Phospho-LAT-Independent Activation of the Ras-Mitogen-Activated Protein Kinase Pathway: A Differential Recruitment Model of TCR Partial Agonist Signaling

Luan A. Chau and Joaquín Madrenas

*J Immunol* 1999; 163:1853-1858;
http://www.jimmunol.org/content/163/4/1853

**References** This article cites 48 articles, 27 of which you can access for free at:
http://www.jimmunol.org/content/163/4/1853.full#ref-list-1

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

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

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Phospho-LAT-Independent Activation of the Ras-Mitogen-Activated Protein Kinase Pathway: A Differential Recruitment Model of TCR Partial Agonist Signaling

Luan A. Chau* and Joaquin Madrenas2*†

Stimulation of mature T cells with agonist ligands of the Ag receptor (TCR) causes rapid phosphorylation of tyrosine-based activation motifs in the intracellular portion of TCR-ζ and CD3 and activation of several intracellular signaling cascades. Coordinate activation of these pathways is dependent on Lck- and ZAP-70-mediated tyrosine phosphorylation of a 36-kDa linker for activation of T cells and subsequent recruitment of phospholipase C-γ1, Grb2-SOS, and SLP-76-vav. Here, we show that TCR partial agonist ligands can selectively activate one of these pathways, the Ras/mitogen-activated protein kinase pathway, by inducing recruitment of Grb2-SOS complexes to incompletely phosphorylated p21 phospho-TCR-ζ. This bypasses the need for activation of Lck and ZAP-70, and for phosphorylation of the linker for activation of T cells to activate Ras. We propose a general model in which differential recruitment of activating complexes away from transmembrane linker proteins may determine selective activation of a given signaling pathway.

The Journal of Immunology, 1999, 163: 1853–1858.

Signaling transduction through the TCR is initiated by phosphorylation of tyrosine-based activation motifs (ITAMs) in the intracellular portion of the TCR-ζ and CD3 chains by a Src kinase (Lck or Fyn) (1, 2). In the presence of sustained engagement of the TCR, ITAM phosphorylation is followed by recruitment, phosphorylation, and activation of a Syk family kinase (ZAP-70), and formation of multimolecular complexes that signal through different intracellular pathways (3). Significant attention has been focused on the link between the early phosphorylation events occurring in the engaged TCR complex and the downstream signaling cascades emanating from that receptor. The emerging paradigm is that the two steps are linked by a heterogeneous group of transmembrane molecules known as linkers (4). Two of these have already been characterized: the linker for activation of T cells (LAT) (5, 6); and the TCR-interacting molecule (TRIM) (7). Both are type III transmembrane proteins that are tyrosine phosphorylated by coordinate action of a Src kinase and ZAP-70 after TCR-mediated signaling. On phosphorylation, they recruit multiple signaling molecules either directly (e.g., phospholipase C-γ1 (PLCγ1) or phosphatidylinositol 3-kinase (PI3-kinase), or indirectly (e.g., SOS, vav, and Cbl), through Grb2 or SLP-76 (SH2 domain-containing 76-kDa leukocyte protein) adapters. Thus, LAT and TRIM have a common function in redistributing molecules to the cell membrane on early receptor signaling, facilitating the interaction between enzymes and its substrates.

One of the intracellular signaling pathways activated by TCR engagement is the Ras/mitogen-activated protein kinase (MAPK) pathway (8, 9). Productive activation of this cascade causes translocation of several transcription factors and up-regulation of transcription of several genes that are required for proliferation and/or differentiation of activated T cells (10). Current evidence suggests that TCR-induced Ras activation requires cell membrane recruitment of SOS, a guanine-nucleotide-exchange factor (GEF) that converts the GDP-bound form of Ras into its active GTP-bound counterpart (8). Such a recruitment is likely determined by interaction between the SH2 domain in the Grb2 or Grb2-like Grap adapters with phosphorylated tyrosine residues in LAT (5, 11). Consistent with this model, it has been proposed that signaling through Ras in the context of coordinate activation of other signaling pathways (as induced by agonists of the TCR) requires tyrosine-phosphorylated LAT (5, 12). However, two observations suggest that Ras activation involves additional interactions: 1) phospho-LAT-dependent Grb2-SOS recruitment to the cell membrane is not sufficient for activation of Ras as seen in SLP-76-deficient cells after TCR ligation (13); 2) the Ras-MAPK pathway can be selectively activated in the absence of Lck and ZAP-70 kinase activities (Refs. 14 and 15; M. L. Baroja and J. Madrenas, manuscript in preparation), e.g., after T cell stimulation with partial agonists of the TCR (14). Because phosphorylation of LAT and SLP-76 in mature T cells requires the activities of Lck and ZAP-70, it is logical to propose that TCR partial agonist-induced Ras-MAPK activation can occur through an alternative pathway independently of LAT phosphorylation. These alternative interactions between signaling molecules may determine distinct signaling patterns.

Although coordinate activation of different signaling cascades is necessary for the development of effector functions by T cells, selective activation of signaling pathways may have some biological implications such as regulation of T cell survival (16). In this sense, revealing the molecular basis of alternative pathways for Ras activation may help us to understand how T cells can dissociate signaling cascades and mount selective responses to different

*Transplantation and Immunobiology Group, John P. Robarts Research Institute, and Departments of Microbiology and Immunology and of Medicine, University of Western Ontario, London, Ontario, Canada

Received for publication April 8, 1999. Accepted for publication June 7, 1999.

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

† This work has been funded by the Medical Research Council of Canada, the Kidney Foundation of Canada, the Cancer Research Society, and the Juvenile Diabetes Foundation International.

2 Address correspondence and reprint requests to J. Madrenas, John P. Robarts Research Institute, Room 2.05, P.O. Box 5015, 100 Perth drive, London, Ontario, Canada N6A 5K8. E-mail address: madrenas@rri.on.ca

3 Abbreviations used in this paper: ITAM, immune receptor, tyrosine-based activation motif; LAT, linker for activation of T cells; MAPK, mitogen-activated protein kinase; PLCγ1, phospholipase C-γ1; ERK, extracellular signal-related kinase.

Copyright © 1999 by The American Association of Immunologists 0022-1767/99/$02.00
types of Ag receptor ligands. Here, we report that TCR partial agonist ligands utilize a phospho-LAT-independent pathway to activate the Ras-MAPK pathway selectively. This occurs by recruitment of Grb2-SOS complexes to an incompletely phosphorylated (p21) form of TCR-ζ. On the basis of these data, we propose a differential recruitment model for TCR partial agonist signaling. According to this model, partial agonists of the TCR induce incomplete phosphorylation of TCR-ζ but no phosphorylation of LAT. Under these conditions, Ras-activating complexes are recruited to phospho-TCR-ζ, and this correlates with selective activation of the MAPK pathway. In contrast, the full agonists of the TCR induce activation of Lck and ZAP-70 and subsequent phosphorylation of LAT. This correlates with recruitment of Ras-activating complexes to phospho-LAT and coordinate activation of the MAPK pathway with other downstream signaling cascades.

Materials and Methods

Cells

3C6 and A,E7 are CD4+ Th1 clones specific for pigeon cytochrome c fragment 81–104 bound to I-Ek molecules. These clones were kept in culture with repeated rounds of Ag stimulation followed by IL-2-driven expansion and rest for 10 to 14 days (17, 18). For consistency, all the values in this paper are representative of results using 3C6 T cells.

Monoclonals

The following mAbs were used in these studies: 4G10 (a gift from Dr. B. Dukker, Oregon Health Sciences University, Portland, OR) is a mouse IgG2b mAb against phosphotyrosine; 6B10.2 is a mouse IgG1 mAb against TCR-ζ (Santa Cruz Biotechnology, Santa Cruz, CA); 81 is a mouse IgG1 mAb against Grb2 (Transduction Laboratories, Lexington, KY) used for immunoblotting of Grb2; and PE-labeled H1.2F3 is an Armenian hamster IgG Ab against mouse CD69 (PharMingen Canada, Mississauga, Canada). Rabbit polyclonal IgG sera against Grb2 (for immunoprecipitation) and against SOS were purchased from Santa Cruz Biotechnology. Anti-ACTIVE MAPK (Promega, Madison, WI) is a rabbit antiserum against dual phosphorylated MAPK-derived peptide and recognizes extracellular signal-related kinase (ERK) bands at 42/44 kDa after cell activation. Dual phosphorylation of ERK-1 and ERK-2 correlates with activation of these enzymes (14). SHC was immunoprecipitated or immunoblotted with a rabbit polyclonal immune serum purchased from Transduction Laboratories. A sheep polyclonal IgG serum against mouse SLP-76 was purchased from Upstate Biotechnology (Lake Placid, NY). The rabbit immune sera against TCR-ζ, ZAP-70, and LAT used in these studies were kindly provided by Dr. L. E. Samelson (Lymphocyte Signaling Unit, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD). The chimeric anti-CD3-fos and anti-CD4-jun molecules were previously described (14) and were generated by Dr. J. Tso (Protein Design Labs, Palo Alto, CA).

Cell stimulation and protein biochemistry

T cells (10–20 × 10^6/group) were stimulated with hetero-functional Abs at a final concentration of 10 μg/ml, at 37°C, for 2, 5, or 10 min. Cells were pelleted in PBS containing sodium-β-agarose (400 μM) and EDTA (400 μM) and lysed in lysis buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris (pH 7.6), 5 mM EDTA, 1 mM sodium-β-agarose, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 25 μg/ml p-nitrophenyl-p′-guanidinobenzoate), at 4°C for 30 min. Lysates were cleared of debris (14,000 rpm, 4°C, 10 min) followed by immunoprecipitation of target molecules using Ab-coated protein A- or G-Sepharose or agarose beads, for 2 h at 4°C. The chimeric Abs cannot bind protein A because they lack an Fc portion. Nevertheless, they may show some binding to protein G through the CH1 domain of the Fab fragment (19–21). However, the effect of Fab binding on the specificity of TCR subunit immunoprecipitations is minimal, as shown by controls in which the Abs were added to the lysates before immunoprecipitation, by precleaving with protein G-Sepharose or agarose beads alone, and by using protein A-Sepharose or agarose beads for immunoprecipitations with appropriate Abs (see Fig. 3). Beads were pelleted and resuspended in sample buffer, run in SDS-PAGE, and immunoblotted with the indicated Abs. Signal detection was performed by chemiluminescence (Roche Diagnostics, Laval, Canada). Signal-quantitation was done using a Bio-Rad GS-700 Image densitometer and the MultiAnalyst version 1.0.2 software package (Bio-Rad, Hercules, CA). Densitometry units for each band are expressed as OD adjusted by surface (OD × mm^2). Semiquantitative analysis of protein association was reported as the ratio of densitometric readings between specific treatments over background levels in nonstimulated cells and corrected by cell number. As controls for nonspecific reactivity of the blotting Ab, we used protein G-Sepharose or agarose beads coated with the immunoprecipitating Ab in the absence of cell lysate.

Flow cytometry

Expression of the very early activation marker CD69 was examined on T cell clones (1.2 × 10^6 cells/group) after overnight culture without or with hetero-functional Abs at a final concentration of 10 μg/ml. Flow cytometric analysis was performed after cell staining for 45 min with PE-labeled mAb against mouse CD69 at 4°C.

Results and Discussion

We have previously reported that coengagement of CD3 and CD4 on T cells leads to an agonist type of signaling characterized by complete phosphorylation of TCR-ζ chains (p21 and p23 forms of phospho-ζ) and CD3-ε, and activation of Lck and ZAP-70 kinase activities (14, 18). In contrast, bivalent engagement of CD3 leads to TCR-mediated signaling in a partial agonist mode, with incomplete tyrosine phosphorylation of TCR-ζ (only the p21 form of phospho-ζ is present) without phosphorylation of CD3-ε and without activation of Lck and ZAP-70 (14). Despite the notable differences in early signaling events, both agonist and partial agonist signaling through the TCR lead to significant activation of the Ras-MAPK pathway as illustrated by the appearance of dual phosphorylation and kinase activity of ERK-1 and ERK-2 (Fig. 1a). As we have previously reported (14), activation of ERK-1/2 is more transient under partial agonist conditions when compared with that after agonist signaling from the TCR and is undetectable after 30 min of stimulation. Functionally, activation of the Ras-MAPK pathway under both patterns of early TCR-mediated signaling

![FIGURE 1. Activation of the Ras-MAPK pathway under agonist and partial agonist conditions of TCR-mediated signaling.](http://www.jimmunol.org/...)}
translates into similar up-regulation of CD69 expression, a downstream response that is dependent only on Ras activation (12, 13, 22) (Fig. 1b). Therefore, these data confirm that partial agonist ligands of the TCR induce a biologically significant activation of the Ras-MAPK pathway.

A distinctive feature of TCR partial agonist signaling is that it activates the Ras-MAPK pathway without activating Lck and ZAP-70 (14). Given that these two kinases are required for optimal phosphorylation of LAT, one would expect that TCR partial agonists fail to induce LAT phosphorylation as previously suggested (23). We found that agonist ligands induced substantial ERK activation without significant phosphorylation of LAT. One of these could be Cbl, which can interact with Grb2 and with CrkL, potentially interfering with Ras activation (25–29). Under partial agonist conditions, ZAP-70 is recruited to the TCR but is not activated (17), LAT is not phosphorylated, and Cbl is not recruited/phosphorylated on LAT (Fig. 2a) (30). This may allow for recruitment of Ras-activating Grb2-SOS complexes to an alternative site such as phosphorylated TCR-ζ. According to our model, phospho-LAT would be required for agonist-induced Ras activation but not for partial agonist-induced Ras activation. We have recently obtained further evidence supporting this model using Jurkat T cells that lack functional ZAP-70 (p116 cells). In these cells, dimerization of CD3 induces substantial ERK activation without significant phosphorylation of LAT (M. L. Baroja and J. Madrenas, manuscript in preparation).

Where could Ras-activating complexes be recruited in the absence of phospho-LAT? A primary candidate as an alternative recruitment site of Grb2-SOS complexes in the absence of phospho-LAT is the differentially phosphorylated TCR-ζ chain induced by partial agonists (17, 24, 31–33). An association between phospho-TCR-ζ and Grb2 after TCR ligation has been reported previously (32, 34), and the association of Grb2 with TCR-ζ has been shown in vivo with constitutive expression of the p21 form of phospho-TCR-ζ and after abolishing the effect of CTLA-4 (35). Thus, we examined the recruitment of Grb2-SOS to phospho-TCR-ζ induced by agonists and partial agonists of the TCR. As shown in Fig. 3, TCR-mediated signaling in agonist pattern induced association between TCR-ζ and Grb2. However, under partial agonist conditions, there was a higher association between TCR-ζ and Grb2 than the one seen under agonist conditions (Fig. 3a). This was consistently detected after 10 min of T cell stimulation. Semi-quantitative analysis of three different experiments for the amount of Grb2 associated to TCR-ζ suggests that Grb2 associates to TCR-ζ twice as much under partial agonist conditions as under agonist conditions, and ~3 times above background levels. The difference could not be explained by differences in the amount of TCR-ζ immunoprecipitated because the amount of TCR-ζ immunoprecipitated from nonstimulated samples and from stimulated samples was similar (Fig. 3a). In addition, no detectable amount of TCR-ζ was immunoprecipitated by the stimulating chimeric anti-CD3e Abs in the absence of immunoprecipitating Ab coating agarose beads (Fig. 3b), indicating that detection of TCR-ζ in these samples is dependent on the immunoprecipitating Ab. The TCR-ζ-Grb2 association was also detectable after performing Grb2 immunoprecipitations and immunoblots for TCR-ζ (Fig. 3c). The increased association between Grb2 and TCR-ζ seen under partial agonist conditions correlated with predominant induction of the p21 form of phospho-TCR-ζ (Fig. 3d). In some experiments, we
found a low level of TCR-ζ-Grb2 association in nonstimulated cells that correlated with a low level of p21 phospho-ζ present in some batches of T cells. Thus, Grb2 is recruited to phospho-TCR-ζ when phosphorylated after TCR ligation either by Lck, by a Syk kinase (ZAP-70 or Syk) (36), or by Fyn (37). It can also interact with ZAP-70 (38), and potentially recruit Grb2 through its SH2 domain. The involvement of SHC as an intermediate molecule for the TCR-ζ-Grb2 interaction on TCR signaling by partial agonists is very attractive because SHC can bind TCR-ζ directly or indirectly through an intermediate adapter. Ravichandran et al. (34) originally claimed that such an intermediate adapter role could be played by SHC. This protein is tyrosine phosphorylated after TCR ligation either by Lck, by a Syk kinase (ZAP-70 or Syk) (36), or by Fyn (37). It can also interact with ZAP-70 (38), and potentially recruit Grb2 through its SH2 domain. The involvement of SHC as an intermediate molecule for the TCR-ζ-Grb2 interaction on TCR signaling by partial agonists is very attractive because SHC can bind TCR-ζ ITAM3 when phosphorylated only on the second tyrosine residue (39). This is one of the three tyrosine residues that is phosphorylated in response to TCR partial agonist ligands, the other two being the second tyrosine of the first ITAM and the first tyrosine residue on the second ITAM. Thus, we examined whether the association between Grb2 and TCR-ζ occurred through SHC. As shown in Fig. 4a, the magnitude of TCR-ζ-SHC association was similar under agonist and partial agonist conditions. On the other hand, the association between SHC and Grb2 was dependent on tyrosine phosphorylation of SHC (Fig. 4, b and c), with SHC being phosphorylated more under agonist conditions than under partial agonist signaling (32, 34). As expected, the more tyrosine-phosphorylated SHC, the more Grb2 was found in SHC immunoprecipitates (Fig. 4c).

On the basis of the previous data, we believe that the association between Grb2 and incompletely phosphorylated TCR-ζ after partial agonist induced signaling may occur, in part, independently from SHC. This is suggested by higher levels of Grb2 associated to TCR-ζ in partial agonist signaling (Fig. 3a) with a similar amount of SHC associated to TCR-ζ in agonist and partial agonist signaling (Fig. 4a). If the stoichiometry between Grb2 and SHC is 1:1, then this observation implies that some Grb2 may associate directly with monophosphorylated ITAM2 and -3 of TCR-ζ in addition to its binding to monophosphorylated ITAM1 (24). The role of SHC in the association between Grb2 and TCR-ζ and its dependency on tyrosine phosphorylation of SHC seen under partial agonist conditions is consistent with previous observations in TCR agonist signaling (24).

We have shown that Grb2 is proportionally recruited 5 times more to phospho-LAT than to phospho-TCR-ζ under agonist conditions. In contrast, Grb2 is recruited 3 times more to phospho-TCR-ζ than to LAT under partial agonist conditions. Despite these differences, both agonist and partial agonist signaling induced similar increase in the association of SOS to Grb2 (Fig. 5). This association is a necessary step for Ras activation (4, 40–42), further supporting a biological effect for both types of Ras-activating complex recruitment.

An additional molecule required for optimal activation of the PLC-γ1 and the Ras-MAPK pathways following TCR ligation with agonist ligands is SLP-76 (13). This is an adapter protein that is recruited to phospho-LAT, most likely through a Grb2-like adaptor (5, 43, 44). SLP-76 is also phosphorylated on multiple tyrosine residues after T cell stimulation (45), possibly by ZAP-70, likely determining its association with vav and nck and its effects on cytoskeletal reorganization (46). SLP-76-deficient T cells have a defect in PLC-γ1 and Ras-MAPK activation (13), and SLP-76 knockout mice have a severe block in early T cell development at...
FIGURE 4. Recruitment to the TCR complex, and tyrosine phosphorylation of SHC under agonist and partial agonist conditions of TCR-mediated signaling. Cell lysates (10^6 cells per group) from resting 3C6 T cells or after stimulation under agonist and partial agonist conditions were subjected to SHC immunoprecipitation and blotted for TCR-ζ (a). Densitometric units for each band are shown. The immunoprecipitates (ip) from a were subsequently blotted for phosphotyrosine content (b), and Grb2 (c). Values are representative of three independent experiments.

FIGURE 5. Association between Grb2 and SOS under agonist and partial agonist conditions of TCR-mediated signaling. Cell lysates from 3C6 T cells (10^6 cells/group) after stimulation with anti-CD3-fos Abs or anti-CD4-jun Abs were prepared and used for immunoprecipitation of SOS. These immunoprecipitates (ip) were subsequently blotted for phosphotyrosine content (a) and SOS (b) to confirm similar loading.

FIGURE 6. Partial agonist ligands of the TCR induce tyrosine phosphorylation of SLP-76. Cell lysates from 3C6 T cells (10^6 cells/group) after stimulation with anti-CD3-fos:anti-CD4-jun heterofunctional Abs or with anti-CD3-fos Abs or anti-CD4-jun Abs separately were prepared and used for immunoprecipitation of SLP-76. The resulting immunoprecipitates (ip) were immunoblotted sequentially for phosphotyrosine content (a) and SLP-76 (b) to confirm similar loading.

the level of double-negative thymocytes (47, 48). Interestingly, the defective activation of Ras in SLP-76-deficient T cells occurs despite normal LAT phosphorylation and abundant Grb2-SOS relocation to the cell membrane after TCR ligation. The fine mechanism by which this occurs is not clear, but it has been suggested that SLP-76 may contribute to the formation of the multimolecular complexes centered on LAT (13). We found that TCR partial agonists can induce low but significant tyrosine phosphorylation of SLP-76 (Fig. 6). It is unknown at this time how SLP-76 is phosphorylated under partial agonist conditions, and what kinase is responsible for such phosphorylation, because ZAP-70 is not active as a kinase under these conditions. One possibility is that SLP-76 could interact with one of the SH3 domains in Grb2 through a proline-rich domain in its N terminus, fulfilling the requirement of Grb2-SOS-SLP-76 complex formation in the membrane to activate Ras. This would obviate the need for phospho-LAT and be consistent with the absence of phospho-SLP-76 in the LAT immunoprecipitates from T cells under partial agonist conditions of stimulation (Fig. 2a). However, we were not able to demonstrate convincingly the presence of Grb2 in SLP-76 immunoprecipitates. One could argue that this is due to the association of SLP-76 to the Grb2-related adaptor Gads rather than to Grb2 as recently shown under agonist conditions of TCR-mediated signaling (44). Nevertheless, we believe that this scenario is unlikely because the Gads-SLP-76 association requires phospho-LAT.

The functional significance of Grb2-SOS association with phospho-TCR-ζ and its effects on Ras-MAPK activation have remained controversial (24). The discrepancies on these issues reported with the use of T cell clones and hybridomas may reflect distinct early TCR-mediated signaling in response to TCR engagement (24, 32, 34). In agonist type of signaling, phospho-TCR-ζ-Grb2-SOS association would not lead to additional Ras activation if LAT is phosphorylated. On the other hand, under partial agonist conditions of signaling, TCR-ζ-Grb2-SOS association would be able to activate the Ras-MAPK pathway. It is not known whether the involvement of SHC in the recruitment of Grb2 to TCR-ζ has functional implications different from those of direct recruitment of Grb2 to TCR-ζ.

Our data led us to propose that coordinate or selective activation of the Ras-MAPK pathway occurs after recruitment of Grb2-SOS complexes to phospho-LAT or to phospho-TCR-ζ in response to agonists or partial agonists of the TCR, respectively. TCR agonist ligands induce complete phosphorylation of TCR-ζ and subsequent phosphorylation of LAT. Under these circumstances, multimolecular complexes form on phospho-LAT, including Grb2-SOS, Cbl, PLC-γ1, and phosphatidylinositol 3-kinase. These complexes determine coordinate activation of the Ras-MAPK pathway concurrent with activation of other pathways such as the PLC-γ1 pathway. Some alternative recruitment of Ras-activating complexes to the TCR-ζ occurs under these conditions, but it may not be sufficient to activate the Ras-MAPK pathway because of interference/stabilization by ZAP-70-dependent activation of a negative regulator such as cbl (28, 29) (our preliminary observations). In contrast, TCR partial agonist ligands induce incomplete phosphorylation of TCR-ζ and fail to phosphorylate LAT. Under these conditions, there is recruitment of Grb2-SOS complexes only to the incompletely phosphorylated (p21) form of TCR-ζ. Contrary
to agonist conditions of T cell activation, alternative recruitment of Ras-activating complexes to incompletely phosphorylated TCR-ζ would be sufficient for selective activation of the Ras-MAPK pathway because the lack of ZAP-70 activation prevents from negative regulation of TCR-ζ-Grb2 association. This model emphasizes the role of linker molecules in determining the location of specific signaling complexes for coordinate activation of many signaling pathways, leading to the development of effector T cell responses (16). Thus, differential recruitment of some signaling complexes away from linker molecules may be a general regulatory mechanism of the pattern of activation of intracellular signaling pathways and enhance the versatility of signaling from that receptor.

Acknowledgments

We thank Ron Wange (Gerontology Research Center, National Institute of Aging, National Institutes of Health, Baltimore, MD) for critical reading of the manuscript, J. Tso (Protein Design Labs, Palo Alto, CA) for providing us with the heterofunctional Abs, and the members of the Madrenas laboratory for helpful comments and criticisms on the data presented here.

References