EphB6 is the most recently identified member of the Eph receptor tyrosine kinase family. EphB6 is primarily expressed in thymocytes and a subpopulation of T cells, suggesting that it may be involved in regulation of T lymphocyte differentiation and functions. We show here that overexpression of EphB6 in Jurkat T cells and stimulation with the EphB6 ligand, ephrin-B1, results in the selective inhibition of TCR-mediated activation of JNK but not the MAPK pathway. EphB6 appears to suppress the JNK pathway by preventing T cell receptor (TCR)-induced activation of the small GTPase Rac1, a critical event in initiating the JNK cascade. Furthermore, EphB6 blocked anti-CD3-induced secretion of IL-2 and CD25 expression in a ligand-dependent manner. Dominant negative EphB6 suppressed the inhibitory activity of the endogenous receptor and enhanced anti-CD3-induced JNK activation, CD25 expression, and IL-2 secretion, confirming the requirement for EphB6-specific signaling. Activation of the JNK pathway and the establishment of an IL-2/IL-2R autocrine loop have been shown to play a role in the negative selection of CD4+CD8+ self-reacting thymocytes. In agreement, stimulation of murine thymocytes with ephrin-B1 not only blocked anti-CD3-induced CD25 up-regulation and IL-2 production, but also inhibited TCR-mediated apoptosis. Thus, EphB6 may play an important role in regulating thymocyte differentiation and modulating responses of mature T cells.

Eph receptors are the largest family of receptor tyrosine kinases, with at least 14 members (1, 2). Ephs are activated by a group of ligands, all membrane-anchored either by glycosylphosphatidylinositol (ephrin-A1–A5) or a trans-membrane domain (ephrin-B1–B3) (3). Eph receptors are divided into two groups EphA and EphB according to their ligand binding preference; although within a group receptor-ligand specificity is degenerate (4). It is a characteristic of the Eph receptor family that their ligands must be membrane bound to be active (5–7). This requirement for membrane anchorage of the ligand makes cell-cell contact an obligatory event for activation of Eph receptors, and consequently, the activated receptors are concentrated in the area of cell-cell contact. In accordance with their membrane-anchored nature, ephrins are also involved in the process of reverse signaling. They interact with cytoplasmic signaling molecules and upon stimulation with appropriate receptors transmit signals inside the cell (8). Eph receptors and their ligands are typically most highly expressed in neural and endothelial cells (4), and most descriptions of their function concern development of the nervous system and angiogenesis (9–11). Upon the formation of cell-cell contact, signaling through the Eph receptors results in modulation of integrin activity and reorganization of the actin cytoskeleton. As a result, Ephs generate adhesive or repulsive signals, and in the neural system guide the movement of axonal growth cones, cell migration, and synapse formation (12–18).

Uncharacteristically, a recently discovered member of the Eph subfamily, EphB6, is predominantly expressed in the thymus and a subpopulation of mature T lymphocytes (19, 20). While structural analysis of EphB6 reveals conservation of the major Eph receptor autophosphorylation sites (Tyr-638 and Tyr-644), there are several critical alterations in its kinase domain. These include substitution of a crucial lysine residue in the ATP binding site, resulting in a receptor without detectable kinase activity (19, 21). We have previously shown that stimulation with ephrin-B1 induces EphB6 trans-phosphorylation by a catalytically active EphB partner and thus initiates its signaling (22). The predominant expression of EphB6 in the thymus (19) and a subset of T lymphocytes (20) indicates that it may play an important role in regulation of both T cell differentiation and function. Current evidence suggests that Eph receptors may interact with the T cell receptor (TCR) signaling pathways as Eph receptors can regulate the activity of small GTPases (23, 24) and thus control MAPK pathway and integrin activation, as well as cytoskeletal rearrangement (23, 25–27), all crucial in TCR-induced responses (28–35). Moreover, recent observations suggest that EphB6 interacts with a key member of TCR signaling pathways, c-Cbl (22, 36), and that cross-linking of EphB6 and CD3 with antibodies increases apoptotic cell death in Jurkat T cells (36).

In this paper, we demonstrate that stimulation of thymocytes with the EphB6 ligand ephrin-B1 (22) prevents major TCR-induced responses such as IL-2 secretion and up-regulation of IL-2 receptor α-chain (CD25) expression. In agreement with previously published data (37), the blockage of IL-2 receptor (IL-2R) signaling in thymocytes significantly inhibits TCR-
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mediated apoptotic cell death. This response to ephrin-B1 is most likely mediated by the EphB6 receptor as overexpression of EphB6 in Jurkat cells strongly suppresses TCR-mediated IL-2 cytokine secretion and IL-2RI expression, an effect further enhanced by ephrin-B1 stimulation. We show here that stimulation of Jurkat T cells with ephrin-B1 or overexpression of the EphB6 receptor results in the selective inhibition of TCR-mediated activation of JNK but not the MAPK pathway. EphB6 appears to suppress the JNK pathway by preventing TCR-induced amino acid metabolism of the small GTPase Rac1, a critical event in initiating the JNK cascade (38). The requirement for EphB6-specific signaling in modulation of TCR pathways was confirmed by the inability of the EphB6 receptor with its cytoplasmic domain deleted (ΔEphB6) to inhibit TCR-induced JNK activation and cellular responses. Moreover, ΔEphB6 demonstrated dominant negative properties and suppressed the inhibitory influence of the endogenous EphB6 receptor. In sum, these findings strongly suggest that the EphB6 receptor alters TCR-mediated responses through inhibition of the Rac1 GTPase.

MATERIALS AND METHODS

Antibodies and Recombinant Proteins—Monoclonal anti-phospho-tyrosine and PKA-1 PBD-agarose beads were obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). Antibodies to EphB6, Myc, phospho-MAPK, MAPK, JNK, Rac1, and Lck were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-phospho-JNK, anti-phospho-β70 S6 kinase, and anti-β70 S6 kinase were from New England Biolabs (Beverly, MA). Soluble ephrin-B1 and FGFR-Fc were purchased from R&D Systems. Anti-human CD3ε was purchased from Serotec (Oxford, UK) or BD Biosciences (Mississauga, Ontario, Canada).

Immunoprecipitation and Western Blotting—Cells were lysed in ice-cold 1% Triton X-100 lysis buffer. Antibodies and protein G-Sepharose were added to cleared lysates and incubated at 4 °C overnight. Immunoprecipitates were washed, separated by SDS-PAGE, and transferred to nitrocellulose (Amersham Biosciences). Membranes were blocked overnight at 4 °C in 7% non-fat milk (Bio-Rad, Richmond, CA) in PBS. Immunoblotting antibodies were added at optimal dilutions in PBS-T (0.1% Tween 20) at 4 °C. Bound antibodies were detected using horse-radish peroxidase-conjugated donkey anti-rabbit or sheep anti-mouse antibodies (Amersham Biosciences) and LumiGlo chemiluminescent reagents (Kirkgaard and Perry, Gaithersburg, MD).

Cloning and Mutation of EphB6—cDNA for EphB6, was cloned from normal human thymocyte RNA by reverse transcription-PCR into the expression vector pcDNA3 (Invitrogen) and sequenced. ΔEphB6-deletion of the cytoplasmic tail was created by PCR using cloned cDNA as the template. The resulting cDNA was cloned and sequenced to confirm the mutation. Myc-tagged EphB6 was generated by PCR by insertion of a Myc tag, and constructs were verified by sequencing. Expression of wild type proteins and mutants were examined by transfection in COS-7 cells and Western blotting with appropriate antibodies.

Transfection of Jurkat Cells—The human T-cell line Jurkat was transfected with empty pcDNA3, EphB6-M, or ΔEphB6. The Jurkat cells were electroporated in 400 μl complete RPMI medium with 30 μg of DNA by pulsing once for 65 ms at 260 V (BTX electrosorator; BTX, Division of Genetronics Inc., San Diego, CA). Cells were incubated at 37 °C for 24 h before addition of G418 to the medium. After 30 days of selection the resulting oligoclonal cell populations were screened by immunoprecipitation with anti-Myc and Western blotting with anti-Myc or anti-EphB6 and the EphB6- or ΔEphB6-expressing cell population was selected.

Isolation of Murine Thymocytes—Thymuses were obtained from female BALB/c mice, 5–7 weeks old. Mononuclear cells were isolated by Ficoll-Hypaque gradient centrifugation. Adherent cells were removed by incubation to plastic dishes for 60 min at 37 °C. The resulting thymocytes are typically >95% CD3. All animal-involved experiments were conducted according to Canadian federal regulations and Hospital of Sick Children instructions.

Stimulation with Ephrin-B1 and Anti-CD3—Ephrin-B1-Fc and anti-CD3 were immobilized at the concentration of 20 μg/ml, unless otherwise indicated, on plastic tissue culture dishes for 1.5 h at 37 °C and remaining soluble protein was removed by three washes with PBS.

Ephrin-B1 Stimulation Attenuates TCR-mediated Responses in Thymocytes—The EphB6 receptor was reported to be predominantly expressed in thymocytes (19). Complementary expression of its ligands was also detected in the thymus (4), suggesting that EphB6 may play a role in T cell differentiation. CD4+8+ double positive thymocytes that express productively rearranged TCRαβ are susceptible to repertoire selection. Double positive thymocytes reacting with self-MHC/antigen complex with high affinity are negatively selected and die by apoptosis. A TCR-induced IL-2/IL-2R autocrine loop, among other factors, contributes significantly to the induction of apoptosis in self-reacting double positive thymocytes (37). Stimulation of thymocytes with a high concentration of immobilized anti-CD3 can mimic this process and induce apoptosis in thymocytes (39). As expected, stimulation of murine thymocytes with immobilized anti-CD3-induced IL-2 secretion and up-regulation of IL-2R α chain (CD25) expression (Fig. 1, A and B). To examine the possible involvement of Eph receptors in the regulation of these responses we simultaneously stimulated thymocytes with the EphB6 ligand ephrin-B1. Dimeric ephrin-B1 fused to the Fc domain of human IgG was immobilized to mimic its membrane-bound nature. Purified human IgG was used in all points without ephrin-B1 as a specificity control. Co-stimulation with ephrin-B1 strongly inhibited both TCR-mediated IL-2 secretion and CD25 up-regulation (Fig. 1, A and B). The inhibition of T cell response was ephrin-B1 concentration-dependent and ligand immobilization at 2–5 μg/ml was sufficient for significant suppression of TCR-mediated responses (Fig. 1C). An additional specificity control with irrelevant FGFR-Fc fusion protein had no effect even when immobilized at 10 μg/ml (Fig. 1C). Consistent with its inhibitory effect on TCR-mediated induction of the IL-2/IL-2R autocrine loop in thymocytes, ephrin-B1 treatment strongly inhibited TCR-initiated apoptosis (Fig. 1D). Thus, ephrin-B1 and its receptors, potentially including the highly expressed EphB6, can negatively modulate TCR-induced responses in thymocytes.

The EphB6 Receptor Inhibits TCR-induced IL-2 Secretion and CD25 Expression—A high level of EphB6 receptor expression has been reported not only in thymocytes, but also in a subpopulation of mature T cells and in the T cell line Jurkat (20, 36). The ligands for the EphB6 receptor, ephrin-B1 and ephrin-B2 (22, 40), are expressed in most organs and cell types (4), and therefore the potential for T cells to be activated through EphB6 upon cell-cell contact is high. The persistent expression of EphB6 across the T cell lineage, combined with
the practically ubiquitous expression of its ligands, suggests that EphB6 might not be important only during differentiation but also in mature T cell function.

T lymphocyte homeostasis is precisely regulated with numerous TCR co-stimulatory events required for finely tuned control of cell fate, these signals regulating both proliferative and apoptotic pathways (41). In particular, stimulation of the TCR can lead to the induction of IL-2 production and CD25 (IL-2R/α) expression, resulting in an autocrine loop and thus the clonal expansion of activated T cells. To examine whether the EphB6 receptor, specifically, could down-regulate TCR-mediated induction of IL-2 and IL-2R, we generated stable overexpression of Myc-tagged EphB6 in the mature T cell line Jurkat (B6-J cells) (Fig. 2A). We have previously determined that upon stimulation with ephrin-B1, EphB6 undergoes tyrosine phosphorylation by catalytically active Eph receptors (22). As a test for functionality of the transfected EphB6 receptor, the oligoclonal B6-J cell line was stimulated with ephrin-B1, and the overexpressed receptor was indeed observed to undergo tyrosine phosphorylation (Fig. 2B).

To determine if the EphB6 receptor could influence responses to TCR activation, pcDNA3-transfected control and B6-J cells were stimulated with immobilized anti-CD3 and ephrin-B1 and the secretion of IL-2 was examined by ELISA. In agreement with recent observations, immobilized anti-CD3 was sufficient to induce IL-2 expression (38, 42). However, a significant inhibition of anti-CD3-induced IL-2 production was observed upon stimulation of control cells with ephrin-B1 (Fig. 3A) in accordance with the high level of endogenous EphB6.
expression reported in those cells. Furthermore, the overexpression of EphB6 strongly suppressed induction of IL-2 secretion by anti-CD3 in B6-J cells. This inhibitory effect was further enhanced upon ephrin-B1 stimulation.

The induction of CD25 expression in control and B6-J cells was also examined (Fig. 3B). Once again, a significant inhibition of anti-CD3-induced CD25 up-regulation was observed upon both ephrin-B1 stimulation and overexpression of EphB6. In addition to using human IgG as a control for the human Fc portion of the recombinant ephrin-B1, an irrelevant fusion protein control (FGFR-Fc) was included (Fig. 3C). In sum, these findings show that the EphB6 receptor can inhibit TCR-mediated IL-2 secretion and CD25 expression in mature T cells, suggesting that EphB6 may be responsible for the similar pattern of inhibition induced by ephrin-B1 in thymocytes.

Selective Inhibition of JNK Activation by the EphB6 Receptor—The JNK pathway is one of the major signaling cascades initiated upon TCR activation (35, 38, 39, 43). To determine if the EphB6 receptor could down-regulate TCR-mediated activation of the JNK pathway, we treated control pcDNA3 cells and B6-J cells with anti-CD3 alone or in combination with increasing concentrations of ephrin-B1 and determined the activation status of JNK by Western blotting with anti-phospho-JNK. Stimulation with immobilized anti-CD3 induced JNK activation in agreement with recent findings using pre-complexed anti-CD3 (38). Anti-CD3-induced p54Jun kinase phosphorylation was prominent in control cells but reduced upon stimulation with ephrin-B1 in a concentration-dependent manner. Induction of JNK phosphorylation was also significantly inhibited in B6-J cells and further down-regulated upon ephrin-B1 stimulation.
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Fig. 5. Overexpression of EphB6 does not affect MAPK phosphorylation. A, B6-J and pcDNA3-transfected control cells were stimulated with anti-CD3, ephrin-B1 were immobilized at 20 μg/ml as indicated, and phosphorylation of MAPK was detected by Western blotting with anti-phospho-MAPK. The presence of MAPK was confirmed by Western blotting with anti-MAPK. Densitometry analysis of the MAPK phosphorylation blots are presented in arbitrary units. B, phosphorylation of p70 S6 kinase on Thr-421/Ser-424 residues was examined by Western blotting with anti-phospho-p70S6K. Expression of p70S6K was confirmed by reblotting with anti-p70S6K. In all experiments human IgG was immobilized at the appropriate concentrations as a specificity control.

In addition to inducing JNK activation, TCR stimulation also results in the initiation of the MAPK signaling pathway, leading to phosphorylation of MAPK and its consequent activation. As certain Eph receptors have been previously reported to down-regulate activation of the MAPK pathway in non-lymphoid cells (25, 26), we examined the influence of the EphB6 receptor on anti-CD3-induced MAPK phosphorylation. Remarkably, attenuation of anti-CD3-initiated signaling by EphB6 appeared to specifically target the JNK pathway as TCR-mediated MAPK phosphorylation was not affected by the EphB6 receptor either (Fig. 5B).

Thus, EphB6 appears to be a specific negative regulator of TCR-mediated Rac1 and JNK pathway activation, suggesting that the down-regulation of TCR-induced IL-2 secretion upon ephrin-B1 stimulation or EphB6 overexpression probably results from the selective inhibition of the Rac1 GTPase.

EphB6-specific Signaling Is Required for the Suppression of TCR Responses—To confirm that the inhibitory effects of EphB6 overexpression were specific to the EphB6 receptor and did not result from reverse signaling through ephrin-B ligands (potentially expressed in Jurkat cells) or from nonspecific interference with the signaling of other Eph receptors, we compared the consequences of overexpressing wild type and cytoplasmic domain-deleted (ΔEphB6) forms of EphB6 (Fig. 6A). While oligoclonal B6-J populations were utilized in previous experiments, this should also eliminate the possibility that the effects observed with B6-J cells were due to clonal variation. The functional status and expression level of ΔEphB6 was verified by the ability of ΔEphB6-transfected Jurkat cells (ΔB6-J) to bind recombinant ephrin-B1-Fc ligand, which was greater than control cells and equivalent to B6-J cells (not shown). Equal CD3ε expression on B6-J, ΔB6-J, and pcDNA3 cells was confirmed by flow cytometry (not shown). In contrast to the overexpression of wild-type EphB6, expression of ΔEphB6 did not inhibit TCR-mediated responses. Moreover, although we did not detect any increase in Vav-Rac1 association in ΔB6-J (not shown), probably due to low sensitivity of the assays, ΔB6-J cells demonstrated significantly increased responses to anti-CD3 stimulation relative to control cells (Fig. 6, A, C, and D). This suggests that ΔEphB6 may act as dominant negative toward endogenous EphB6 receptors, releasing the cell from their basal inhibitory effects. In agreement, ΔEphB6 also reduced to varying degrees the inhibitory effects of ephrin-B1 stimulation. In sum, these findings strongly suggest that an EphB6-specific signaling cascade is responsible for the attenuation of TCR-mediated responses.

DISCUSSION

Unusually for a receptor tyrosine kinase, and particularly for an Eph receptor, EphB6 is most highly expressed in the T cell lineage (19, 20), and recent findings suggested a potential role for EphB6 in modulating T-cell responses (36). We have shown here that both the EphB6 receptor and its ligand ephrin-B1 can regulate TCR-induced IL-2 secretion and IL-2R α-chain expression in T lymphocytes. Ephrin-B1 treatment and EphB6 overexpression also inhibited stimulation of the JNK pathway in anti-CD3-activated cells. The inhibitory properties of EphB6 were confirmed by the ability of a truncated EphB6 receptor, missing its cytoplasmic portion, to suppress the inhibitory activity of the endogenous EphB6 receptor. The inhibition of TCR responses by EphB6 specifically targeted the JNK pathway as essentially no effect on another major signaling cascade, the MAPK pathway, was observed upon either increased EphB6 expression or ephrin-B1 stimulation. Similarly there was no inhibition of anti-CD3-induced p70 S6 kinase phosphorylation. While there is evidence to suggest that production of IL-2 upon TCR engagement requires activation of the JNK signaling cascade (38, 39, 43), contradictory reports also exist and the precise role of JNK in T cells is currently controversial (35).
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Fig. 6. Cytoplasmic domain-deficient EphB6 does not inhibit TCR-mediated responses. A, stable expression of truncated EphB6. Jurkat T cells were transfected with Myc-tagged ΔEphB6 (EphB6 with the cytoplasmic domain deleted) in the pcDNA3 expression vector. Selection was performed as in Fig. 2A. Expression of the ΔEphB6 receptor in the resulting cell population (ΔB6-J) was confirmed by immunoprecipitation with anti-Myc and Western blotting with anti-EphB6. B, ΔEphB6 does not prevent induction of CD25 expression. B6-J, ΔB6-J, and pcDNA3 cells were stimulated and analyzed as Fig. 2B. C, IL-2 secretion is not inhibited by ΔEphB6. Cells were stimulated as in Fig. 2A, and conditioned media were analyzed for IL-2 by ELISA. D, expression of ΔEphB6 does not inhibit the JNK pathway. Cells were stimulated with immobilized anti-CD3 and ephrin-B1 as indicated, and phosphorylation of JNK was examined by Western blotting of cell lysates with anti-phospho-JNK. Equal protein loading was confirmed by Western blotting with the protein-specific antibodies (not shown). Blots were analyzed by densitometry, and the results for each cell line were presented as a percentage relative to its unstimulated control. Human IgG was immobilized at 20 µg/ml as a specificity control in all ephrin-negative points.

activation. This also raises an alternative model of EphB6 action, whereby suppression of TCR-mediated IL-2 secretion may not be the consequence of blockage of the JNK pathway, but rather IL-2 secretion and JNK activation may be downregulated independently as the result of the inhibition of the Rac1 GTPase. While we were preparing this manuscript findings were published, demonstrating multiple effects of Vav1 deficiency on various anti-CD3-induced responses in Jurkat (44). Interestingly, activation of JNK and the IL-2 promoter were strongly inhibited in Vav1-deficient cells, while anti-CD3-initiated MAPK phosphorylation was unaffected. These observations essentially mimic the effects of ephrin-B1 stimulation and EphB6 overexpression on anti-CD3-induced responses observed in our experiments in Jurkat. This strongly, although indirectly, supports our model suggesting that the EphB6 receptor may inhibit certain TCR-mediated responses through the control over Rac1 activity.

The precise mechanism by which EphB6 regulates Vav-Rac1 interaction remains unclear. Overexpression of the EphB6 receptor did not appear to affect Vav tyrosine phosphorylation or its association with c-Cbl (not shown). However, we and others have demonstrated that EphB6 is constitutively associated with c-Cbl (22, 36), and as Cbl directly interacts with Vav upon TCR engagement and is required for the activation of another small GTPase Rap1 (45), EphB6 could potentially control Vav-Rac1 interaction through regulation of Cbl activity.

In mature T cells blockage of the JNK pathway was reported to enhance TCR-induced Fas-mediated apoptosis (46). In agreement with the EphB6-mediated inhibition of the JNK pathway observed in our experiments, stimulation of Jurkat T cells with an anti-EphB6 antibody was recently reported to increase anti-CD3-induced Fas-mediated apoptosis (36). Supporting this observation, we found that overexpression of EphB6 not only prevented TCR-induced IL-2 secretion but also increased the initiation of Fas-dependent apoptotic cell death in an ephrin-B1-dependent manner (not shown). Thus, one of the functions of EphB6 in mature T cells may be control over the clonal expansion of antigen-activated T lymphocytes through suppression of Rac1 activation and subsequent inhibition of the JNK pathway and IL-2 secretion, leading to the induction of apoptosis without preceding proliferation.

Clearly, whenever an EphB6-positive T lymphocyte encounters and interacts with a cell expressing ephrins complementary to the EphB6 receptor, signaling through the TCR has the potential to be modified as a consequence of simultaneous EphB6-ephrin ligation. The EphB6 ligands ephrin-B1 and ephrin-B2 are widely expressed throughout the body and therefore only EphB6 expression by T cells should be a limiting factor in this interaction. This potentially means that the decision to express EphB6 or not is equivalent to a decision to proliferate or die upon subsequent TCR stimulation. We propose that in mature T cells, EphB6 may either help to eliminate potentially harmful clones or simply control clone expansion.

Thymocytes that reach the double positive (CD4+8+) stage of maturation and express a successfully rearranged TCR undergo positive or negative selection in the cortical area of thymus. Thymocytes that express a TCR that interacts weakly with self-MHC/antigen are positively selected, whereas strongly activated thymocytes undergo negative selection. The formation of an IL-2/IL-2R autocrine loop appears to be one of the crucial events in initiation of apoptosis in negative selection (37). By definition, organization of an autocrine signaling loop depends strictly on both ligand secretion and expression of a complementary receptor. Stimulation with ephrin-B1 appeared to block TCR-mediated induction of both IL-2 secretion and IL-2R α-chain expression in thymocytes. As expected, anti-CD3-induced apoptosis was similarly disrupted. Since each ephrin can activate multiple members of the Eph family, we cannot be absolutely certain that the EphB6 receptor was pre-
dominantly responsible for the transduction of ephrin-B inhibitory signals in thymocytes. However, EphB6 is activated by stimulation with ephrin-B1 (12), and the highest level of EphB6 expression is found in thymocytes (19). Furthermore, overexpression of EphB6 enhances ephrin-B1–induced inhibition of TCR-mediated responses in Jurkat cells, strongly suggesting that EphB6 is likely to be the primary ephrin-B1 effector in thymocytes.

It is possible that one of the functions of the EphB6 receptor and its ephrin-B ligands in the thymus is to modulate TCR signaling in a manner that prevents induction of apoptosis by low affinity TCR interactions but does not affect TCR survival signals. This possibility is supported by our observation that in thymocytes (similarly to Jurkat cells) ephrin-B1 stimulation, although inhibiting IL-2 secretion, does not significantly affect TCR-mediated MAPK pathway activation (not shown), a major survival signal in CD4+ TCR-mediated responses in Jurkat cells, strongly suggesting that EphB6 is likely to be the primary ephrin-B1 effector in thymocytes.

In T cells, survival signals in CD4+ TCR-mediated MAPK pathway activation (not shown), a major survival signal in CD4+ T cells (35). Complementing these observations, expression of the EphB6 receptor was found by in situ hybridization analysis to be restricted to the cortex in both murine and human thymus (36) where CD4+ T cells predominate and selection takes place. In sum, these findings suggest that in thymocytes stimulation with EphB6 receptor ligands may allow suboptimal TCR stimulation to switch on survival signals without triggering apoptotic events. Thus, the ability of the EphB6 receptor to inhibit TCR-mediated JNK activation and IL-2/IL-2R production without a significant effect on the MAPK pathway may serve to mark the fine line between the positive and negative selection of CD4+ T cells.

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REFERENCES
1. van der Geer, P., Hunter, T., and Lindberg, R. A. (1994) Ann. Rev. Cell Biol. 10, 251–297
2. Pasquale, E. B. (1997) Curr. Opin. Cell Biol. 9, 668–615
3. Drescher, U. (1997) Curr Biol. 7(12)
4. Zhou, R. (1998) Pharmacol. Ther. 77, 151–181
5. Davis, S., Gale, N. W., Aldrich, T. H., Maisonnier, P. C., Lhotak, V., Pawson, T., Goldfarb, M., and Yangopoulos, G. D. (1994) Science 266, 816–819
6. Sakanoe, S., Serizawa, R., Isada, T., Iwama, A., Ito, A., Kato, C., Shimizu, Y., Shinoki, F., Shimizu, R., Kondo, S., Ohno, M., and Suda, T. (1996) Oncogene 13, 813–822
7. Winslow, J. W., Morgan, P., Valverde, J., Shih, A., Yuan, J. Q., Wong, S. C., Tsai, S. P., Goddard, A., Henzel, W. J., Hefi, F. et al. (1995) Neuron 14, 973–981
8. Boyd, A. W., and Lackmann, M. (2001) Science’s STKE, http://stke.sciencemag.org/content/full/stke2001112re20
9. Frisen, J., Holmberg, J., and Barbacid, M. (1999) EMBO J. 18, 5159–5165
10. Wilkinson, D. G. (2001) Nat. Rev. Neurosci. 2, 155–164
11. Cheng, N., Brandley, D. M., and Chen, J. (2002) Cytokine Growth Factor Rev.
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