: six GPCRs for LPA, $LPA_{1–6}$, and five GPCRs for S1P, $S1P_{1–5}$.[3,22] Initial findings with both LPA and S1P suggested that these lysophospholipids could induce cell migration, cell

morphological alterations and cell proliferation in a variety of cell types.[3] Notably, S1P signaling via $S1P_1$ is required for the egress of immature T cells from the thymus[23] and manipulation of $S1P_1$ signaling is currently used in the clinic to treat multiple sclerosis.[24] At present, a number of diverse activities have been attributed to LPA signaling in the nervous and vascular systems.[3,17–19] Despite that all mature human and murine lymphocytes and other immune cell types express one or more of the six LPA receptors ($LPA_{1–6}$)[16,25–29], its role in innate and adaptive immunity has been less well studied relative to S1P.

LPA concentration in the blood of healthy individuals and wild type mice has been reported to range from high nM to low μM concentrations.[30–35] This variability in the reporting of LPA levels is dependent not only on assay measurement (mass spectroscopy, ELISA, etc.) but also sample preparation. Accordingly, when samples collected for LPA measurement include active inhibition of both LPA production and degradation it appears the plasma from healthy individuals harbor on the order of 50nM LPA.[3,36,37] However, systemic levels of extracellular LPA and autotaxin (ATX), the enzyme predominantly responsible for extracellularly produced LPA, are often found significantly elevated in human chronic inflammatory disorders such as chronic viral (HCV, HBV and EBV) infections[38–41] autoimmune diseases[42–44], obesity[45–52] and a number of diverse cancers[53–60]. In these settings, serum LPA levels are considerably heightened (~2–10-fold) compared to healthy individuals and concentrations over 50μM have been reported in malignant effusions of ovarian cancer patients.[61] It is further worth noting, however, that the membrane-associated phosphatidic-selective phospholipase A1a, PA-PLA1α, has also been shown to contribute to extracellular LPA production in certain microenvironments.[3] How pathological systemic LPA levels impact diverse infections and disease is an area of active investigation.

Extracellular production of LPA as a bioactive GPCR signaling lipid

The vast majority of extracellular LPA is produced by ATX, a phospholipase D enzyme expressed and secreted by certain cell-types that hydrolyzes the abundantly available lysophosphatidylcholine (LPC) lipid to produce extracellular LPA.[62,63] ATX, encoded by *ENPP2* (*Ectonucleotide Pyrophosphatase/ Phosphodiesterase 2*), was initially isolated from conditioned media of the A2058 melanoma cell line and thought to be a motility factor that signaled in an autocrine manner via an unknown GPCR to promote directed (and random) melanoma migration.[64] ATX was only later identified as a secreted phospholipase D whose enzymatic activity produces LPA through the hydrolysis of LPC. Since LPC is a highly abundant lipid, the rate-determining step of LPA synthesis is considered to be ATX catalysis. As a secreted enzyme, ATX has been shown to associate with surface integrins[65,66] and has led to a current model in which ATX associates with integrins on a cell surface where it subsequently converts LPC to LPA that then signals to the ATX-expressing or nearby cells expressing LPARs (Figure 1). The association of LPA with integrins was initially thought to occur by an exposed RGD (arginine-glycine-aspartic acid) integrin ligand motif. However, structural analyses showed that ATX associates with integrins in an RGD-indpendent manner.[66] Thus, the precise mechanism by which ATX associates with integrins requires further clarification. Regardless, physiological LPA signaling results from localized and directed autocrine/paracrine LPA production and subsequent GPCR-induced signaling[65,67–69] allowing for local LPA concentrations to reach much higher concentrations

than systemic levels. Extracellular LPA is quite labile with an *in vivo* half-life on the order of (3–5) minutes[62,70] as a result of its rapid hydrolysis by lipid phosphate phosphohydrolase type 1 (LPP1) and LPP3 to monoacylglycerols (MAGs).[70] These MAGs can be further catabolized into free fatty acids by MAG lipases. Indeed, the half-life of LPA increases 4 fold when LPA is intravenously introduced into LPP1-deficient mice and *Lpp1*$^{-/-}$ mice harbor elevated levels of LPA[70]. An ATX-deficiency resulting from homozygosity for null *Enpp2* alleles (*Enpp2*$^{-/-}$) results in embryonic lethality largely due to vascular and nervous system defects.[63,71] However, heterozygous *Enpp2*$^{+/-}$ mice are viable and as adults are reported to harbor close to half the levels of systemic LPA as found in wild type mice.[63,71]

While many diverse cell types express one or more of the six LPARs, the expression of the ATX phospholipase D responsible for LPA extracellular production is more restricted. Certain cell types such as fibroblastic reticular stromal cells[72] and high endothelial venule cells associated with lymphoid organs constitutively express high levels of ATX where subsequent LPA production promotes chemokinetic activity and motility of T lymphocytes.[65,67,73]

Our interest in the ability of LPA to regulate adaptive immunity initiated with the appreciation that a structurally similar lysophospholipid, sphingosine-1-phosphate (S1P), was identified as a chemoattractant ligand for the $S1P_1$ G-protein coupled receptor (GPCR) expressed by T and B lymphocytes. In particular, there are notable parallels in the developmental checkpoints that exist for both T and B lymphocytes in the thymus and bone marrow, respectively. Yet, an $S1P_1$-deficiency selectively prevented immature T cells from exiting the thymus but not immature B cells from exiting the bone marrow.[23] This apparent discrepancy led us to consider whether additional lipid GPCRs (e.g., LPARs) were expressed by immature B cells that might be alternatively used by immature B cells to promote marrow egress. While LPARs have not been demonstrated to contribute to B cell egress from the bone marrow, we determined that $S1P_3$ was an additional S1P receptor contributing to immature B cell egress.[74,75] There are five characterized S1P receptors, $S1P_{1–5}$, and all are GPCRs that based on a shared homology belong to the Edg family of GPCRs; named initially as an induced endothelial differentiation gene. $LPAR_{1–3}$ were the first LPARs to be identified and all three were shown to be members of the Edg family of GPCRs. By 2006 it was evident that LPA was a cognate ligand for three additional LPARs, LPA_4, LPA_5 and LPA_6, that did not belong to the Edg family of receptors but nevertheless are cognate GPCRs for LPA. GPCRs signal via $\alpha\beta\gamma$ hetrotrimeric G-proteins and are typically characterized by the associated $G\alpha$ subunit of which there are 4 families: $G\alpha_i$, $G\alpha_s$, $G\alpha_q$ and $G\alpha_{12/13}$. The ability of individual LPARs to associate with a specific $G\alpha$ family member but not others has been demonstrated using cell lines and overexpression approaches. However, whether these associations promote physiological *in vivo* LPAR signaling is less certain. In broad terms, the three Edg family members, $LPA_{1–3}$, often associate with $G\alpha_i$ and $G\alpha_q$ heterotrimeric proteins and the non-Edg LPAR4–6 most often are found to associate with $G\alpha_{12/13}$ heterotrimeric G-proteins.

The identification and molecular cloning of all six LPARs allowed us to survey the expression of the LPAR-encoding genes by quantitative real-time PCR and to determine that immature and mature B and T lymphocytes from both mice and humans expressed the

same set of GPCRs specific for the LPA lysophospholipid, LPA_2, LPA_5 and LPA_6.[76–78] This review will highlight work from our group demonstrating the ability of LPA_5 to antagonize antigen receptor signaling and function of T and B lymphocytes.

LPA suppresses lymphocyte antigen receptor-induced calcium stores release and signaling

S1P is a lysophospholipid chemoattractant that upon engaging the $S1P_1$ GPCR signals directed cell migration via a $G\alpha_i$ associated heterotrimer signaling axis, similar to chemokine GPCRs. Despite similarities between S1P and LPA receptors, our initial experiments did not reveal any significant role for LPA as a chemoattractant for B or T lymphocytes *ex vivo* (although LPA has been shown to promote chemokinesis of T cells via LPA_2 signaling[65,67,73,79]). Thus, we explored how LPA might influence other aspects of lymphocyte function. These studies soon revealed that when the antigen receptor on B and T cells were stimulated in the presence of pathological concentrations of LPA (1–20μM), antigen receptor-induced intracellular calcium mobilization was significantly attenuated indicating that LPA had the potential to suppress lymphocyte activation (Figure 2).[76–78,80] As mentioned, a number of early studies had reported plasma LPA levels to be in the range of high nM to low μM concentrations.[30–35] With the relatively recent understanding that LPA catabolism and production post-collection accounted for this variability in LPA concentrations[37], it will be important to further determine if normal concentrations of 50–100nM LPA are able to impinge on antigen receptor signaling. In this regard, however, we note that in our studies (discussed below) we have found that an approximate 50% reduction of wild type LPA levels, as produced in *Enpp2*$^{+/-}$ mice, significantly improve *in vivo* T cell cytotoxicity suggesting that wild type LPA concentrations indeed normally influence adaptive immunity.

Antigen receptor signaling by T and B lymphocytes initiates with tyrosine phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) within the cytoplasmic domains of CD3 and CD79, respectively. T and B cell-specific tyrosine kinases phosphorylate ITAMs that recruit Zap70 and Syk kinases, respectively, that in turn are also activated by tyrosine phosphorylation. Activated Zap70/Syk subsequently promote canonical signaling cascades that lead to the tyrosine phosphorylation and activation of PLCγ1 in T cells and PLCγ2 in B cells by Tec-family kinases.[81] Activated PLCγ hydrolyzes phosphatidylinositol 4,5 bisphosphate producing diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). IP_3 subsequently engages IP_3R calcium release channels expressed on the surface of the end reticulum (ER) to release intracellular stores of calcium into the cytosol (Figure 2). Calcium levels within the ER are monitored by the ER resident proteins STIM1 and STIM2, which upon depletion of ER calcium stores, oligomerize and stimulate the ORAI calcium channel within the plasma membrane to facilitate extracellular calcium entry.[82–84] Resulting overall elevated levels of cytosolic calcium are required for a number of important lymphocyte biological processes such as cytokine secretion and cytolytic granule exocytosis but also the activation of transcription factors (e.g, NFAT) that initiate maturational transcriptional programs. Our finding that LPA impaired intracellular calcium

mobilization initiated by antigen receptor signaling indicated that this lysophospholipid may contribute to the regulation of these processes.

Antigen receptor-proximal kinase signaling is not perturbed by LPA

To determine more precisely where LPA, signaling via an LPAR (LPA_2, LPA_5 and/or LPA_6), suppresses antigen receptor-induced calcium intracellular stores release, primary mouse splenic B cells and human T cells isolated from healthy peripheral blood were stimulated via the antigen receptor in the presence and absence of LPA and evaluated by western blot analyses for activation of lymphocyte-specific kinases and PLCγ. The results of these experiments demonstrated that in the presence of LPA, LPAR signaling did not perturb TCR or BCR receptor-proximal signaling events. For mouse B cells stimulated with anti-IgM $F(ab')_2$, we found equivalent amounts of pSyk, pBtk and pPLCγ2 in the presence and absence of 20μM LPA[76]; for human T cells we found that stimulation with anti-CD3 and anti-CD28 in the presence and absence of LPA led to equivalent increases in total tyrosine phosphorylation of cellular proteins over 5 minutes and similar increases in pCD3ζ and pPLCγ1[80]. Thus, LPA treatment of antigen receptor-stimulated mouse and human lymphocytes did not alter antigen receptor-proximal kinase signaling events, including the activation of PLCγ.

LPAR signaling impairs IP3R activity and inhibits intracellular calcium stores release in lymphocytes

PLCγ activity produces IP_3 which engages the IP_3R calcium channel subsequently promoting the release of ER calcium stores into the cytosol. Cytosolic calcium release is rapidly followed by extracellular calcium influx via ORAI channels with resulting cytosolic calcium levels reaching low micromolar concentrations critical for efficient antigen receptor signaling. PLCγ activation was found to be intact in the presence of LPA, as indicated by tyrosine phosphorylation, suggesting that LPAR signaling was inhibiting elevation in cytosolic calcium either by impeding IP_3R-mediated calcium release from intracellular stores and/or the ORAI-mediated extracellular calcium entry through the plasma membrane. To evaluate these two possibilities, we measured calcium mobilization using flow cytometry after antigen receptor stimulation in the absence and presence of LPA with the A20 B cell line and primary mouse splenic T cells, with both cell types displaying similar results. Specifically, when lymphocytes were stimulated via the antigen receptor in the absence or presence of 1–20μM LPA, and ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) was present in the medium to prevent extracellular calcium influx, we observed a dose-dependent inhibition of calcium release from intracellular stores which strongly suggested that LPAR signaling was impeding IP_3R activity.[76,80] Because we were concerned that incomplete calcium ER stores release may not fully activate ORAI activity at the plasma membrane, we directly evaluated whether LPAR signaling impaired ORAI activity. To accomplish this, we used thapsigargin, a plant toxin that specifically inhibits the sarcoplasmic-ER calcium ATPase (SERCA), which is an ER calcium pump whose function is to constitutively replenish ER calcium stores with cytosolic calcium.[85] Lymphocytes treated with thapsigargin fully release calcium into the cytosol from ER stores (albeit with slower kinetics than antigen-receptor stimulation) and promote STIM1/2 to fully activate the ORAI channel. Once calcium stores release is

complete, additional calcium given in the medium overcomes EGTA chelation and permits extracellular calcium entry via ORAI channels. Neither the A20 B cell line nor primary splenic T cells demonstrate any impairment of the ORAI channel when LPA is included in these analyses, suggesting that LPAR signaling impaired intracellular calcium mobilization via selectively inhibiting IP_3R activity.[76,80]

The results from two additional experiments further confirmed IP_3R activity induced by antigen receptor engagement is inhibited by LPAR signaling. In the first, A20 B cells were loaded with a membrane-permeable caged-IP_3 that was released upon exposure to UV light. When, A20 B cells were loaded with caged-IP_3 and either treated or not with 5μM LPA immediately prior to UV excitation, LPA treated cells displayed reduced calcium release from intracellular stores, demonstrating that LPA inhibition of calcium mobilization occurs downstream of IP_3 generation.[76] Further, with primary splenic T cells, adenophostin A, an IP_3R-specific agonist more potent than IP_3[86], was able to fully rescue the IP_3R-mediated calcium release from intracellular stores that was otherwise significantly reduced in the presence of LPA.[80] Considered in aggregate, these data provide strong evidence that the presence of LPA impairs antigen receptor-induced IP_3R activity leading to reduced intracellular calcium mobilization and subsequent signaling in lymphocytes. Cell biological and molecular studies described below address how LPAR signaling impacts cytotoxic CD8 T cell immune synapse formation and demonstrate that as a consequence of LPAR-mediated cytoskeletal reorganization, IP_3R localization during synapse formation is perturbed possibly accounting for LPA-induced suppression of IP_3R activity.

Importantly, we recognize that LPAR signaling in different cell types can mobilize calcium from internal stores including in immune cells such as NK cells, eosinophils, dendritic cells and the Jurkat leukemia cell line[87–96] but is not observed with T or B lymphocytes,[76,77] likely reflecting differences in expression of PLC isoforms and Gα family heterotrimeric G-proteins between cell types. In particular, lymphocytes express relatively high levels of PLCγ isoforms (PLCγ1 by T cells and PLCγ2 by B cells) whereas PLCβ isoforms are more broadly expressed and display reduced levels in T and B lymphocytes relative to PLCγ.[97,98]

TCR-mediated stimulation of ERK activity is inhibited by LPA

TCR signaling activates PLCγ1 which hydrolyzes membrane-bound PIP_2 to produce IP_3 and DAG resulting in a bifurcation of the signaling pathway. As discussed above, IP_3 production leads to elevated cytosolic calcium levels necessary to promote transcriptional activity and maturational programs. DAG production as a product of TCR signaling promotes the activation of the mitogen-activated protein kinase (MAPK) pathway involving Ras-Erk pathway via inducing PKCθ and RasGRP.[99–102] Thus, it was of interest to understand if LPA also regulated TCR signaling downstream of DAG production since both calcium mobilization and Erk activity are required for important lymphocyte-specific processes such as cytotoxicity[103–105] and cytokine secretion[106–111]. Accordingly, we also evaluated Erk activity as measured by levels of pERK (Erk1/2; Thr202/Tyr204) after stimulation of the TCR with and without LPA present. Both primary mouse and human T cells isolated from peripheral blood stimulated with anti-CD3 in the presence of 10μM LPA revealed a diminution in the activation of Erk that, for mouse T cells, was shown

to depend on LPA_5 expression[78] as described below. Thus, as further demonstrated in the next section, LPA_5 signaling negatively impacts acute TCR-induced signaling pathways that include the calcium mobilization and ERK activation that bifurcate after PLCγ1 hydrolysis of PIP_2.

An LPA_5-Gα13-Arhgef1 signaling axis inhibits antigen receptor mediated signaling

To determine the LPAR(s) responsible for the observed LPA-induced suppression of antigen receptor signaling, short hairpin RNAs (shRNAs) specific for *Lpar* genes were initially used and expressed in mature primary B cells to demonstrate that LPA-mediated suppression of BCR signaling was dependent on LPA_5 as this inhibition was lost when *Lpar5* expression was silenced by RNA interference[76]. The conclusion that an LPA-LPA_5 axis suppresses antigen receptor signaling of intracellular calcium was further genetically confirmed in mouse T lymphocytes using splenic T cells individually deficient for LPA_2, LPA_5 and LPA_6. That is, LPA-mediated antagonism of TCR-induced calcium mobilization was maintained in *Lpar2*$^{-/-}$ and *Lpar6*$^{-/-}$ T cells but lost in *Lpar5*$^{-/-}$ T cells.[76–78] Importantly, human T cell TCR-mediated calcium mobilization was also inhibited by LPA and this inhibition was relieved with an LPA_5-specific antagonist indicating that LPA impairment of human TCR-induced calcium mobilization is also dependent on LPA_5 signaling.[78]

LPA_5 receptor-proximal signaling events leading to antigen receptor suppression was characterized using both biochemical analyses of the A20 mature B cell line and genetic *in vivo* analyses of mature B cells from mouse mutants.[76] Specifically, retroviral-mediated expression of shRNAs specific for the Gα proteins that associate with LPA_5, Gαq and $G\alpha_{12/13}$[3,17], in A20 B cells revealed that $G\alpha_{12/13}$, but not $G\alpha_q$, expression was required for LPAR-mediated suppression of BCR-induced calcium mobilization.[76] Furthermore, Arhgef1, a hematopoietically-restricted protein able to regulate GPCR signaling and activate the RhoA GTPase, was also further determined to be required for LPA_5 suppression as evidenced by the inability of LPA to suppress BCR signaling in *Arhgef1*$^{-/-}$ B cells.[76] In particular, Arhgef1 harbors a **r**egulator of **G** protein **s**ignaling (RGS) domain previously shown to associate with (active) GTP-bound $G\alpha_{12}$ and $G\alpha_{13}$ G-proteins and accelerate their GTPase activity thereby aiding in return to the inactive GDP-bound state and terminating GPCR signaling.[112] In addition, Arhgef1 is also a guanine nucleotide exchange factor (GEF) and association with $G\alpha_{13}$ stimulates its RhoA GEF activity.[113] As a consequence of LPA_5-$G\alpha_{12/13}$ mediated signaling, Arhgef1 is expected to attenuate LPA_5 signaling and subsequently activate RhoA; a well-characterized and critical regulator of the actin cytoskeleton. Indeed, LPA treatment alone of primary human T cells leads to activation of RhoA.[80]

LPA suppresses T lymphocyte activation, proliferation and effector functions

TCR-mediated calcium mobilization and ERK activation are important for cytokine production by T cells[109,114,115] and cytotoxic activity by CD8 T cell effectors.[103–105]

The ability of LPA to suppress both TCR-induced ERK activity and calcium mobilization suggested that LPA suppression of CD8 T cell TCR signaling would be observed in *in vitro* and *in vivo* assays of lymphocyte activation, proliferation and function. The ability of LPA to suppress TCR-mediated cell activation and function was characterized using primary mouse T cells stimulated by antigen presenting cells (APCs) presenting peptide in the context of major histocompatibility complex (MHC). To accomplish this, we relied on OT-I TCR transgenic T cells with specificity for the chicken ovalbumin (Ova)-derived SIINFEKL peptide presented by MHC class I H-2^b-expressing APCs.[116] Furthermore, LPA is a relatively labile lipid that is quickly degraded *in vitro* and physiologically.[62,70] Accordingly, many of these *in vitro* and *in vivo* experiments exploited a metabolically stable LPA mimic, octodecenyl thiophosphate (OTP)[117–119], developed and provided by a long-standing collaborator in our LPA studies, Gabor Tigyi, PhD at the University of Tennessee Health Sciences Center.

Antigen-specific TCR-mediated induction of activation marker expression and cell proliferation are suppressed by LPA in vitro and in vivo

Initial *in vitro* experiments evaluated whether LPA, or the metabolically-stable LPA analog, OTP, suppressed the ability of SIINFEKL-presenting splenocytes to stimulate naïve OT-I CD8 T cells.[77] Experimental readouts in these diverse experiments were TCR-induced changes in the surface expression of the CD25, CD69, CD62L and CD44 activation markers in addition to proliferation.[77,78] Consistent with the ability of LPA_5 to inhibit TCR-induced calcium mobilization and Erk activation, in each case we found the presence of LPA/OTP significantly diminished expression of the CD25, CD69 and CD44 activation markers, impaired down-regulation of CD62L and potently suppressed proliferation by *in vitro* TCR-stimulated naïve CD8 T cells. Importantly, OTP did not perturb *in vitro* T cell viability over the range of concentrations used in these studies, suggesting that OTP *in vivo* treatment did not impact T cell survival.[77,78]

To promote *in vivo* LPAR signaling by OT-I CD8 T cells during antigen-specific TCR stimulation, we delivered OTP (or a vehicle control) subcutaneously every 8 hours for 3 days (OTP has an approximate 5.5 hour *in vivo* half-life; G. Tigyi, personal communication). OTP treatment was initiated one day after the intravenous adoptive transfer of naïve CFSE-labeled OT-I CD8 T cells and one hour before the subcutaneous transfer of SIINFEKL-pulsed bone marrow-derived dendritic cells (BMDCs).[77] As expected, in the absence of experimentally-induced LPA signaling by OTP, three days after peptide-pulsed BMDC transfer, OT-I CD8 T cells in the draining lymph node were $CD25^+$, had extensively diluted CFSE and accumulated to considerable numbers. Strikingly, OTP treatment during this period suppressed CD25 (mean fluorescent intensity) expression, and CFSE dilution by OT-I CD8 T cells and their cumulative numbers were 10-fold reduced compared to vehicle treated mice. Further, compared to wild type, virtually all LPA_5-deficient OT-I CD8 T cells stimulated *in vivo* by peptide-pulsed BMDCs had divided and accumulated to six-fold higher numbers in the draining lymph node three days after stimulation.[77] These findings provide abundant demonstration that LPA_5 signaling inhibits CD8 T cell antigen-specific TCR signaling, activation and proliferation *in vitro* and *in vivo*.

LPA suppresses in vitro *and* in vivo *CD8 T cell cytotoxic function*

LPA_5 *in vivo* signaling suppresses antigen-specific T cell activation leading to a reduced accumulation of antigen-specific T cells in the draining lymph node[77] and we were interested to understand if LPA signaling via LPA_5 further altered effector CD8 T cell function. Initial assessment of effector CD8 T cell function relied on intracellular expression of IFNγ and TNFα after *in vitro* antigen-specific stimulation and did not reveal any substantial expression differences between *Lpar5*$^{-/-}$ and wild type T cells.[77] A similar cytokine analysis of *in vivo* tumor-specific CD8 T cells harvested from a subcutaneously implanted melanoma tumor revealed comparable expression levels between *Lpar5*$^{-/-}$ and wild type T cells.[77] Thus, early findings did not suggest that LPA_5 signaling regulated effector CD8 T cell function as measured by induced intracellular cytokine expression, however, as described in a later section, LPA is found to impair cytokine secretion as a result of LPA-driven modulation of the cytoskeleton. Further, when effector CD8 T cell cytotoxic activity was directly tested, LPAR signaling again suppressed T cell killing activity *in vitro* and, *in vivo*, this suppression was shown to be LPA_5-dependent.[120]

The ability of LPA_5 to regulate cytotoxic killing by CD8 T cells was first investigated with *in vitro* killing assays relying on quantitative real time imaging (Incucyte Live-Cell analysis) of effector CD8 T cells actively killing antigen-specific B16 melanoma cells over 24 hours.[78] Specifically, primary naïve OT-I CD8 T cells were stimulated *in vitro* with SIINFEKL-pulsed APCs to generate Ova-specific cytotoxic effector CD8 T cells that were assayed for killing activity towards B16 melanoma cells either stably expressing chicken Ova (B16.cOVA) or pulsed with SIINFEKL peptide.[78] Here, in a dose-dependent manner, OTP inhibited both cytotoxic OT-I CD8 T cell Ova-specific killing and peripheral blood-derived human CD8 T cell allogeneic killing of a breast cancer cell line.[78]

The ability of LPAR signaling to impair CD8 cytotoxic T cell target cell killing *in vitro* is supported by *in vivo* experiments and further provided strong evidence that LPA_5 inhibitory signaling is driven by endogenous systemic LPA levels[78,120]. This was quantitatively demonstrated using an *in vivo* cytotoxic killing assay[121] which measured the ability of Ova-specific CD8 T cells, generated *in vivo* after Ova immunization, to eliminate adoptively-transferred (antigen-specific) SIINFEKL peptide-presenting APCs. In one approach, CD8 T cell *in vivo* killing was measured in *Enpp2*$^{+/-}$ mice, which harbor 50% LPA systemic levels compared to wild type.[63,71] Here, wild type and *Enpp2*$^{+/-}$ mice were immunized with Ova and found to elicit comparable frequencies of SIINFEKL-tetramer-positive Ova-specific CD8 T cells 4 days later.[78] SIINFEKL-pulsed and non-pulsed fluorescently-distinguishable target cells were co-transferred into immunized mice and specific killing measured (compared to killing of irrelevant (HSV1) peptide-pulsed and non-pulsed target cell transfers) one day later as measured as loss of the antigen-specific target cell population. These experiments revealed that endogenously-generated Ova-specific CD8 T cells in *Enpp2*$^{+/-}$ mice, harboring reduced LPA systemic levels, but (presumably) the same wild type T cell repertoire, killed twice the frequency of target cells compared with Ova-specific CD8 T cells from wild type mice.[78] This indicates that reducing the systemic levels of LPA by half results in a two-fold increase *in vivo* antigen-specific killing. In additional approaches, wild type and *Lpar5*$^{-/-}$ mice were immunized with Ova

(again generating comparable frequencies of Ova-tetramer-specific CD8 T cells four days post-immunization) and SIINFEKL-pulsed and irrelevant peptide-pulsed antigen presenting cells transferred to immunized mice. Here, Ova-specific *Lpar5*$^{-/-}$ CD8 T cell *in vivo* killing activity was significantly better than wild type.[120] The final iteration of this approach involved the adoptive transfer of either wild type or *Lpar5*$^{-/-}$ OT-I CD8 T cells into a wild type host immediately prior to Ova immunization. Five days later peptide-pulsed and non-pulsed target cells were transferred and two hours later cytotoxic *Lpar5*$^{-/-}$ OT-I CD8 T cells were found to display significantly better target cell killing compared to wild type OT-I CD8 T cells. [120] In aggregate these *in vitro* and *in vivo* studies not only show that systemic LPA levels limit antigen-specific CD8 T cell killing via LPA_5 but also provide strong evidence that elevated systemic LPA levels, as occurs with cancers and chronic viral infections, would robustly impair adaptive cellular immunity.

As discussed above, the extracellular LPA lysophospholipid signals to T cells via LPA_5 to inhibit CD8 T cell TCR signaling, activation, proliferation and cytotoxic activity, at least in part, by impairing TCR-induced IP_3R activity.[76,80] Mature T (and B) lymphocytes in both humans and mice express LPA_2, LPA_5, and LPA_6, but LPAR expression is not restricted to lymphocytes or immune cells as LPARs are widely expressed by diverse cell types.[122,123] This raises the question of why does LPAR signaling regulate CD8 T cell TCR signaling and cytotoxic activity and how is this accomplished? Currently, we propose systemic LPA signaling via LPA_5 expressed by lymphocytes acts as a form of peripheral tolerance that contributes to the suppression of basal and potentially energetically-wasteful polyreactive or weak self-reactive T and B cell antigen receptor signaling. In the following sections, we discuss in greater detail how specifically LPA serves as a tolerizing mechanism in CD8 T cells by impairing cytoskeletal reorganization and bioenergetics, which are both required for antigen-specific killing.

LPA obstructs TCR signaling and CD8 T cell effector cell killing ability by subverting the actin and microtubule cytoskeleton

An LPA_5-$G\alpha_{13}$-Arhgef1 signaling axis impedes IP_3R activity and Arhgef1, as a RhoGEF, would be expected to promote RhoA activation, possibly to regulate the actin cytoskeleton. Thus, an immunofluorescence microscopy study was undertaken to understand how LPAR signaling altered the cytoskeleton during the formation of an immunological synapse (IS) between a cytotoxic CD8 T cell and antigen-specific target cell. Here, attention was focused on the formation and function of the IS that not only defines the location of TCR recognition of target cells but also where cytolytic granules (and cytokines) are secreted to kill a target cell.[80] The ability of CD8 T cells to kill an infected or transformed cell requires that killing activity be very carefully and specifically controlled to be directed precisely towards the target cell and avoiding collateral damage of other nearby host cells.[124,125] Further, naïve CD8 T cells do not exert cytotoxic activity on initial specific antigen encounter but instead become activated, proliferate and develop into effector cytotoxic CD8 T cells expressing perforin and granzyme B stored within cytolytic granules. Thus, IS formation and function were assessed in an antigen-specific manner using primary effector CD8 T cells:target cells. This study revealed that the presence of LPA, at the time antigen-specific CD8 T cell:target

cell conjugates were established, significantly perturbed IS formation in several important features that rely on the actin and microtubule cytoskeleton (Figure 3).[80]

LPA and TCR induction of the RhoA GTPase and actin polymerization in CD8 T cells

The rapid reorganization of both the actin and microtubule cytoskeleton in effector CD8 T cells upon target cell contact has long been appreciated[126–132] and is of particular importance during physiological CD8 T cell TCR signaling and killing initiated by immune synapse formation with an antigen-presenting target cell.[125,133–135] TCR signaling initiated via synapse formation with an APC promotes actin and microtubule polymerization and reorientation of the centrosome, or microtubule-organizing center (MTOC),[134–136] which is necessary for the transport of cytolytic granules and cytokines to the IS for secretion.[136,137] The RhoA GTPase is considered to play a major role in regulating the actin cytoskeleton, but RhoA also contributes to regulating microtubule dynamics by stabilizing microtubules through its effector, Diaphanous-related formin-1 (mDia1).[138–143] It is thus not surprising, but also not well appreciated, that TCR signaling induced by anti-CD3 stimulation of purified single T cells in suspension promotes RhoA activation[80,144] as well as actin polymerization, which has been more broadly characterized.[125,144–147] Furthermore, both RhoA activity and polymerized actin are not only required for antigen-specific APC-induced TCR signaling but also for a number of important T cell biological processes that include integrin adhesion, immune synapse formation and target cell killing.[135,145–150]

On IS formation between a T cell and target cell, filamentous (F-) actin quickly accumulates within the T cell along the juxtaposed plasma membranes and is clearly observed by immunofluorescence microscopy.[80,134] TCR signaling also induces RhoA activation and both total and active (GTP-bound) RhoA are not only found at the antigen-specific IS but also at the uropod of the polarized cytotoxic T cell.[80] In agreement with the ability of T cell-expressed LPARs (LPA_2, LPA_5 and LPA_6) to signal via a $G\alpha_{13}$-RhoA axis[151], LPA alone activated RhoA and the presence of LPA during the formation of T cell:target cell conjugates led to significant changes in the cellular locations of both RhoA and polymerized actin.[80] Although cytotoxic T cell:target cell conjugates established in the presence of LPA revealed a similar localization of RhoA and F-actin along the IS, when LPA was present, F-actin further localized along the cell body accumulating at the uropod whereas RhoA (total and active) expression was precluded from uropod positioning in cell conjugates and was restricted to the IS.[80] Thus, concurrent LPAR signaling at the time of conjugate formation perturbed the localization of both RhoA and polymerized actin in cytotoxic CD8 T cells.

Again, consistent with an LPA_5-$G\alpha_{13}$-Arhgef1 axis, LPA treatment of human primary T cells alone in the absence of TCR signaling induces significant levels of (active) GTP-RhoA that are considerably elevated compared with those induced by TCR stimulation alone.[80] Intriguingly, simultaneous TCR and LPAR signaling by naïve T cells results in active RhoA levels that are slightly but significantly elevated relative to TCR signaling alone but not near the levels induced by LPAR signaling alone. This suggests that when both TCR and LPAR signaling occurs simultaneously, the cellular levels of RhoA required by TCR signaling supersede those needed for LPAR signaling. Regardless, TCR signaling promotes both

RhoA activity and actin polymerization whereas LPAR signaling induces RhoA activation but fails to induce rapid actin polymerization in T cells.[80] This suggests that early induction of RhoA activity in T cells by LPAR signaling is not devoted to actin polymerization.

LPA regulates the microtubule cytoskeleton and impairs mDia1 and IP_3R positioning to the IS

In T cells, microtubules have been shown to be acetylated and detyrosinated, post-translational modifications associated with microtubule stabilization to facilitate the transport of the TCR, ZAP70, and other cargo to the immunological synapse.[152–154] In other cell types LPA has been shown to activate RhoA and its effector mDia, a formin which not only promotes actin polymerization but also regulates microtubule stability through detyrosination.[138,142,155–157] mDia is expressed by T cells and, in its absence, T cells display impaired chemotaxis and trafficking to secondary lymphoid organs in addition to reduced TCR-induced actin polymerization, cell polarity and proliferation.[158,159] When we evaluated microtubule acetylation and detyrosination in T cell conjugates established in the presence or absence of LPA, we found LPA did not appear to influence microtubule acetylation but did impair microtubule detyrosination. Furthermore, in wild type CD8 T cell:target cell conjugates, mDia1 is found in the central region of the IS. However, in the presence of LPA, mDia1 was not centrally located but was instead enriched at the periphery of the IS.[80] Together these observations reveal that LPAR signaling at the time of effector CD8 T cell and target cell immune synapse formation leads to altered localization of RhoA, mDia1, actin polymerization and microtubule detyrosination.[80] Thus, LPAR signaling alters the regulation of both the actin and microtubule cytoskeleton leading to impaired TCR signaling and immune synapse formation.

As discussed, LPA signaling through LPA_5 suppresses TCR signaling by impairing IP_3R activity.[76,80] IP_3R1 has previously been shown to co-localize with the TCR in anti-CD3 stimulated Jurkat T cells where the Fyn tyrosine kinase phosphorylates IP_3R1 (Tyr 353), thereby enhancing its activity and increasing sensitivity to IP_3.[160,161] Thus, it was not surprising to find that the IP_3R1 calcium channel also rapidly and exclusively positioned at the immune synapse in antigen-specific wild type CD8 T cell:target cell conjugates.[80] The positioning of the IP_3R close to the IS at the site of TCR-peptide/MHC interaction would be advantageous for several reasons. Not only would this allow the IP_3R calcium channel to provide intracellular calcium in proximity to the TCR signalosome and effectors that are dependent on calcium for activity, but this positioning would also be important for maximizing IP_3R activation by Fyn and placing the IP_3R in close proximity to where PLCγ1 is generating IP_3. Notably, however, when LPA was present during conjugate formation, IP_3R1 location to the IS was considerably diminished and instead IP_3R1 was now enriched at the periphery of the IS and at the distal uropod end of the cell. The positioning of IP_3R1 at the synapse is dependent on TCR-induced mDia1 activity as IP_3R1 was also aberrantly positioned in *mDia1*$^{-/-}$ OT-I CD8 T cell:target cell conjugates, but not in Fmnl1-deficient OT-I CD8 T cells that lack a different T cell formin.[80] Indeed, LPAR signaling during IS formation led to altered positioning not only of IP_3R1 but also mDia and IP_3R1 positioning was similar whether conjugates were established in the presence of LPA or with T cells lacking mDia1. Finally, positioning of the IP_3R to the IS in wild type

T cell:target cell conjugates was dependent on both actin and microtubule polymerization as pharmacological inhibitors of polymerization impaired the position of the IP_3R to the IS.[80] These findings show that LPAR signaling during T cell target cell killing subverts mDia1 localization and activity needed for IP_3R positioning at the IS.

Considered together, these data suggest that full IP_3R activity depends on its location requiring the calcium channel to be in close proximity of the IS at the plasma membrane where TCR-associated Fyn phosphorylates and fully activates IP_3R1 and where PLCγ1 is generating IP_3 via hydrolysis of membrane PIP_2. LPA_5 regulation of the cytoskeleton prevents efficient IP_3R positioning to the IS where its ligand is being produced and, likely as a result of inefficient IP_3R activation and IP_3 diffusion, leads to inefficient signaling by those IP_3Rs not at the synapse. Whether LPA_5-induced signaling effectors further impair IP_3R activity by a more direct mechanism cannot be ruled out. However, LPA_5 inhibition of calcium stores release is overcome with a (potent) IP_3R agonist that has a higher affinity for IP_3R1 than IP_3. This argues that LPA-driven impairment of IP_3R activity is likely not regulated by post-translational or protein-association inhibitory mechanisms but is compatible with LPA-mediated cytoskeletal changes that physically constrain IP_3R from being fully activated and with full access to lP_3.

LPA-mediate reorganization of the cytoskeleton impedes cytolytic granule and cytokine secretion at the IS

A critical functional consequence of LPA-mediated subversion of the actin and microtubule cytoskeleton during target cell killing is that perforin-containing cytolytic granules are not delivered efficiently to the IS[78] (Figure 3) and ultimately impair both *in* vitro and *in* vivo CD8 T cells effector cytotoxicity.[78,120] Furthermore, IL-2 and IFNγ are directionally secreted in CD4 T cells[137] and in cytotoxic CD8 T cell:target cell conjugates where both IL-2 and IFNγ are transported to the IS for secretion[80]. In contrast, TNFα is secreted in a multi-dimensional direction in both CD4 and CD8 T cells[80,137]. Importantly, LPAR signaling by effector CD8 T cells does not impact the expression of either IL-2, IFNγ or TNFα as determined by intracellular cytokine staining.[77,80] However, LPAR signaling impedes the directional secretion of IL-2 and IFNγ, but not the secretion of TNFα.[80]

LPA regulation of ciliogenesis and CD8 T cell immune synapse formation

It is worth noting that immune synapse formation by cytotoxic CD8 T cells has been recognized to have possible evolutionary origins in primary cilia formation.[162–165] Primary (non-motile) cilia are microtubule-based structures that emanate from the plasma membrane and serve as sensory receptors or ‘antennae’ for most vertebrate cells, but evidently are not formed in T cells.[166] Similar to cilia formation where the centrosome, or MTOC, docks close to the plasma membrane and directs microtubule assembly of the cilium, on establishing an IS the MTOC of the T cell also polarizes close to the plasma membrane at the immune synapse where it directs microtubule assembly for the release of cytolytic granules and secretory vesicle cargo, such as cytokines, at the cell-cell interface.[166] Moreover, the intraflaggelar transport protein, IFT20, characterized in contributing to cilia assembly also positions to the IS in cytotoxic T cell conjugates.[163] Of interest, as sensory receptors, a number different receptors are located on the primary cilium including a number

of GPCRs[167] that respond and integrate information from diverse stimuli such as chemical, mechanical, light, temperature and osmolality to regulate tissue homeostasis. Defects in primary ciliogenesis lead to multisystemic genetic disorders, known as ciliopathies. Recently, variants in *CCDC28B* (Coiled-coil domain-containing protein 28B), a gene encoding a cilia-associated protein, was found in a subset of common-variable immune deficiency patients whose T cells were impaired in IS formation.[168] Further, an LPA-LPA_1 axis has been reported by several groups to promote disassembly of cilium in cultured human retinal pigment epithelial cells or human primary astrocytes thereby promoting cilia disassembly.[169–171] Thus, LPAR regulation of IS formation has evolutionary origins in LPA regulation of the primary cilium.

LPA_5 expression and signaling by CD8 T cells impedes tumor immunity

Diverse cancer cells often aberrantly turn on expression of *ENPP2*[172] and systemic levels of LPA are often elevated with certain cancers.[173] Indeed, a large body of literature has provided evidence that LPA is able to promote cell proliferation and facilitate tumor growth via different mechanisms that largely depend on signaling by $LPA_{1–3}$ Edg family members.[174,175] The notion that ATX expression and $LPA_{1–3}$ signaling promote tumorigenesis is strongly supported by a mouse model of enforced transgenic expression of either ATX or LPA_1, LPA_2, or LPA3 in mammary breast epithelium.[174] Transgenic lines for all three transgenes frequently developed late-onset, estrogen receptor (ER)-positive, invasive, and metastatic mammary cancer.[174] Thus, abundant data supports the notion that an ATX-LPA axis promotes tumorigenesis that, in turn, can increase systemic levels of LPA. In our studies we have observed that physiological levels of LPA (i.e., not intentionally manipulated) signaling via LPA_5 interferes with both cellular and humoral antigen-specific immunity.[76,78,120] As example, T cell immunization of *Enpp2*$^{+/-}$ mouse mutants, which express approximately half the levels of systemic LPA as wild type mice[63,71], leads to a two-fold increase of *in vivo* antigen-specific target cell killing[78], and Ova-specific *Lpar5*$^{-/-}$ CD8 T cells, when directly compared to Ova-specific wild type CD8 T cells, kill adoptively-transferred Ova peptide presenting target cells at higher and significant frequencies.[120] Further, immunization of *Lpar5*$^{-/-}$ and wild type mice with a model antigen results in heighted antigen-specific antibody responses by LPA_5-deficient B cells.[76] With this in mind, we addressed whether LPA_5-mediated *in vivo* signaling tempers tumor-specific CD8 T cell anti-tumor immunity using mouse models of tumor growth and metastasis.

For these tumor-specific *in vivo* experiments, OT-I TCR transgenic T cells and B16 melanoma cells stably-expressing chicken ovalbumin (B16.cOva) were employed. B16.cOva melanoma cells express and process ovalbumin followed by the presentation of SIINFEKL with MHC class I that is recognized by adoptively transferred mature ‘tumor-specific’ wild type and *Lpar5*$^{-/-}$ OT-I CD8 T cells. B16.cOva cells were implanted subcutaneously in the flank of mouse cohorts and allowed to grow for 5 days and measured daily. At day five, when tumors were palpable, either C57BL/6 or *Lpar5*$^{-/-}$ OT-I CD8 T cells were adoptive-transferred intravenously and mice monitored for an additional 8 days[77] and again one week later[78] (day 20 of tumor implantation). Beginning five days after implant and at the time of adoptive T cell transfer, B16.cOva tumor size increased at a nearly linear rate over the next eight days in wild type hosts and this growth rate was not changed with

the transfer of wild type OT-I CD8 T cells reflecting the inability of a C57BL/6 wild type host to control this aggressive syngeneic melanoma cell line.[176] Transfer of *Lpar5*$^{-/-}$ OT-I CD8 T cells 5 days after tumor implant, in contrast, significantly reduced tumor growth at six and eight days post T cell transfer and B16.cOva tumors were significantly reduced in mass while harboring higher numbers of OT-I CD8 T cells compared to wild type T cell transfers.[77]

In another approach, B16.cOva melanoma cells were intravenously injected into C57BL/6 wild type hosts followed immediately with the adoptive transfer of either wild type or *Lpar5*$^{-/-}$ OT-I CD8 T cells. Twenty days after tumor cells were injected and T cells transferred, mice that received LPA_5-deficient tumor-specific T cells harbored significantly fewer B16.cOva-derived tumors in the lungs that were not only significantly smaller in size but also contained considerably more LPA_5-deficient OT-I CD8 T cells relative to than tumors transferred with wild type CD8 T cells (Figure 4).[120] Impressively, *Lpar5*$^{-/-}$ cytotoxic CD8 T cells also expressed reduced levels of the Tim-3 and Lag-3 inhibitory receptors and the Tox transcription factor[120], which taken together with the observed differences in tumor burden and improved tumor immunity, strongly suggest that *Lpar5*$^{-/-}$ tumor-specific CD8 T cells were less dysfunctional.

These proof-of-principle studies, while contrived, provide strong *in vivo* evidence that LPA_5 inhibitory signaling by *bona fide* cytotoxic CD8 T cells, and in response to endogenously produced LPA, impairs tumor immunity. Further, ATX is often aberrantly expressed by diverse cancers to promote cell proliferation and tumorigenesis and is often accompanied by heightened systemic LPA levels. Thus, this suggests that the ATX-LPA axis serves to promote tumorigenesis (via induced ATX and LPA_{1-3} expression and resulting LPA autocrine activity) while simultaneously this tumor-derived LPA exerts paracrine activity suppresses cytotoxic CD8 T cell tumor immunity (Figure 5).

LPA_5 signaling by effector CD8 T cells modifies cellular metabolic energy source and efficiency

This review has highlighted *in vitro* and *in vivo* studies documenting CD8 T cell TCR signaling and killing activity are dysregulated if LPA simultaneously engages LPA_5 during T cell recognition of tumor-specific antigen. However, as a bioactive lysophospholipid, LPA levels are not only increased with obesity but LPA has also been reported to alter mitochondrial metabolism in skeletal muscle and induce de novo lipid synthesis in ovarian cancer cells.[45,46,50,177] Thus, it was hypothesized that LPA may also signal to T cells to regulate metabolic fitness and, consequently, functional activity. Consistent with this notion, treatment of primary cytotoxic OT-I CD8 T cells with a physiological concentration (1μM) of LPA for up to four hours significantly modulated T cell metabolism.[120] This is illustrated, in part, by the evaluation of cytotoxic CD8 T cells treated *in vitro* with LPA followed by the measurement of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) using the Seahorse Cell Mito Stress test to assess mitochondrial function. These results revealed that LPA treatment of OT-I primary CD8 T cells led to significant increases in both basal T cell respiration and maximal respiratory capacity in effector CD8 T cells and, importantly, the ability to achieve maximal respiratory capacity was dependent on LPA_5

(Figure 6 and ref. 119). Intriguingly, while promoting an increase in respiratory capacity of effector CD8 T cells, LPA treatment also promoted an increase in extracellular acidification of the media indicating that glycolysis was ongoing and generating and releasing lactate from the cell further suggesting an alternate non-glycolytic endogenous energy source was being used to increase mitochondrial metabolism (Figure 6). One likely non-glycolytic energy source are fatty-acid lipids, which are often stored in lipid droplets, a cell reservoir of neutral lipids produced by most cells and whose presence can reflect metabolic changes and access to nutrients.[178] Whether LPA-mediated regulation of T cell metabolism also changed the presence of lipid droplets was tested with BODIPY staining of neutral lipids and revealed a significant loss of lipid droplet content after 30 min – four hours of LPA treatment.[120] Moreover, preventing fatty-acid transport into the T cell mitochondria with etomoxir (1μM), a pharmacological inhibitor of carnitine palmitoyltransferase-1 (CPT-1), also led to an immediate reduction in maximal respiratory capacity.[120] These findings thus show that despite ongoing aerobic glycolysis, LPA signaling via LPA_5 shifts CD8 T cell metabolism to consume fatty-acids for mitochondrial respiration,[120] and consistent with a previous finding showing that PD-1 promotes fatty-acid oxidation of endogenous lipids.[179] Moreover, as a ubiquitous bioactive lipid signaling via GPCR cognate receptors, LPA participates in regulating the cytoskeleton and metabolism of CD8 T cells in a manner that also would be predicted to negatively impact T cell adaptive immunity.[180]

LPA, tumor immunity and cancer

There is considerable interest in the ATX-LPA axis from the cancer field where different cancers are often found to aberrantly express *ENPP2* thus producing extracellular LPA for autocrine LPAR signaling by tumor cells to promote cell proliferation and facilitate tumorigenesis. Evaluation of all curated non-redundant studies in cBioPortal[181,182] (a cancer database harboring multidimensional genomics data sets from individuals with different cancers) reveals that individuals with cancers in which *ENPP2* has been amplified have significantly worse progression-free survival and also when compared to patients with *MYC* amplification (Figure 7)[120], a gene amplification known to be associated with poor outcomes.[183] Notably, plasma LPA levels (16:0 species) are significantly higher in melanoma patients that do not respond to checkpoint blockade immunotherapy compared to melanoma patients that positively respond to checkpoint blockade therapy (Figure 7).[120] Within this data, there was a single outlier in the non-responder group that did not respond to upfront immunotherapy treatment. However, when followed-up it was determined this patient was later treated with a bispecific anti-PD/ICOS antibody and is currently disease free.[120] Thus, plasma LPA levels may not only serve as a prognostic indicator for therapy treatment, but antagonism of an ATX-LPA-LPAR axis may be an attractive therapeutic intervention for certain cancers.

Our studies document the ability of LPA_5 signaling to suppress acute *in vivo* TCR signaling by naïve CD4 and CD8 T cells and in response to physiological endogenous LPA levels. Based on this, *in vivo* LPA_5 antagonism would be expected to lower the threshold for activation and proliferation of naïve CD8 T cells responding to tumor antigen resulting in a higher number of responding tumor-specific T cells, as shown in mouse tumor models and with LPA_5-deficient CD8 T cells.[77,120] This is also consistent with the ability of a

high affinity antigen to elicit an increased number of antigen-specific CD8 T cells when compared to a lower affinity antigen.[184] Further, unmutated tumor antigens are self-antigens and any endogenous naïve CD8 T cell capable of recognizing tumor self-antigen will almost certainly express low-affinity TCRs[185] as high-affinity CD8 T cells are eliminated during central tolerance induction in the thymus. If, as we hypothesize, LPA_5-mediated suppression of TCR and BCR signaling has evolved as a mechanism to restrain activation of lymphocytes with self- and poly-reactive antigen receptors, then LPA_5 antagonism might prove further useful as a cancer therapeutic in promoting anti-tumor responses from self (tumor) antigen-specific CD8 T cells. Of course, this would need to be carefully considered as autoimmune T cell responses may also be facilitated. Along these lines, it should nevertheless be noted that for studies presented here, the OT-I TCR affinity for the SIINFEKL peptide (K_D ~ 6μM) is more reflective of TCR affinities that are usually higher for pathogen-derived peptides compared to the weaker affinities normally demonstrated for *bona fide* tumor antigens.[185]

LPA, through its regulation of the actin and microtubule skeleton also impedes TCR signaling at the immunological synapse as cytotoxic effector CD8 T cells are actively engaged in killing antigen-specific tumor cells or virally infected cells. LPA_5 antagonism could thus feasibly not only promote TCR signaling by naïve T cells on initial encounter with tumor antigen but also enhance tumor killing by weak-affinity cytotoxic effector CD8 T cells. Notably, current approved checkpoint blockade immunotherapy in the clinic is focused on promoting killing activity from tumor-specific effector T cells as most of the targeted inhibitory receptors (PD-1[186,187], CTLA-4[188,189], Tim3[190,191], Lag3[192,193] and TIGIT[194,195]) are not expressed by naïve T cells but instead their expression is induced only after initial T cell recognition of tumor antigen.

Finally, the LPA_1 Edg family member GPCR has been shown to promote the disassembly of primary cilium in cultured human retinal epithelial cells and primary astrocytes.[169–171] Primary cilium are found in non-cycling quiescent cells as cilia assembly has been long-recognized to be incompatible with cell proliferation,[196] and primary cilia are often not expressed by cancer cells.[197] Indeed, it has been suggested that newly transformed cells may need to disassemble primary cilia in order to proliferate, thus, LPA antagonism might additionally prevent cilia disassembly and proliferation by a number of different cancer cells.[173]

Summary and concluding remarks

The studies described in this review demonstrate that active LPA_5 signaling during CD8 T cell antigen-specific recognition of a target cell competes with TCR signaling for reorganization of the actin and microtubule cytoskeleton and thus restrains the recruitment of critical effector molecules to the immunological synapse. As a result, TCR-induced intracellular calcium stores release and transport of cytolytic granules and cytokines to the IS are impaired. Further, LPA induced LPA_5 signaling also regulates T cell metabolism by promoting fatty-acid oxidation for mitochondrial respiration and in a manner independent from LPA_5 regulation of TCR signaling. Thus, LPA signaling, at least in part via LPA_5,

regulates both the cytoskeleton and metabolism of effector CD8 T cells in a manner that ultimately impinges on CD8 T cell antigen-receptor signaling and killing activity.

Our findings suggest that LPA, under normal physiological conditions, acts as an inhibitory signal to restrain T cell activation thus contributing to peripheral immunological tolerance. Tumor- and chronic infection-induced increases in systemic LPA levels would be expected to raise the TCR signaling threshold to limit CD8 T cell adaptive immunity by not only inhibiting the activation of naïve T cells to tumor or viral antigens but also by directly impeding killing activity. LPA also promotes T cell chemokinesis and movement into, and within, the lymph node [72,73,79] that likely contributes to the ability of T cells to scan APCs for possible antigen-specific interactions. Accordingly, the ability of a T cell to be fully activated is dependent on the levels of LPA present in the environment as TCR and LPAR signaling compete for the same signaling molecules to achieve their specific end.

Most LPA cognate GPCRs are able to associate with $G\alpha_{12/13}$ and thus regulate RhoA GTPase activity in reorganizing the actin and microtubule cytoskeleton. Accordingly, a major function of systemic LPA signaling via LPA receptors may lie in cell type-specific regulation of the cytoskeleton necessary not only for cell shape but also cell division, polarity, migration and secretion. In particular, LPA receptors that associate with and signal via $G\alpha_{12/13}$ heterotrimeric G-proteins have also been shown to engage the Hippo pathway[198], an evolutionary conserved signaling pathway that responds to the cell microenvironment and regulates cell proliferation and tissue homeostasis, including organ size.[199] Thus, the ability of LPA to also further participate in controlling cellular metabolism is not surprising and further suggests that LPA-LPAR cell type specific signaling may not only reorganize the cytoskeleton in a variety of diverse cells but also may regulate metabolism in response to the environment.

The therapeutic interference of the ATX-LPA axis has attracted industry attention and currently there are 14 phase I and II clinical trials evaluating ATX and LPA_1 inhibitors for the treatment of systemic sclerosis, rheumatoid arthritis, pulmonary fibrosis, chronic liver disease, ovarian cancer and metastatic pancreatic cancer. Our findings would suggest ATX inhibition, which quickly reduces systemic LPA levels, would also promote tumor-specific CD8 T cell immunity. The studies presented here would also suggest LPA_5 antagonism may be a useful therapeutic approach for promoting T cell immunity to certain cancers and would require the identification of effective small molecule inhibitors for this receptor. Indeed, LPA_5 antagonists have been evaluated for alleviating microglia inflammatory cascades.[200] Notably, LPARs are all GPCRs, which currently comprise over one-third of all small molecule drugs approved for use by the US Food and Drug Administration[201,202] and drugs targeting both $S1P_1$ and $S1P_5$ GPCRs are in current use in the clinic for the treatment of multiple sclerosis.[24] It may also be useful to investigate the potential for LPA_5 agonism to suppress autoreactive lymphocyte responses in autoimmune diseases.[43]

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References

1. Aoki J, Inoue A, Okudaira S. Two pathways for lysophosphatidic acid production. Biochim Biophys Acta. 2008;1781(9):513–518. [PubMed: 18621144]
2. Xu Y Targeting Lysophosphatidic Acid in Cancer: The Issues in Moving from Bench to Bedside. Cancers (Basel). 2019;11(10).
3. Kano K, Aoki J, Hla T. Lysophospholipid Mediators in Health and Disease. Annu Rev Pathol. 2022;17:459–483. [PubMed: 34813354]
4. McIntyre TM, Pontsler AV, Silva AR, et al. Identification of an intracellular receptor for lysophosphatidic acid (LPA): LPA is a transcellular PPARgamma agonist. Proc Natl Acad Sci U S A. 2003;100(1):131–136. [PubMed: 12502787]
5. Zhang C, Baker DL, Yasuda S, et al. Lysophosphatidic acid induces neointima formation through PPARgamma activation. J Exp Med. 2004;199(6):763–774. [PubMed: 15007093]
6. Lipton JH, McMurray WC. Mitochondrial biogenesis in cultured mammalian cells. III. Synthesis of mitochondrial phospholipids by subcellular fractions isolated from normal and chloramphenicol-treated BHK-21 cells. Biochim Biophys Acta. 1977;486(2):228–242. [PubMed: 836855]
7. Tokumura A, Fukuzawa K, Tsukatani H. Effects of synthetic and natural lysophosphatidic acids on the arterial blood pressure of different animal species. Lipids. 1978;13(8):572–574. [PubMed: 703535]
8. Gerrard JM, Kindom SE, Peterson DA, Peller J, Krantz KE, White JG. Lysophosphatidic acids. Influence on platelet aggregation and intracellular calcium flux. Am J Pathol. 1979;96(2):423–438. [PubMed: 112871]
9. Gerrard JM, Clawson CC, White JG. Lysophosphatidic acids: III. Enhancement of neutrophil chemotaxis. Am J Pathol. 1980;100(3):609–618. [PubMed: 7416233]
10. Tokumura A, Fukuzawa K, Yamada S, Tsukatani H. Stimulatory effect of lysophosphatidic acids on uterine smooth muscles of non-pregant rats. Arch Int Pharmacodyn Ther. 1980;245(1):74–83. [PubMed: 6902643]
11. Lapetina EG, Billah MM, Cuatrecasas P. Lysophosphatidic acid potentiates the thrombin-induced production of arachidonate metabolites in platelets. J Biol Chem. 1981;256(23):11984–11987. [PubMed: 6795196]
12. Watson SP, McConnell RT, Lapetina EG. Decanoyl lysophosphatidic acid induces platelet aggregation through an extracellular action. Evidence against a second messenger role for lysophosphatidic acid. Biochem J. 1985;232(1):61–66. [PubMed: 3853461]
13. Moolenaar WH, van der Bend RL, van Corven EJ, Jalink K, Eichholtz T, van Blitterswijk WJ. Lysophosphatidic acid: a novel phospholipid with hormone- and growth factor-like activities. Cold Spring Harb Symp Quant Biol. 1992;57:163–167. [PubMed: 1339655]
14. Hecht JH, Weiner JA, Post SR, Chun J. Ventricular zone gene-1 (vzg-1) encodes a lysophosphatidic acid receptor expressed in neurogenic regions of the developing cerebral cortex. J Cell Biol. 1996;135(4):1071–1083. [PubMed: 8922387]
15. Hla T, Lee MJ, Ancellin N, Paik JH, Kluk MJ. Lysophospholipids--receptor revelations. Science. 2001;294(5548):1875–1878. [PubMed: 11729304]
16. Georas SN. Lysophosphatidic acid and autotaxin: emerging roles in innate and adaptive immunity. Immunol Res. 2009;45(2–3):229–238. [PubMed: 19184538]
17. Sheng X, Yung YC, Chen A, Chun J. Lysophosphatidic acid signalling in development. Development. 2015;142(8):1390–1395. [PubMed: 25852197]
18. Yung YC, Stoddard NC, Mirendil H, Chun J. Lysophosphatidic Acid signaling in the nervous system. Neuron. 2015;85(4):669–682. [PubMed: 25695267]
19. Smyth SS, Kraemer M, Yang L, Van Hoose P, Morris AJ. Roles for lysophosphatidic acid signaling in vascular development and disease. Biochim Biophys Acta Mol Cell Biol Lipids. 2020;1865(8):158734. [PubMed: 32376340]

20. Lee SC, Dacheux MA, Norman DD, et al. Regulation of Tumor Immunity by Lysophosphatidic Acid. Cancers (Basel). 2020;12(5).
21. Tang X, Hou Y, Schwartz TW, Haeggstrom JZ. Metabolite G-protein coupled receptor signaling: Potential regulation of eicosanoids. Biochem Pharmacol. 2022;204:115208. [PubMed: 35963340]
22. Kihara Y, Maceyka M, Spiegel S, Chun J. Lysophospholipid receptor nomenclature review: IUPHAR Review 8. Br J Pharmacol. 2014;171(15):3575–3594. [PubMed: 24602016]
23. Matloubian M, Lo CG, Cinamon G, et al. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Nature. 2004;427(6972):355–360. [PubMed: 14737169]
24. Burg N, Salmon JE, Hla T. Sphingosine 1-phosphate receptor-targeted therapeutics in rheumatic diseases. Nat Rev Rheumatol. 2022;18(6):335–351. [PubMed: 35508810]
25. Goetzl EJ, Kong Y, Voice JK. Cutting edge: differential constitutive expression of functional receptors for lysophosphatidic acid by human blood lymphocytes. J Immunol. 2000;164(10):4996–4999. [PubMed: 10799850]
26. Goetzl EJ, Rosen H. Regulation of immunity by lysosphingolipids and their G protein-coupled receptors. J Clin Invest. 2004;114(11):1531–1537. [PubMed: 15578083]
27. Wang L, Knudsen E, Jin Y, Gessani S, Maghazachi AA. Lysophospholipids and chemokines activate distinct signal transduction pathways in T helper 1 and T helper 2 cells. Cell Signal. 2004;16(9):991–1000. [PubMed: 15212760]
28. Blaho VA, Hla T. Regulation of mammalian physiology, development, and disease by the sphingosine 1-phosphate and lysophosphatidic acid receptors. Chem Rev. 2011;111(10):6299–6320. [PubMed: 21939239]
29. Choi JW, Herr DR, Noguchi K, et al. LPA receptors: subtypes and biological actions. Annu Rev Pharmacol Toxicol. 2010;50:157–186. [PubMed: 20055701]
30. Eichholtz T, Jalink K, Fahrenfort I, Moolenaar WH. The bioactive phospholipid lysophosphatidic acid is released from activated platelets. Biochem J. 1993;291 (Pt 3)(Pt 3):677–680. [PubMed: 8489494]
31. Chen YL, Xu Y. Determination of lysophosphatidic acids by capillary electrophoresis with indirect ultraviolet detection. J Chromatogr B Biomed Sci Appl. 2001;753(2):355–363. [PubMed: 11334351]
32. Baker DL, Morrison P, Miller B, et al. Plasma lysophosphatidic acid concentration and ovarian cancer. JAMA. 2002;287(23):3081–3082.
33. Kishimoto T, Matsuoka T, Imamura S, Mizuno K. A novel colorimetric assay for the determination of lysophosphatidic acid in plasma using an enzymatic cycling method. Clin Chim Acta. 2003;333(1):59–67. [PubMed: 12809736]
34. Murph M, Tanaka T, Pang J, et al. Liquid chromatography mass spectrometry for quantifying plasma lysophospholipids: potential biomarkers for cancer diagnosis. Methods Enzymol. 2007;433:1–25. [PubMed: 17954226]
35. Hosogaya S, Yatomi Y, Nakamura K, et al. Measurement of plasma lysophosphatidic acid concentration in healthy subjects: strong correlation with lysophospholipase D activity. Ann Clin Biochem. 2008;45(Pt 4):364–368. [PubMed: 18583620]
36. Nakamura K, Kishimoto T, Ohkawa R, et al. Suppression of lysophosphatidic acid and lysophosphatidylcholine formation in the plasma in vitro: proposal of a plasma sample preparation method for laboratory testing of these lipids. Anal Biochem. 2007;367(1):20–27. [PubMed: 17568554]
37. Kano K, Matsumoto H, Kono N, Kurano M, Yatomi Y, Aoki J. Suppressing postcollection lysophosphatidic acid metabolism improves the precision of plasma LPA quantification. J Lipid Res. 2021;62:100029. [PubMed: 33524376]
38. Baumforth KR, Flavell JR, Reynolds GM, et al. Induction of autotaxin by the Epstein-Barr virus promotes the growth and survival of Hodgkin lymphoma cells. Blood. 2005;106(6):2138–2146. [PubMed: 15933052]
39. Watanabe N, Ikeda H, Nakamura K, et al. Both plasma lysophosphatidic acid and serum autotaxin levels are increased in chronic hepatitis C. J Clin Gastroenterol. 2007;41(6):616–623. [PubMed: 17577119]

40. Joshita S, Ichikawa Y, Umemura T, et al. Serum autotaxin is a useful liver fibrosis marker in patients with chronic hepatitis B virus infection. Hepatol Res. 2018;48(4):275–285. [PubMed: 29114991]
41. Kostadinova L, Shive CL, Anthony DD. Elevated Autotaxin and LPA Levels During Chronic Viral Hepatitis and Hepatocellular Carcinoma Associate with Systemic Immune Activation. Cancers (Basel). 2019;11(12).
42. Nikitopoulou I, Oikonomou N, Karouzakis E, et al. Autotaxin expression from synovial fibroblasts is essential for the pathogenesis of modeled arthritis. J Exp Med. 2012;209(5):925–933. [PubMed: 22493518]
43. Schmitz K, Brunkhorst R, de Bruin N, et al. Dysregulation of lysophosphatidic acids in multiple sclerosis and autoimmune encephalomyelitis. Acta Neuropathol Commun. 2017;5(1):42. [PubMed: 28578681]
44. Nojiri T, Kurano M, Araki O, et al. Serum autotaxin levels are associated with Graves' disease. Endocr J. 2019;66(5):409–422. [PubMed: 30814442]
45. Ferry G, Tellier E, Try A, et al. Autotaxin is released from adipocytes, catalyzes lysophosphatidic acid synthesis, and activates preadipocyte proliferation. Up-regulated expression with adipocyte differentiation and obesity. J Biol Chem. 2003;278(20):18162–18169. [PubMed: 12642576]
46. Kulkarni P, Getzenberg RH. High-fat diet, obesity and prostate disease: the ATX-LPA axis? Nat Clin Pract Urol. 2009;6(3):128–131. [PubMed: 19204739]
47. Federico L, Ren H, Mueller PA, et al. Autotaxin and its product lysophosphatidic acid suppress brown adipose differentiation and promote diet-induced obesity in mice. Mol Endocrinol. 2012;26(5):786–797. [PubMed: 22474126]
48. Reeves VL, Trybula JS, Wills RC, et al. Serum Autotaxin/ENPP2 correlates with insulin resistance in older humans with obesity. Obesity (Silver Spring). 2015;23(12):2371–2376. [PubMed: 26727116]
49. Orosa B, Garcia S, Conde C. The autotaxin-lysophosphatidic acid pathway in pathogenesis of rheumatoid arthritis. Eur J Pharmacol. 2015;765:228–233. [PubMed: 26297977]
50. D'Souza K, Nzirorera C, Cowie AM, et al. Autotaxin-LPA signaling contributes to obesity-induced insulin resistance in muscle and impairs mitochondrial metabolism. J Lipid Res. 2018;59(10):1805–1817. [PubMed: 30072447]
51. D'Souza K, Paramel GV, Kienesberger PC. Lysophosphatidic Acid Signaling in Obesity and Insulin Resistance. Nutrients. 2018;10(4).
52. Brandon JA, Kraemer M, Vandra J, et al. Adipose-derived autotaxin regulates inflammation and steatosis associated with diet-induced obesity. PLoS One. 2019;14(2):e0208099. [PubMed: 30730895]
53. Sasagawa T, Okita M, Murakami J, Kato T, Watanabe A. Abnormal serum lysophospholipids in multiple myeloma patients. Lipids. 1999;34(1):17–21. [PubMed: 10188592]
54. Mills GB, Moolenaar WH. The emerging role of lysophosphatidic acid in cancer. Nat Rev Cancer. 2003;3(8):582–591. [PubMed: 12894246]
55. Chun J, Rosen H. Lysophospholipid receptors as potential drug targets in tissue transplantation and autoimmune diseases. Curr Pharm Des. 2006;12(2):161–171. [PubMed: 16454733]
56. Cooper AB, Wu J, Lu D, Maluccio MA. Is autotaxin (ENPP2) the link between hepatitis C and hepatocellular cancer? J Gastrointest Surg. 2007;11(12):1628–1634; discussion 1634–1625. [PubMed: 17902023]
57. Masuda A, Nakamura K, Izutsu K, et al. Serum autotaxin measurement in haematological malignancies: a promising marker for follicular lymphoma. Br J Haematol. 2008;143(1):60–70. [PubMed: 18710386]
58. Braddock DT. Autotaxin and lipid signaling pathways as anticancer targets. Curr Opin Investig Drugs. 2010;11(6):629–637.
59. Panupinthu N, Lee HY, Mills GB. Lysophosphatidic acid production and action: critical new players in breast cancer initiation and progression. Br J Cancer. 2010;102(6):941–946. [PubMed: 20234370]
60. Gotoh M, Fujiwara Y, Yue J, et al. Controlling cancer through the autotaxin-lysophosphatidic acid receptor axis. Biochem Soc Trans. 2012;40(1):31–36. [PubMed: 22260662]

61. Xiao YJ, Schwartz B, Washington M, et al. Electrospray ionization mass spectrometry analysis of lysophospholipids in human ascitic fluids: comparison of the lysophospholipid contents in malignant vs nonmalignant ascitic fluids. Anal Biochem. 2001;290(2):302–313. [PubMed: 11237333]
62. Albers HM, Dong A, van Meeteren LA, et al. Boronic acid-based inhibitor of autotaxin reveals rapid turnover of LPA in the circulation. Proc Natl Acad Sci U S A. 2010;107(16):7257–7262. [PubMed: 20360563]
63. Pamuklar Z, Federico L, Liu S, et al. Autotaxin/lysopholipase D and lysophosphatidic acid regulate murine hemostasis and thrombosis. J Biol Chem. 2009;284(11):7385–7394. [PubMed: 19139100]
64. Stracke ML, Krutzsch HC, Unsworth EJ, et al. Identification, purification, and partial sequence analysis of autotaxin, a novel motility-stimulating protein. J Biol Chem. 1992;267(4):2524–2529. [PubMed: 1733949]
65. Kanda H, Newton R, Klein R, Morita Y, Gunn MD, Rosen SD. Autotaxin, an ectoenzyme that produces lysophosphatidic acid, promotes the entry of lymphocytes into secondary lymphoid organs. Nat Immunol. 2008;9(4):415–423. [PubMed: 18327261]
66. Hausmann J, Kamtekar S, Christodoulou E, et al. Structural basis of substrate discrimination and integrin binding by autotaxin. Nat Struct Mol Biol. 2011;18(2):198–204. [PubMed: 21240271]
67. Nakasaki T, Tanaka T, Okudaira S, et al. Involvement of the lysophosphatidic acid-generating enzyme autotaxin in lymphocyte-endothelial cell interactions. Am J Pathol. 2008;173(5):1566–1576. [PubMed: 18818380]
68. Moolenaar WH, Perrakis A. Insights into autotaxin: how to produce and present a lipid mediator. Nat Rev Mol Cell Biol. 2011;12(10):674–679. [PubMed: 21915140]
69. Tabchy A, Tigyi G, Mills GB. Location, location, location: a crystal-clear view of autotaxin saturating LPA receptors. Nat Struct Mol Biol. 2011;18(2):117–118. [PubMed: 21289650]
70. Tomsig JL, Snyder AH, Berdyshev EV, et al. Lipid phosphate phosphohydrolase type 1 (LPP1) degrades extracellular lysophosphatidic acid in vivo. Biochem J. 2009;419(3):611–618. [PubMed: 19215222]
71. van Meeteren LA, Ruurs P, Stortelers C, et al. Autotaxin, a secreted lysophospholipase D, is essential for blood vessel formation during development. Mol Cell Biol. 2006;26(13):5015–5022. [PubMed: 16782887]
72. Takeda A, Kobayashi D, Aoi K, et al. Fibroblastic reticular cell-derived lysophosphatidic acid regulates confined intranodal T-cell motility. Elife. 2016;5:e10561. [PubMed: 26830463]
73. Zhang Y, Chen YC, Krummel MF, Rosen SD. Autotaxin through lysophosphatidic acid stimulates polarization, motility, and transendothelial migration of naive T cells. J Immunol. 2012;189(8):3914–3924. [PubMed: 22962684]
74. Donovan EE, Pelanda R, Torres RM. S1P3 confers differential S1P-induced migration by autoreactive and non-autoreactive immature B cells and is required for normal B-cell development. Eur J Immunol. 2010;40(3):688–698. [PubMed: 20039302]
75. Pereira JP, Xu Y, Cyster JG. A role for S1P and S1P1 in immature-B cell egress from mouse bone marrow. PLoS One. 2010;5(2):e9277. [PubMed: 20174580]
76. Hu J, Oda SK, Shotts K, et al. Lysophosphatidic acid receptor 5 inhibits B cell antigen receptor signaling and antibody response. J Immunol. 2014;193(1):85–95. [PubMed: 24890721]
77. Oda SK, Strauch P, Fujiwara Y, et al. Lysophosphatidic acid inhibits CD8 T cell activation and control of tumor progression. Cancer Immunol Res. 2013;1(4):245–255. [PubMed: 24455753]
78. Mathew D, Kremer KN, Strauch P, Tigyi G, Pelanda R, Torres RM. LPA(5) Is an Inhibitory Receptor That Suppresses CD8 T-Cell Cytotoxic Function via Disruption of Early TCR Signaling. Front Immunol. 2019;10:1159. [PubMed: 31231367]
79. Knowlden SA, Capece T, Popovic M, et al. Regulation of T cell motility in vitro and in vivo by LPA and LPA2. PLoS One. 2014;9(7):e101655. [PubMed: 25003200]
80. Kremer KN, Buser A, Thumkeo D, et al. LPA suppresses T cell function by altering the cytoskeleton and disrupting immune synapse formation. Proc Natl Acad Sci U S A. 2022;119(15):e2118816119. [PubMed: 35394866]
81. Berg LJ, Finkelstein LD, Lucas JA, Schwartzberg PL. Tec family kinases in T lymphocyte development and function. Annu Rev Immunol. 2005;23:549–600. [PubMed: 15771581]

82. Cahalan MD. STIMulating store-operated Ca(2+) entry. Nat Cell Biol. 2009;11(6):669–677. [PubMed: 19488056]
83. Hogan PG, Lewis RS, Rao A. Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. Annu Rev Immunol. 2010;28:491–533. [PubMed: 20307213]
84. Hogan PG, Rao A. Store-operated calcium entry: Mechanisms and modulation. Biochem Biophys Res Commun. 2015;460(1):40–49. [PubMed: 25998732]
85. Trebak M, Putney JW Jr. ORAI Calcium Channels. Physiology (Bethesda). 2017;32(4):332–342. [PubMed: 28615316]
86. Mak DO, McBride S, Foskett JK. ATP-dependent adenophostin activation of inositol 1,4,5-trisphosphate receptor channel gating: kinetic implications for the durations of calcium puffs in cells. J Gen Physiol. 2001;117(4):299–314. [PubMed: 11279251]
87. An S, Bleu T, Zheng Y, Goetzl EJ. Recombinant human G protein-coupled lysophosphatidic acid receptors mediate intracellular calcium mobilization. Mol Pharmacol. 1998;54(5):881–888. [PubMed: 9804623]
88. Young KW, Bootman MD, Channing DR, et al. Lysophosphatidic acid-induced Ca2+ mobilization requires intracellular sphingosine 1-phosphate production. Potential involvement of endogenous EDG-4 receptors. J Biol Chem. 2000;275(49):38532–38539. [PubMed: 10954727]
89. Panther E, Idzko M, Corinti S, et al. The influence of lysophosphatidic acid on the functions of human dendritic cells. J Immunol. 2002;169(8):4129–4135. [PubMed: 12370341]
90. Hasegawa Y, Erickson JR, Goddard GJ, et al. Identification of a phosphothionate analogue of lysophosphatidic acid (LPA) as a selective agonist of the LPA3 receptor. J Biol Chem. 2003;278(14):11962–11969. [PubMed: 12554733]
91. Jin Y, Knudsen E, Wang L, Maghazachi AA. Lysophosphatidic acid induces human natural killer cell chemotaxis and intracellular calcium mobilization. Eur J Immunol. 2003;33(8):2083–2089. [PubMed: 12884281]
92. Idzko M, Laut M, Panther E, et al. Lysophosphatidic acid induces chemotaxis, oxygen radical production, CD11b up-regulation, Ca2+ mobilization, and actin reorganization in human eosinophils via pertussis toxin-sensitive G proteins. J Immunol. 2004;172(7):4480–4485. [PubMed: 15034064]
93. Lee CW, Rivera R, Dubin AE, Chun J. LPA(4)/GPR23 is a lysophosphatidic acid (LPA) receptor utilizing G(s)-, G(q)/G(i)-mediated calcium signaling and G(12/13)-mediated Rho activation. J Biol Chem. 2007;282(7):4310–4317. [PubMed: 17166850]
94. Lichte K, Rossi R, Danneberg K, et al. Lysophospholipid receptor-mediated calcium signaling in human keratinocytes. J Invest Dermatol. 2008;128(6):1487–1498. [PubMed: 18172456]
95. Choi JW, Lim S, Oh YS, et al. Subtype-specific role of phospholipase C-beta in bradykinin and LPA signaling through differential binding of different PDZ scaffold proteins. Cell Signal. 2010;22(7):1153–1161. [PubMed: 20227493]
96. Jans R, Mottram L, Johnson DL, et al. Lysophosphatidic acid promotes cell migration through STIM1- and Orai1-mediated Ca2+(i) mobilization and NFAT2 activation. J Invest Dermatol. 2013;133(3):793–802. [PubMed: 23096711]
97. Miscia S, Di Baldassarre A, Cataldi A, et al. Immunocytochemical localization of phospholipase C isozymes in cord blood and adult T-lymphocytes. J Histochem Cytochem. 1999;47(7):929–936. [PubMed: 10375381]
98. Heng TS, Painter MW, Immunological Genome Project C. The Immunological Genome Project: networks of gene expression in immune cells. Nat Immunol. 2008;9(10):1091–1094. [PubMed: 18800157]
99. Kortum RL, Rouquette-Jazdanian AK, Samelson LE. Ras and extracellular signal-regulated kinase signaling in thymocytes and T cells. Trends Immunol. 2013;34(6):259–268. [PubMed: 23506953]
100. Roose JP, Mollenauer M, Gupta VA, Stone J, Weiss A. A diacylglycerol-protein kinase C-RasGRP1 pathway directs Ras activation upon antigen receptor stimulation of T cells. Mol Cell Biol. 2005;25(11):4426–4441. [PubMed: 15899849]
101. Dower NA, Stang SL, Bottorff DA, et al. RasGRP is essential for mouse thymocyte differentiation and TCR signaling. Nat Immunol. 2000;1(4):317–321. [PubMed: 11017103]

102. Izquierdo M, Leevers SJ, Marshall CJ, Cantrell D. p21ras couples the T cell antigen receptor to extracellular signal-regulated kinase 2 in T lymphocytes. J Exp Med. 1993;178(4):1199–1208. [PubMed: 8376929]
103. Lyubchenko TA, Wurth GA, Zweifach A. Role of calcium influx in cytotoxic T lymphocyte lytic granule exocytosis during target cell killing. Immunity. 2001;15(5):847–859. [PubMed: 11728345]
104. Fierro AF, Wurth GA, Zweifach A. Cross-talk with Ca(2+) influx does not underlie the role of extracellular signal-regulated kinases in cytotoxic T lymphocyte lytic granule exocytosis. J Biol Chem. 2004;279(24):25646–25652. [PubMed: 15060074]
105. Pores-Fernando AT, Zweifach A. Calcium influx and signaling in cytotoxic T-lymphocyte lytic granule exocytosis. Immunol Rev. 2009;231(1):160–173. [PubMed: 19754896]
106. Feske S, Wulff H, Skolnik EY. Ion channels in innate and adaptive immunity. Annu Rev Immunol. 2015;33:291–353. [PubMed: 25861976]
107. Oh-Hora M, Yamashita M, Hogan PG, et al. Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. Nat Immunol. 2008;9(4):432–443. [PubMed: 18327260]
108. Koike T, Yamagishi H, Hatanaka Y, et al. A novel ERK-dependent signaling process that regulates interleukin-2 expression in a late phase of T cell activation. J Biol Chem. 2003;278(18):15685–15692. [PubMed: 12595531]
109. Egerton M, Fitzpatrick DR, Kelso A. Activation of the extracellular signal-regulated kinase pathway is differentially required for TCR-stimulated production of six cytokines in primary T lymphocytes. Int Immunol. 1998;10(2):223–229. [PubMed: 9533450]
110. Fitzpatrick DR, Kelso A. Independent regulation of cytokine genes in T cells: the paradox in the paradigm. Transplantation. 1998;65(1):1–5. [PubMed: 9448135]
111. Egerton M, Fitzpatrick DR, Catling AD, Kelso A. Differential activation of T cell cytokine production by the extracellular signal-regulated kinase (ERK) signaling pathway. Eur J Immunol. 1996;26(10):2279–2285. [PubMed: 8898934]
112. Kozasa T, Jiang X, Hart MJ, et al. p115 RhoGEF, a GTPase activating protein for Galpha12 and Galpha13. Science. 1998;280(5372):2109–2111. [PubMed: 9641915]
113. Hart MJ, Jiang X, Kozasa T, et al. Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by Galpha13. Science. 1998;280(5372):2112–2114. [PubMed: 9641916]
114. Jorritsma PJ, Brogdon JL, Bottomly K. Role of TCR-induced extracellular signal-regulated kinase activation in the regulation of early IL-4 expression in naive CD4+ T cells. J Immunol. 2003;170(5):2427–2434. [PubMed: 12594266]
115. Whitehurst CE, Geppert TD. MEK1 and the extracellular signal-regulated kinases are required for the stimulation of IL-2 gene transcription in T cells. J Immunol. 1996;156(3):1020–1029. [PubMed: 8557975]
116. Hogquist KA, Jameson SC, Heath WR, Howard JL, Bevan MJ, Carbone FR. T cell receptor antagonist peptides induce positive selection. Cell. 1994;76(1):17–27. [PubMed: 8287475]
117. Deng W, Shuyu E, Tsukahara R, et al. The lysophosphatidic acid type 2 receptor is required for protection against radiation-induced intestinal injury. Gastroenterology. 2007;132(5):1834–1851. [PubMed: 17484878]
118. Kiss GN, Fells JI, Gupte R, et al. Virtual screening for LPA2-specific agonists identifies a nonlipid compound with antiapoptotic actions. Mol Pharmacol. 2012;82(6):1162–1173. [PubMed: 22968304]
119. Kosanam H, Ma F, He H, et al. Development of an LC-MS/MS assay to determine plasma pharmacokinetics of the radioprotectant octadecenyl thiophosphate (OTP) in monkeys. J Chromatogr B Analyt Technol Biomed Life Sci. 2010;878(26):2379–2383.
120. Turner J, Fredrickson M, D'Antonio M, et al. Lysophosphatidic acid modulates CD8 T cell immunosurveillance, metabolism, and anti-tumor immunity. 2023, Nat Comm, In press, 10.21203/rs.3.rs-2058360/v1
121. Barber DL, Wherry EJ, Ahmed R. Cutting edge: rapid in vivo killing by memory CD8 T cells. J Immunol. 2003;171(1):27–31. [PubMed: 12816979]

122. Choi JW, Herr DR, Noguchi K, et al. LPA receptors: subtypes and biological actions. Annu Rev Pharmacol Toxicol. 2010;50:157–186. [PubMed: 20055701]
123. Ishii S, Noguchi K, Yanagida K. Non-Edg family lysophosphatidic acid (LPA) receptors. Prostaglandins Other Lipid Mediat. 2009;89(3–4):57–65. [PubMed: 19524700]
124. Cassioli C, Baldari CT. The Expanding Arsenal of Cytotoxic T Cells. Front Immunol. 2022;13:883010. [PubMed: 35514977]
125. Ritter AT, Angus KL, Griffiths GM. The role of the cytoskeleton at the immunological synapse. Immunol Rev. 2013;256(1):107–117. [PubMed: 24117816]
126. Geiger B, Rosen D, Berke G. Spatial relationships of microtubule-organizing centers and the contact area of cytotoxic T lymphocytes and target cells. J Cell Biol. 1982;95(1):137–143. [PubMed: 6982900]
127. Ryser JE, Rungger-Brandle E, Chaponnier C, Gabbiani G, Vassalli P. The area of attachment of cytotoxic T lymphocytes to their target cells shows high motility and polarization of actin, but not myosin. J Immunol. 1982;128(3):1159–1162. [PubMed: 7035558]
128. Kupfer A, Dennert G. Reorientation of the microtubule-organizing center and the Golgi apparatus in cloned cytotoxic lymphocytes triggered by binding to lysable target cells. J Immunol. 1984;133(5):2762–2766. [PubMed: 6384372]
129. Pardi R, Inverardi L, Rugarli C, Bender JR. Antigen-receptor complex stimulation triggers protein kinase C-dependent CD11a/CD18-cytoskeleton association in T lymphocytes. J Cell Biol. 1992;116(5):1211–1220. [PubMed: 1346786]
130. DeBell KE, Conti A, Alava MA, Hoffman T, Bonvini E. Microfilament assembly modulates phospholipase C-mediated signal transduction by the TCR/CD3 in murine T helper lymphocytes. J Immunol. 1992;149(7):2271–2280. [PubMed: 1388187]
131. Valitutti S, Dessing M, Aktories K, Gallati H, Lanzavecchia A. Sustained signaling leading to T cell activation results from prolonged T cell receptor occupancy. Role of T cell actin cytoskeleton. J Exp Med. 1995;181(2):577–584. [PubMed: 7836913]
132. Kuhn JR, Poenie M. Dynamic polarization of the microtubule cytoskeleton during CTL-mediated killing. Immunity. 2002;16(1):111–121. [PubMed: 11825570]
133. Vicente-Manzanares M, Sanchez-Madrid F. Role of the cytoskeleton during leukocyte responses. Nat Rev Immunol. 2004;4(2):110–122. [PubMed: 15040584]
134. Ritter AT, Asano Y, Stinchcombe JC, et al. Actin depletion initiates events leading to granule secretion at the immunological synapse. Immunity. 2015;42(5):864–876. [PubMed: 25992860]
135. Hammer JA, Wang JC, Saeed M, Pedrosa AT. Origin, Organization, Dynamics, and Function of Actin and Actomyosin Networks at the T Cell Immunological Synapse. Annu Rev Immunol. 2019;37:201–224. [PubMed: 30576253]
136. Stinchcombe JC, Majorovits E, Bossi G, Fuller S, Griffiths GM. Centrosome polarization delivers secretory granules to the immunological synapse. Nature. 2006;443(7110):462–465. [PubMed: 17006514]
137. Huse M, Lillemeier BF, Kuhns MS, Chen DS, Davis MM. T cells use two directionally distinct pathways for cytokine secretion. Nat Immunol. 2006;7(3):247–255. [PubMed: 16444260]
138. Palazzo AF, Cook TA, Alberts AS, Gundersen GG. mDia mediates Rho-regulated formation and orientation of stable microtubules. Nat Cell Biol. 2001;3(8):723–729. [PubMed: 11483957]
139. Wen Y, Eng CH, Schmoranzer J, et al. EB1 and APC bind to mDia to stabilize microtubules downstream of Rho and promote cell migration. Nat Cell Biol. 2004;6(9):820–830. [PubMed: 15311282]
140. Gundersen GG, Wen Y, Eng CH, et al. Regulation of microtubules by Rho GTPases in migrating cells. Novartis Found Symp. 2005;269:106–116; discussion 116–126, 223–130. [PubMed: 16358406]
141. Eng CH, Huckaba TM, Gundersen GG. The formin mDia regulates GSK3beta through novel PKCs to promote microtubule stabilization but not MTOC reorientation in migrating fibroblasts. Mol Biol Cell. 2006;17(12):5004–5016. [PubMed: 16987962]
142. Yamana N, Arakawa Y, Nishino T, et al. The Rho-mDia1 pathway regulates cell polarity and focal adhesion turnover in migrating cells through mobilizing Apc and c-Src. Mol Cell Biol. 2006;26(18):6844–6858. [PubMed: 16943426]

143. DeWard AD, Alberts AS. Microtubule stabilization: formins assert their independence. Curr Biol. 2008;18(14):R605–608. [PubMed: 18644336]

144. Gomez TS, Billadeau DD. T cell activation and the cytoskeleton: you can't have one without the other. Adv Immunol. 2008;97:1–64. [PubMed: 18501768]

145. Beemiller P, Krummel MF. Mediation of T-cell activation by actin meshworks. Cold Spring Harb Perspect Biol. 2010;2(9):a002444. [PubMed: 20702599]

146. Kumari S, Curado S, Mayya V, Dustin ML. T cell antigen receptor activation and actin cytoskeleton remodeling. Biochim Biophys Acta. 2014;1838(2):546–556. [PubMed: 23680625]

147. Comrie WA, Burkhardt JK. Action and Traction: Cytoskeletal Control of Receptor Triggering at the Immunological Synapse. Front Immunol. 2016;7:68. [PubMed: 27014258]

148. Angkachatchai V, Finkel TH. ADP-ribosylation of rho by C3 ribosyltransferase inhibits IL-2 production and sustained calcium influx in activated T cells. J Immunol. 1999;163(7):3819–3825. [PubMed: 10490980]

149. Lang P, Guizani L, Vitte-Mony I, et al. ADP-ribosylation of the ras-related, GTP-binding protein RhoA inhibits lymphocyte-mediated cytotoxicity. J Biol Chem. 1992;267(17):11677–11680. [PubMed: 1601841]

150. Joseph N, Reicher B, Barda-Saad M. The calcium feedback loop and T cell activation: how cytoskeleton networks control intracellular calcium flux. Biochim Biophys Acta. 2014;1838(2):557–568. [PubMed: 23860253]

151. Xiang SY, Dusaban SS, Brown JH. Lysophospholipid receptor activation of RhoA and lipid signaling pathways. Biochim Biophys Acta. 2013;1831(1):213–222. [PubMed: 22986288]

152. Andres-Delgado L, Anton OM, Alonso MA. Centrosome polarization in T cells: a task for formins. Front Immunol. 2013;4:191. [PubMed: 23874337]

153. Hashimoto-Tane A, Yokosuka T, Sakata-Sogawa K, et al. Dynein-driven transport of T cell receptor microclusters regulates immune synapse formation and T cell activation. Immunity. 2011;34(6):919–931. [PubMed: 21703543]

154. Serrador JM, Cabrero JR, Sancho D, Mittelbrunn M, Urzainqui A, Sanchez-Madrid F. HDAC6 deacetylase activity links the tubulin cytoskeleton with immune synapse organization. Immunity. 2004;20(4):417–428. [PubMed: 15084271]

155. Nagasaki T, Gundersen GG. Depletion of lysophosphatidic acid triggers a loss of oriented detyrosinated microtubules in motile fibroblasts. J Cell Sci. 1996;109 (Pt 10):2461–2469. [PubMed: 8923207]

156. Cook TA, Nagasaki T, Gundersen GG. Rho guanosine triphosphatase mediates the selective stabilization of microtubules induced by lysophosphatidic acid. J Cell Biol. 1998;141(1):175–185. [PubMed: 9531557]

157. Bartolini F, Gundersen GG. Formins and microtubules. Biochim Biophys Acta. 2010;1803(2):164–173. [PubMed: 19631698]

158. Eisenmann KM, West RA, Hildebrand D, et al. T cell responses in mammalian diaphanous-related formin mDia1 knock-out mice. J Biol Chem. 2007;282(34):25152–25158. [PubMed: 17595162]

159. Sakata D, Taniguchi H, Yasuda S, et al. Impaired T lymphocyte trafficking in mice deficient in an actin-nucleating protein, mDia1. J Exp Med. 2007;204(9):2031–2038. [PubMed: 17682067]

160. deSouza N, Cui J, Dura M, McDonald TV, Marks AR. A function for tyrosine phosphorylation of type 1 inositol 1,4,5-trisphosphate receptor in lymphocyte activation. J Cell Biol. 2007;179(5):923–934. [PubMed: 18056410]

161. Liang J, Lyu J, Zhao M, et al. Tespa1 regulates T cell receptor-induced calcium signals by recruiting inositol 1,4,5-trisphosphate receptors. Nat Commun. 2017;8:15732. [PubMed: 28598420]

162. Stinchcombe JC, Griffiths GM. Secretory mechanisms in cell-mediated cytotoxicity. Annu Rev Cell Dev Biol. 2007;23:495–517. [PubMed: 17506701]

163. Finetti F, Paccani SR, Riparbelli MG, et al. Intraflagellar transport is required for polarized recycling of the TCR/CD3 complex to the immune synapse. Nat Cell Biol. 2009;11(11):1332–1339. [PubMed: 19855387]

164. de la Roche M, Asano Y, Griffiths GM. Origins of the cytolytic synapse. Nat Rev Immunol. 2016;16(7):421–432. [PubMed: 27265595]
165. Cassioli C, Baldari CT. A Ciliary View of the Immunological Synapse. Cells. 2019;8(8).
166. Douanne T, Stinchcombe JC, Griffiths GM. Teasing out function from morphology: Similarities between primary cilia and immune synapses. J Cell Biol. 2021;220(6).
167. Hilgendorf KI, Johnson CT, Jackson PK. The primary cilium as a cellular receiver: organizing ciliary GPCR signaling. Curr Opin Cell Biol. 2016;39:84–92. [PubMed: 26926036]
168. Capitani N, Onnis A, Finetti F, et al. A CVID-associated variant in the ciliogenesis protein CCDC28B disrupts immune synapse assembly. Cell Death Differ. 2022;29(1):65–81. [PubMed: 34294890]
169. Loskutov YV, Griffin CL, Marinak KM, et al. LPA signaling is regulated through the primary cilium: a novel target in glioblastoma. Oncogene. 2018;37(11):1457–1471. [PubMed: 29321663]
170. Walia V, Cuenca A, Vetter M, et al. Akt Regulates a Rab11-Effector Switch Required for Ciliogenesis. Dev Cell. 2019;50(2):229–246 e227. [PubMed: 31204173]
171. Hu HB, Song ZQ, Song GP, et al. LPA signaling acts as a cell-extrinsic mechanism to initiate cilia disassembly and promote neurogenesis. Nat Commun. 2021;12(1):662. [PubMed: 33510165]
172. Willier S, Butt E, Grunewald TG. Lysophosphatidic acid (LPA) signalling in cell migration and cancer invasion: a focussed review and analysis of LPA receptor gene expression on the basis of more than 1700 cancer microarrays. Biol Cell. 2013;105(8):317–333. [PubMed: 23611148]
173. Balijepalli P, Sitton CC, Meier KE. Lysophosphatidic Acid Signaling in Cancer Cells: What Makes LPA So Special? Cells. 2021;10(8).
174. Liu S, Umezu-Goto M, Murph M, et al. Expression of autotaxin and lysophosphatidic acid receptors increases mammary tumorigenesis, invasion, and metastases. Cancer Cell. 2009;15(6):539–550. [PubMed: 19477432]
175. Panupinthu N, Lee HY, Mills GB. Lysophosphatidic acid production and action: critical new players in breast cancer initiation and progression. Br J Cancer. 2010;102(6):941–946. [PubMed: 20234370]
176. Fidler IJ. Biological behavior of malignant melanoma cells correlated to their survival in vivo. Cancer Res. 1975;35(1):218–224. [PubMed: 1109790]
177. Mukherjee A, Wu J, Barbour S, Fang X. Lysophosphatidic acid activates lipogenic pathways and de novo lipid synthesis in ovarian cancer cells. J Biol Chem. 2012;287(30):24990–25000. [PubMed: 22665482]
178. Olzmann JA, Carvalho P. Dynamics and functions of lipid droplets. Nat Rev Mol Cell Biol. 2019;20(3):137–155. [PubMed: 30523332]
179. Patsoukis N, Bardhan K, Chatterjee P, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. Nat Commun. 2015;6:6692. [PubMed: 25809635]
180. Reina-Campos M, Scharping NE, Goldrath AW. CD8(+) T cell metabolism in infection and cancer. Nat Rev Immunol. 2021;21(11):718–738. [PubMed: 33981085]
181. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401–404. [PubMed: 22588877]
182. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6(269):pl1.
183. Kalkat M, De Melo J, Hickman KA, et al. MYC Deregulation in Primary Human Cancers. Genes (Basel). 2017;8(6).
184. Zehn D, Lee SY, Bevan MJ. Complete but curtailed T-cell response to very low-affinity antigen. Nature. 2009;458(7235):211–214. [PubMed: 19182777]
185. Aleksic M, Liddy N, Molloy PE, et al. Different affinity windows for virus and cancer-specific T-cell receptors: implications for therapeutic strategies. Eur J Immunol. 2012;42(12):3174–3179. [PubMed: 22949370]
186. Agata Y, Kawasaki A, Nishimura H, et al. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. Int Immunol. 1996;8(5):765–772. [PubMed: 8671665]

187. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 1992;11(11):3887–3895. [PubMed: 1396582]

188. Brunet JF, Denizot F, Luciani MF, et al. A new member of the immunoglobulin superfamily--CTLA-4. Nature. 1987;328(6127):267–270. [PubMed: 3496540]

189. Freeman GJ, Lombard DB, Gimmi CD, et al. CTLA-4 and CD28 mRNA are coexpressed in most T cells after activation. Expression of CTLA-4 and CD28 mRNA does not correlate with the pattern of lymphokine production. J Immunol. 1992;149(12):3795–3801. [PubMed: 1281186]

190. Monney L, Sabatos CA, Gaglia JL, et al. Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. Nature. 2002;415(6871):536–541. [PubMed: 11823861]

191. Hastings WD, Anderson DE, Kassam N, et al. TIM-3 is expressed on activated human CD4+ T cells and regulates Th1 and Th17 cytokines. Eur J Immunol. 2009;39(9):2492–2501. [PubMed: 19676072]

192. Baixeras E, Roman-Roman S, Jitsukawa S, et al. Cloning and expression of a lymphocyte activation gene (LAG-1). Mol Immunol. 1990;27(11):1091–1102. [PubMed: 2247088]

193. Triebel F, Jitsukawa S, Baixeras E, et al. LAG-3, a novel lymphocyte activation gene closely related to CD4. J Exp Med. 1990;171(5):1393–1405. [PubMed: 1692078]

194. Boles KS, Vermi W, Facchetti F, et al. A novel molecular interaction for the adhesion of follicular CD4 T cells to follicular DC. Eur J Immunol. 2009;39(3):695–703. [PubMed: 19197944]

195. Levin SD, Taft DW, Brandt CS, et al. Vstm3 is a member of the CD28 family and an important modulator of T-cell function. Eur J Immunol. 2011;41(4):902–915. [PubMed: 21416464]

196. Kasahara K, Inagaki M. Primary ciliary signaling: links with the cell cycle. Trends Cell Biol. 2021;31(12):954–964. [PubMed: 34420822]

197. Plotnikova OV, Golemis EA, Pugacheva EN. Cell cycle-dependent ciliogenesis and cancer. Cancer Res. 2008;68(7):2058–2061. [PubMed: 18381407]

198. Yu FX, Zhao B, Panupinthu N, et al. Regulation of the Hippo-YAP pathway by G-protein-coupled receptor signaling. Cell. 2012;150(4):780–791. [PubMed: 22863277]

199. Yu FX, Zhao B, Guan KL. Hippo Pathway in Organ Size Control, Tissue Homeostasis, and Cancer. Cell. 2015;163(4):811–828.

200. Plastira I, Joshi L, Bernhart E, et al. Small-Molecule Lysophosphatidic Acid Receptor 5 (LPAR5) Antagonists: Versatile Pharmacological Tools to Regulate Inflammatory Signaling in BV-2 Microglia Cells. Front Cell Neurosci. 2019;13:531. [PubMed: 31849616]

201. Congreve M, de Graaf C, Swain NA, Tate CG. Impact of GPCR Structures on Drug Discovery. Cell. 2020;181(1):81–91. [PubMed: 32243800]

202. Hauser AS, Attwood MM, Rask-Andersen M, Schioth HB, Gloriam DE. Trends in GPCR drug discovery: new agents, targets and indications. Nat Rev Drug Discov. 2017;16(12):829–842. [PubMed: 29075003]

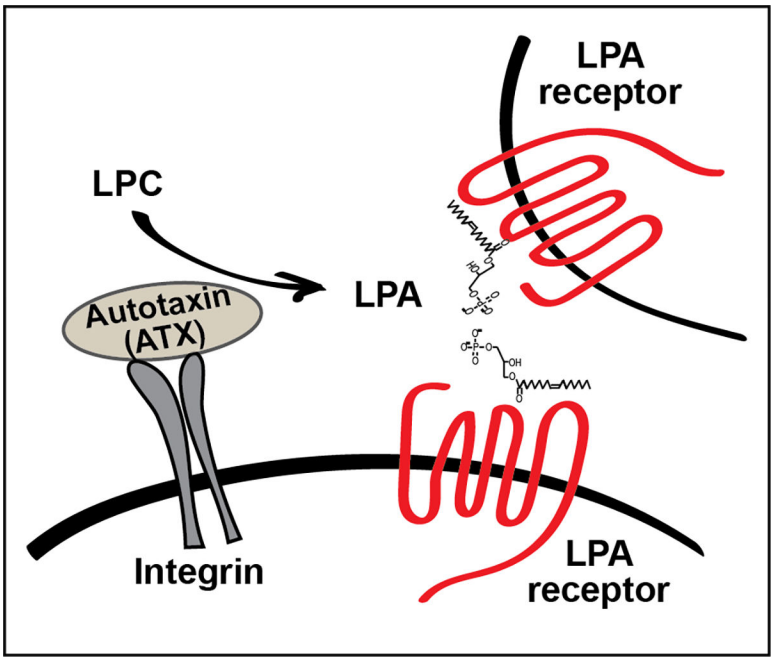

Figure 1.

Model of autocrine/paracrine LPA action. Autotaxin (ATX) is secreted by select cells and binds to integrins on the cell surface and hydrolyzes LPC to produce LPA. LPA then signals via nearby LPA G-protein-coupled receptors on the same or nearby cells.

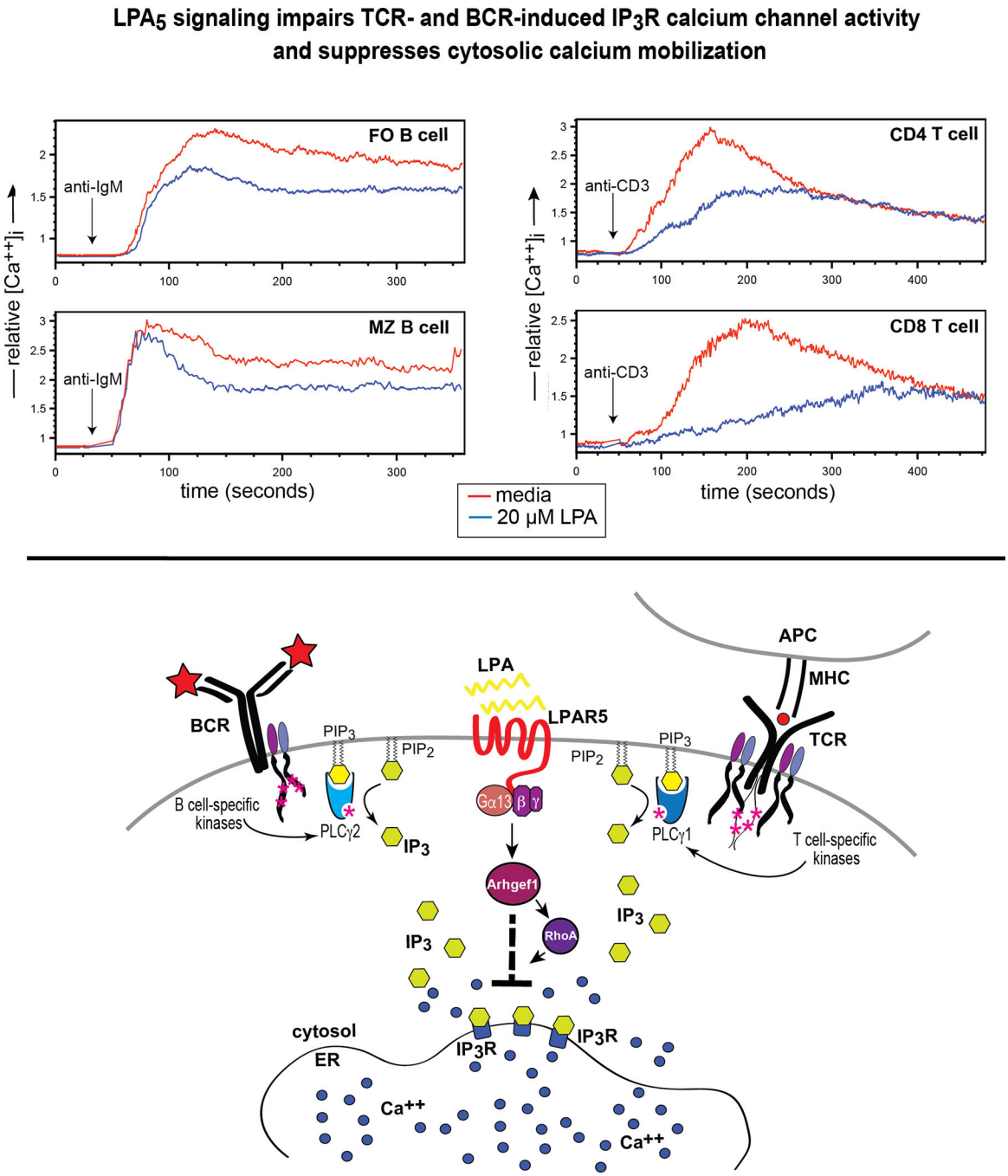

Figure 2.

LPA signals via LPA_5 to impair IP_3R activity and resulting in reduced intracellular calcium stores release and inhibited lymphocyte antigen receptor signaling. Top: The presence of LPA at the time of BCR- and TCR-induced signaling suppresses intracellular calcium stores release for follicular (FO) and marginal zone (MZ) B cells (left) and CD4 and CD8 T cells (right). Bottom: Schematic illustrating TCR- and BCR-proximal kinase and PLCg activity is intact in the presence of LPA_5 signaling while IP_3R activity is depressed resulting in reduced antigen receptor-induced cytosolic calcium levels.

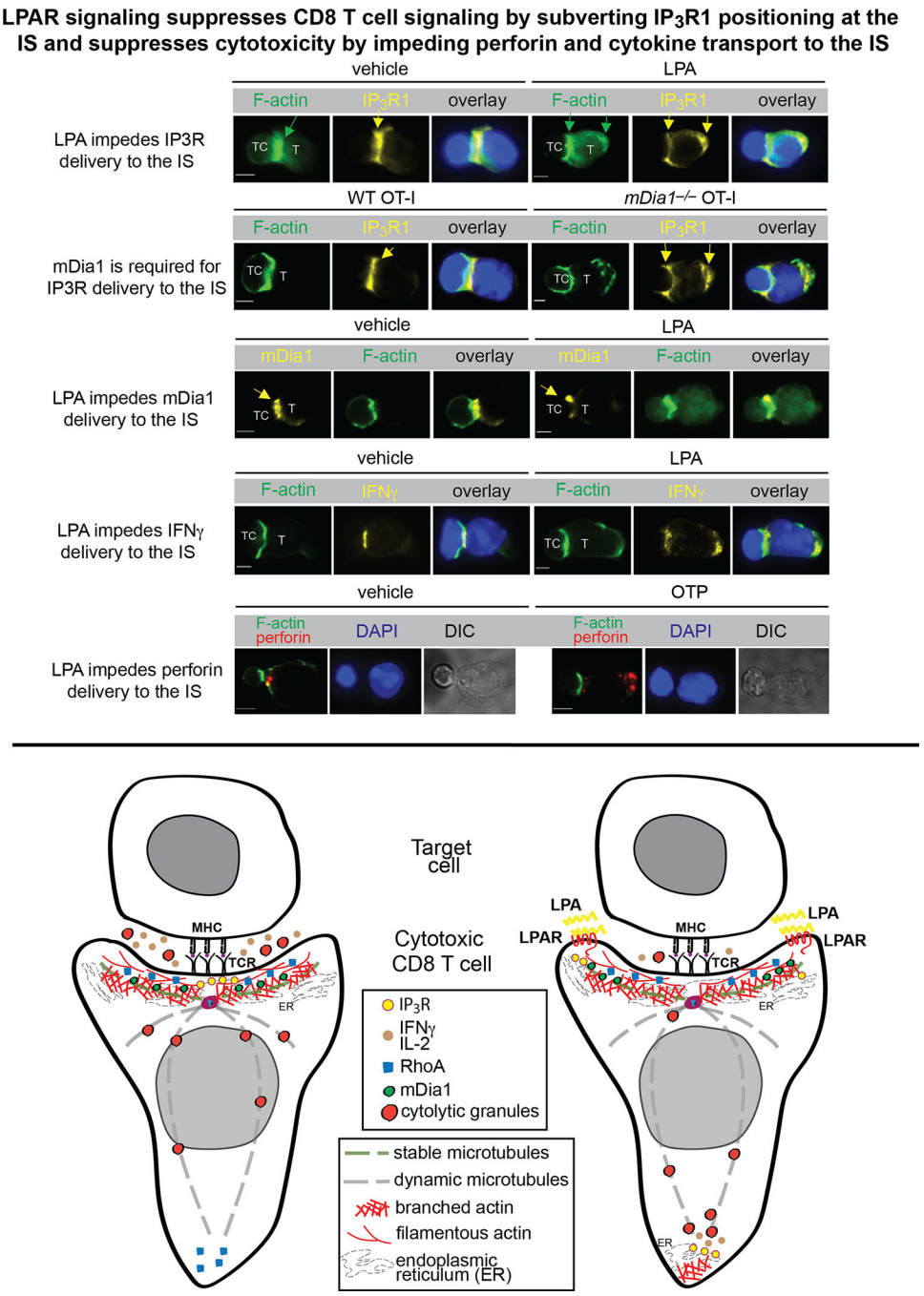

Figure 3.

LPA alters the regulation of the cytoskeleton during CD8 T cell:target cell IS formation in manner feasibly accounting for the resulting impairment in TCR signaling and killing activity. Top: Immunofluorescence microscopy of primary cytotoxic CD8 T cells (T; cell on right) shortly after immune synapse formation with antigen-specific target B cell (TC: cell on left). IS were established in the presence of vehicle or with wild type cells (left 3 images) or in the presence of LPA (OTP) or with *mDia1*$^{-/-}$ T cells (right 3 images). Staining: DAPI (blue) F-actin (green), perforin (red) and IP$_3$R1, mDia1, IFN**γ** (yellow in

top 4 rows). Bottom left: Schematic of an IS between an antigen-specific CD8 T cell:target cell showing IP_3R, RhoA mDia1, polymerized actin and stable microtubules positioned at the IS where cytotoxic granules and cytokines are transported to be secreted. Bottom right: Presence of LPA results in inefficient IP_3R localization to the IS and altered localization of RhoA and F-actin and perturbed microtubule detyrosination that impairs TCR signaling (see text), perforin and cytokine targeted release.[80]

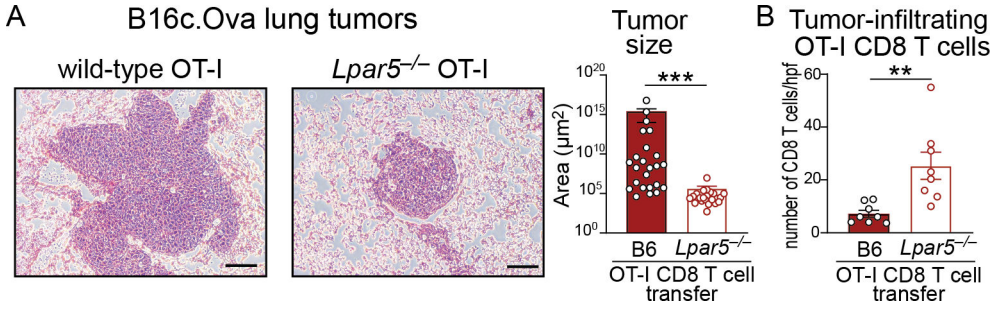

Figure 4.

Lpar5$^{-/-}$ tumor-specific CD8 T cells provide better control of B16 lung tumors than wild type CD8 T cells. A) Representative hematoxylin & eosin (H&E) histology images of day 20 B16.cOVA tumors after i.v. transfer together with either wild type (left) or *Lpar5*$^{-/-}$ (right) OT-I CD8 T cells. Scale bars=100 μm. Right: Quantification of B16.cOva tumor size after transfer of B6 (filled bar) or *Lpar5*$^{-/-}$ (open bar) OT-I CD8 T cells. B) Number of B6 (filled bar) or *Lpar5*$^{-/-}$ (open bar) OT-I CD8 T cells found in lung tumors. Student's t test *p < 0.05 and **p < 0.005.[120]

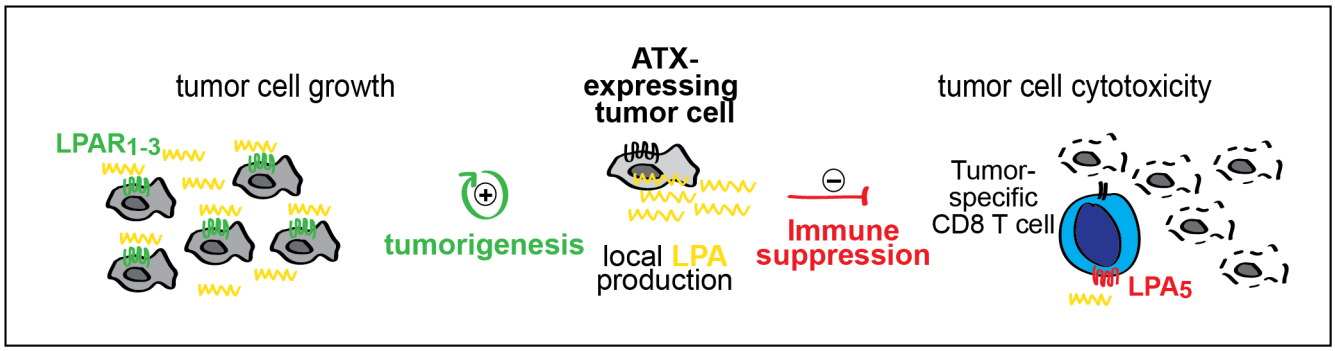

Figure 5.

Schematic of induction of *ENPP2* expression and subsequent ATX production by a transformed cell leading to local LPA production. LPA then signals via $LPA_{1–3}$ expressed by malignant cells to promote autocrine cell proliferation and tumorigenesis (left) or LPA_5 expressed by tumor-specific CD8 T cells to suppress tumor immunity.

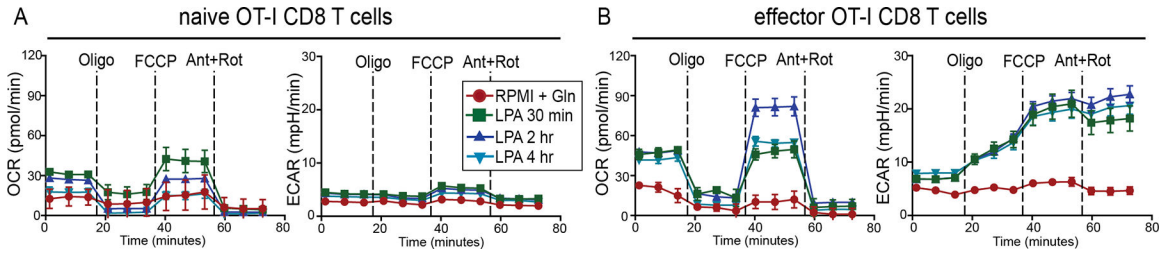

Figure 6.

LPA promotes an increase in basal T cell respiration, maximal respiratory capacity, proton leak and transiently elevates ATP production. Naïve (A) and effector (B) OT-I CD8 T cells were cultured *in vitro* with media (red) or 1 μM LPA for 30 minutes (green), 2 hrs (blue) or 4 hrs (teal) and oxygen consumption rate (OCR; left graphs) and extracellular acidification rate (ECAR; right graphs) measured. Assay was performed with injections of oligomycin (oligo), (4-(trifluoromethoxy) phenyl) carbonohydrazonoyl dicyanide (FCCP), antimycin A (ant), and rotenone (rot) at 18-minute intervals in media supplemented with 25 mM glucose. Data are n=6 technical replicates.[120]

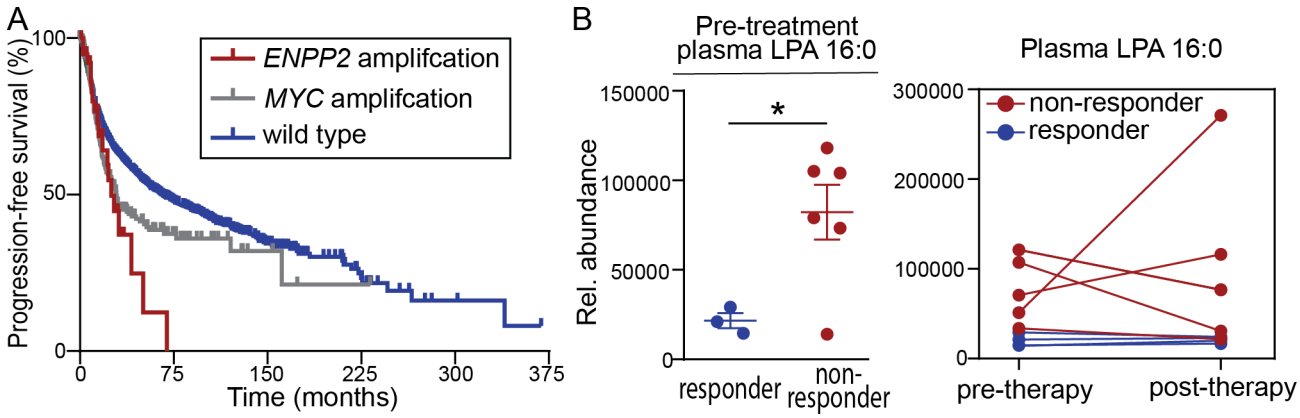

Figure 7.

Lysophosphatidic acid as a potential prognostic marker in melanoma. A) Data analysis from The Cancer Genome Atlas (TCGA) on progression-free survival. Data was from pan-cancer data from all solid tumors in cBioPortal from the complete curated non-redundant studies (as of June 18, 2021). Cohorts were stratified based on genomic status of amplification of *ENNP2*, *MYC*, or wild type for both genes. B) Relative abundance of LPA 16:0 in stage IV melanoma responder patients (blue; complete and partial response) and non-responder patients (red; stable disease and progressive disease) measured both pre- and post-therapy treatment. Unpaired Student's t-test where $p<0.05$.[120]