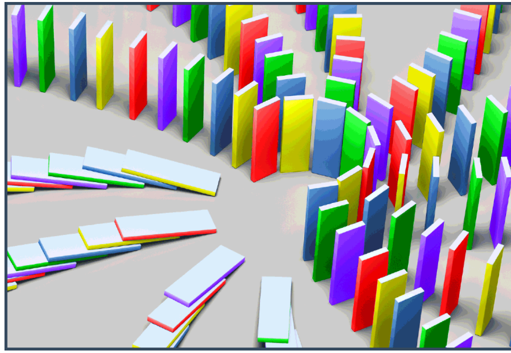

HUMAN & MOUSE CELL LINES

Engineered to study multiple immune signaling pathways.

Transcription Factor, PRR, Cytokine, Autophagy and COVID-19 Reporter Cells
ADCC , ADCC and Immune Checkpoint Cellular Assays

The Journal of Immunology

RESEARCH ARTICLE | FEBRUARY 01 2001

p59^{fyn} (Fyn) Promotes the Survival of Anergic CD4$^-$CD8$^-$ αβ TCR$^+$ Cells but Negatively Regulates Their Proliferative Response to Antigen Stimulation[1]

FREE

Oliver Utting; ... et. al

J Immunol (2001) 166 (3): 1540–1546.
https://doi.org/10.4049/jimmunol.166.3.1540

Related Content

Anergic Th1 cells express altered levels of the protein tyrosine kinases p56lck and p59fyn.

J Immunol (November,1992)

Prostaglandin E_2 Modulation of p59^{fyn} Tyrosine Kinase in T Lymphocytes During Sepsis

J Immunol (January,1998)

The proliferative in vivo activities of lpr double-negative T cells and the primary role of p59fyn in their activation and expansion.

J Immunol (September,1997)

Downloaded from http://journals.aai.org/jimmunol/article-pdf/166/3/1540/1138831/1540.pdf by guest on 01 March 2024

p59^{fyn} (Fyn) Promotes the Survival of Anergic CD4$^-$CD8$^-$ $\alpha\beta$ TCR$^+$ Cells but Negatively Regulates Their Proliferative Response to Antigen Stimulation[1]

Oliver Utting, John J. Priatel, Soo-Jeet Teh, and Hung-Sia Teh[2]

T cell anergy is characterized by alterations in TCR signaling that may play a role in controlling the unresponsiveness of the anergic cell. We have addressed questions regarding the importance of the Src kinase p59^{fyn} (Fyn) in this process by using Fyn null mice. We demonstrate that a mature population of CD4$^-$CD8$^-$ $\alpha\beta$ TCR$^+$ anergic T cells lacking Fyn have a substantial recovery of their proliferation defect in response to Ag stimulation. This recovery cannot be explained by ameliorated production of IL-2, and the improved proliferation correlates with an enhanced ability of the Fyn$^{-/-}$ anergic T cells to up-regulate the high affinity IL-2 receptor. We also observe that anergic CD4$^-$CD8$^-$ $\alpha\beta$ TCR$^+$ T cells have a heightened survival ability that is partially dependent on the elevated levels of Fyn and IL-2 receptor β-chain expressed by these cells. The enhanced survival correlates with an increased capacity of the anergic cells to respond to IL-15. We conclude that Fyn plays an important role in aspects of T cell anergy pertaining to TCR signaling and to cell survival. ***The Journal of Immunology,* 2001, 166: 1540–1546.**

Downloaded from http://journals.aai.org/jimmunol/article-pdf/166/3/1540/1138831/1540.pdf by guest on 01 March 2024

Maintenance of immune system self tolerance is essential, with tolerance being induced either in the thymus or in the periphery. Failures in self tolerance are critical to the induction of autoimmune diseases; therefore, an understanding of tolerance is key to disease prevention and treatment. The processes of peripheral tolerance encompass either the deletion (1–3) or inactivation of autoreactive cells (4, 5), with clonal ignorance also potentially playing a role (6). The inactivation of T cells is termed anergy and involves the induction of a state characterized by failure to proliferate or produce IL-2 in response to antigenic ligand (7).

The mechanisms controlling T cell anergy in conventional CD4$^+$CD8$^-$ and CD4$^-$CD8$^+$ $\alpha\beta$ TCR$^+$ cells are not well understood. Signaling defects associated with the anergy of these cells seem to vary depending on the precise protocol used for its induction. Alterations in activation of either the Ras pathway or calcium pathway typify T cell anergy, and anergic T cells generally have signaling defects proximal to the TCR (reviewed in Ref. 8). In vivo anergized T cells exhibit defects in the phosphorylation of 70-kDa zeta-associated protein (ZAP-70),[3] recruitment of ZAP-70 to the TCR, and phosphorylation of a 36-kDa protein (9). These alterations in TCR signaling have been shown to correlate with changes in the constitutive protein expression levels of p56^{lck} (Lck) and p59^{fyn} (Fyn), with Lck decreased by between 50 and 90% and Fyn increasing 3- to 4-fold (10, 11). Recent demonstrations have shown that preferential TCR signaling via Fyn results in reduced ZAP-70 activation, reduced phosphorylation of linker for the activation of T cell (LAT), normal 76-kDa SH2-containing leukocyte phosphoprotein phosphorylation and CD69 induction, and impaired IL-2 production and cell growth (12). The association of Fyn with the CD3 ζ-chains has also been shown to be important in anergy induction (13), with Fyn phosphorylating the CD3 ζ-chains but not the γ-, δ-, or ϵ-chains.

Recently, we described an in vivo system for the generation of anergic CD4$^-$CD8$^-$$\alpha\beta$ TCR$^+$ T cells (14, 15). This system is based on the transgenic $\alpha\beta$ 2C TCR (16, 17). The 2C TCR is specific for the p2Ca peptide (derived from the mitochondrial protein 2-oxoglutarate dehydrogenase) presented by L^d MHC class I molecules (18, 19) and is positively selected by K^b MHC class I molecules (20). The 2C TCR binds to the p2Ca/L^d ligand with a relatively high affinity ($K_A = 2 \times 10^6$ M^{-1}) (21) and to the p2Ca/K^b ligand with a very low affinity ($K_A = 3 \times 10^3$ M^{-1}) (21). This anergy model is based on a CD4$^-$CD8$^-$ T cell population of peripheral T cells expressing the 2C TCR (herein referred to as DN cells). The development of these DN cells is thymus dependent (22) and does not require the expression of class I MHC molecules (22, 23). They are resistant to clonal deletion in Ag-expressing mice (23, 24). The DN cells recovered from Ag-expressing mice are defined as anergic because they hypoproliferate and produce little or no IL-2 in response to Ag stimulation when compared with DN cells from Ag-free mice (14). Biochemical analysis (15) of the early intracellular signaling events of these anergized DN T cells indicates they have a signaling defect at the level of ZAP-70 and LAT phosphorylation. They also exhibit a defect in mobilization of intracellular calcium upon TCR engagement. However, in contrast to conventional CD4 or CD8 anergic $\alpha\beta$ TCR$^+$ T cells, these anergic DN cells demonstrate no phosphorylation defects in 76-kDa SH2-containing leukocyte phosphoprotein or extracellular signal-regulated kinase 1/2 (ERK1/2).

We have demonstrated that Fyn expression is elevated in this in vivo anergy model (15). To determine the importance of Fyn in the

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

Received for publication August 14, 2000. Accepted for publication October 31, 2000.

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

[1] This work was supported by grants from the Medical Research Council of Canada and the Arthritis Society of Canada. O.U. is a recipient of a studentship from the Medical Research Council of Canada. J.J.P. is a recipient of a Canadian Institute of Health Research Fellowship.

[2] Address correspondence and reprint requests to Dr. Hung-Sia Teh, Department of Microbiology and Immunology, University of British Columbia, Room 300, 6174 University Boulevard, Vancouver, British Columbia, Canada V6T 1Z3. E-mail address: teh@interchange.ubc.ca

[3] Abbreviations used in this paper: ZAP-70, 70-kDa zeta-associated protein; Fyn, p59^{fyn}; Lck, p56^{lck}; LAT, linker for the activation of T cell; DN cells, CD4$^-$CD8$^-$ T cell population of peripheral T cells expressing the 2C TCR; 7-AAD, 7-amino actinomycin D; γ_c, common γ-chain; IL-2Rβ, IL-2 receptor β-chain.

Copyright © 2001 by The American Association of Immunologists 0022-1767/01/$02.00

induction of anergy and maintenance in DN cells, we compared the function of anergized DN cells recovered from wild-type or Fyn$^{-/-}$ mice. Our results indicate that Fyn plays a role in T cell anergy, as anergized T cells lacking Fyn partially recover from their proliferation defect. Fyn also appears to play a role in the survival of anergic T cells. Loss of Fyn from these cells results in decreased survival that is correlated with a failure to up-regulate IL-2 receptor β-chain (IL-2Rβ) and respond to IL-15. Our data suggest a dual function for Fyn in regulating Ag responsiveness and survival of anergic DN cells.

Materials and Methods

Mice

Breeders for the H-2^b 2C TCR-transgenic mice (16, 17) were provided by Dr. Denis Loh (then at Washington University, St. Louis, MO). The H-2^b 2C TCR-transgenic mice were backcrossed to the C57BL/6 background. DBA/2 (H-2^d) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). H-2$^{b/d}$ 2C mice were F_1 mice obtained by mating DBA/2 mice with H-2^b 2C TCR mice. Fyn$^{-/-}$ mice, on a mixed 129 (H-2^b) and C57BL/6 background, were provided by Dr. R. Perlmutter (then of the Howard Hughes Medical Institute, University of Washington, Seattle, WA) (25). TCR-transgenic mice and Fyn$^{-/-}$ mice were mated to produce transgenic animals with the Fyn mutation. All animals were maintained in the animal facility at the University of British Columbia in the Microbiology and Immunology building.

Cells

Lymph node cells were harvested from transgenic mice. Purification of DN cells was as described previously (14). The purified DN cells were typically >95% CD4$^-$CD8$^-$Ig$^-$ and expressed exclusively the 2C TCR, which was detected by the 1B2 mAb (26). The peptide transporter-deficient cell lines T2-L^d and T2-K^b (27) were derived by transfecting the human (T × B) hybridoma T2 with L^d or K^b. The T2-L^d or T2-K^b cells were used as APC for the p2Ca peptide. The p2Ca peptide (LSPFPFDL) was synthesized by the Nucleic Acid Service Laboratory at the University of British Columbia. Cells were cultured in IMDM (Life Technologies, Burlington, Ontario, Canada) supplemented with 5×10^{-5} M 2-ME, 10% FCS (Life Technologies), and antibiotics.

Proliferation assays

Proliferation assays were performed by culturing 1×10^4 purified DN cells with 5×10^4 mitomycin C-treated T2-L^d or T2-K^b cells with indicated concentrations of the p2Ca peptide. Cells were cultured in triplicate in a volume of 0.20 ml in 96-well round-bottom plates. For assessment of proliferation, 1 μCi of [^{3}H]thymidine was added to each culture well in the last 6 h of a 72-h culture period.

Abs and cytokines

Abs used include the following: Anti-CD4 (GK1.5), anti-CD8 (53.67), and anti-CD25 (PC61) were obtained from American Type Culture Collection (Manassas, VA). Anti-CD69 (catalog no. 01502D) and anti-IL-2Rβ (catalog no. 55336) were obtained from PharMingen (San Diego, CA). For blocking experiments the anti-IL-2Rβ Ab was first extensively dialyzed against PBS to remove sodium azide. The 1B2 hybridoma, which produces the idiotypic mAb to the 2C TCR, was a gift from Dr. Eisen (Massachusetts Institute of Technology, Cambridge, MA) (26). Recombinant mouse IL-2 was purchased from PharMingen (catalog no. 19211T; San Diego, CA). Recombinant human IL-15 (catalog no. 247-IL) was purchased from R&D Systems (Minneapolis, MN).

CD69, CD25, and IL-2Rβ flow cytometry

Single-cell suspensions of lymph node cells were prepared. Purified DN cells (1×10^5) were stimulated with 1×10^5 mitomycin C-treated T2-L^d or T2-K^b cells plus the indicated concentration of the p2Ca peptide in a 96-well plate in a volume of 0.20 ml. No exogenous IL-2 was added. After a culture period of 18 h, the cells were collected and stained with biotinylated anti-CD69 or anti-CD25 mAb followed by streptavidin-PE Cy5 (Cedarlane Laboratories, Hornby, Ontario, Canada) and analyzed with the FACScan flow cytometer using CellQuest software (Becton Dickinson, Mountain View, CA). A total of 15,000 events were analyzed for each sample.

7-Amino actinomycin D (7-AAD) staining

7-AAD (catalog no. 129935) was purchased from Calbiochem-Novabiochem (La Jolla, CA). Purified DN cells were cultured for the indicated time, washed and stained with 7-AAD, and analyzed with the FACScan flow cytometer using CellQuest software (Becton Dickinson). Samples were collected in triplicate, and the percentage of live cells (cells not stained with 7-AAD) was calculated. Cell survival was scored as (% live cells/% live cells at time 0) × 100. SD values were calculated as the square root of the variance. The variance was calculated as $((x_1 - \text{xbar})^2 + (x_2 - \text{xbar})^2 + (x_3 - \text{xbar})^2/(n - 1)$ where xbar is equal to the average of x_1 through x_3. The z test was used to determine whether results were statistically significant. This was calculated as follows: $(x_1 - x_2)/\text{root}\ ((s_1{}^2/n_1) + (s_2{}^2/n_2))$ where s_1 equals the SD of set 1, and n_1 equals the number of values in this set. The values were deemed statistically different with a z score of greater than 4.5, indicating 99.99% confidence.

RT-PCR

RNA was prepared from DN cells stimulated for 9 h. Briefly, the stimulation was as follows: 1×10^5 DN cells were stimulated with 5×10^4 T2-L^d APCs + 1 μM p2Ca in 0.2-ml wells. Twenty wells were pooled, and RNA was prepared using the Qiagen RNeasy kit (catalog no. 74104; Valencia, CA) according to the manufacturer's instructions. RNA was quantified and reverse transcribed, and cDNAs were normalized for HPRT content using competitive PCR (28). Using normalized samples, IL-2 message was quantified using the pPQRS competitors (28). The following primers were used: 5′ IL-2, 5′-CCACTTCAAGCTCTACAGCGGAAG-3′; 3′ IL-2, 5′-GAGTCAAATCCAGAACATGCCGCA-3′; 5′ HPRT, 5′-GTTGGATACAGGCCAGACTTTGTTG-3′; and 3′ HPRT, 5′-GAGGGTAGGCTGGCCTATAGGCT-3′.

Results

The deletion of Fyn from anergic DN cells leads to partial recovery of their proliferation defect

T cell anergy is generally defined as the failure of T cells to proliferate and produce IL-2 in response to stimulation with their cognate antigenic ligand (7). We compared the proliferative responses of H-2^b and H-2$^{b/d}$ DN cells with and without Fyn to H-2L^d and the p2Ca peptide in a standard proliferation assay (Fig. 1). The response of H-2^b and H-2^b Fyn$^{-/-}$ DN cells reveals that cells lacking Fyn were at a disadvantage in the proliferation assay, requiring between 100 and 1000 times more peptide to yield the same cpm as their Fyn-expressing counterparts, a result similar to what we have observed for CD4$^-$CD8$^+$1B2$^+$ T cells (29). Conversely, the H-2$^{b/d}$ Fyn$^{-/-}$ cells clearly outperform the Fyn-expressing H-2$^{b/d}$ cells. The phenomenon also held true when the cells were stimulated with the low affinity p2Ca/K^b ligand. These results demonstrate that Fyn does play a role in in vivo induced T cell anergy. To address whether the role of Fyn in T cell anergy is restricted to TCR signaling or whether it extends to other aspects of T cell biology, we designed the following experiments.

H-2$^{b/d}$ Fyn$^{-/-}$ DN cells do not have improved IL-2 production

We have previously demonstrated that the H-2$^{b/d}$ DN cells have impaired IL-2 production compared with DN cells from H-2^b mice (14). One possible explanation for the improved proliferation of the H-2$^{b/d}$ Fyn$^{-/-}$ DN cells could be an improved ability to produce IL-2 compared with their Fyn-expressing counterpart. Therefore, we examined the ability of the H-2$^{b/d}$ Fyn$^{-/-}$ cells to produce IL-2 mRNA using a competitive RT-PCR method (28). However, contrary to this prediction, we were unable to detect any difference in IL-2 transcription between the H-2$^{b/d}$ cells with or without Fyn (Fig. 2). Interestingly, we did observe a reduction in the ability of the H-2^b Fyn$^{-/-}$ cells to produce IL-2 mRNA (Fig. 2) which may, at least in part, explain their decreased proliferation observed in Fig. 1. As we were unable to detect any increased production of IL-2 by the H-2$^{b/d}$ Fyn$^{-/-}$ DN cells we sought alternative explanations for their enhanced proliferation.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/166/3/1540/1138831/1540.pdf by guest on 01 March 2024

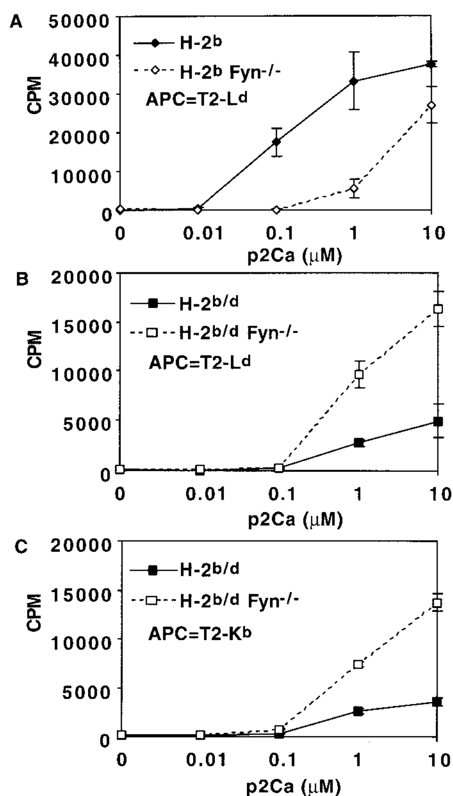


FIGURE 1. Deletion of Fyn results in enhanced proliferation of anergic H-2$^{b/d}$ DN cells. DN cells from H-2^{b}, H-2^{b} Fyn$^{-/-}$, H-2$^{b/d}$, and H-2$^{b/d}$ Fyn$^{-/-}$ mice were stimulated in a standard proliferation assay for 72 h and assayed for [^{3}H]thymidine incorporation. Stimulation was performed either with p2Ca presented by mitomycin C-treated T2-L^{d} cells (*A* and *B*) or p2Ca presented by T2-K^{b} cells (*C*) (see *Materials and Methods* for details). The error bars represent SD values of triplicate cultures. One representative experiment of three is shown.

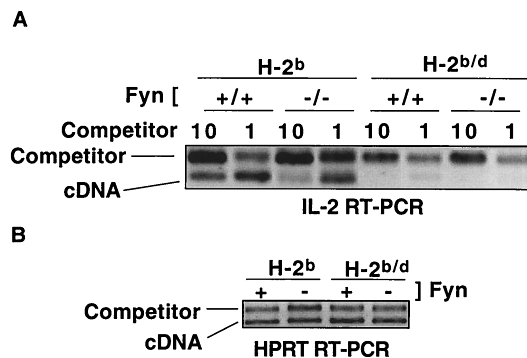


FIGURE 2. Defective IL-2 production by H-2$^{b/d}$ DN cells is not rescued by deletion of Fyn. Competitive RT-PCR (see *Materials and Methods* for details) was used to assess IL-2 production (*A*) by DN cells after 9 h of stimulation with mitomycin C-treated T2-L^{d} cells and 1 μM p2Ca. *B*, HPRT cDNA normalization for the amount of template. One representative experiment of three is shown.

H-2$^{b/d}$ Fyn$^{-/-}$ DN cells display enhanced up-regulation of the high affinity IL-2 receptor

Expression of the high affinity IL-2 receptor is essential for optimal T cell proliferation, and up-regulation of the high affinity IL-2 receptor is a critical step in the T cell activation process. Naive T cells express a low affinity heterodimeric receptor consisting of the common γ chain (γ_c) and IL-2Rβ (reviewed in Ref. 30). After activation-induced expression of the α-chain (CD25), it pairs with γ_c and IL-2Rβ, and forms the high affinity IL-2 receptor. This heterotrimeric receptor has a 100-fold greater affinity for IL-2 than the IL-2R$\beta\gamma_c$ pair (K_d = 10 pM vs K_d = 1 nM). As IL-2Rα is a key component of the high affinity IL-2R and is only expressed in activated T cells, it seems reasonable to propose that activated cells that are able to rapidly up-regulate IL-2Rα would surpass cells that do not. Interestingly, IL-2Rβ has a dual function serving also as the β subunit in the IL-15 receptor and binding IL-15 (31).

Previously, we observed that CD25 and CD69 are up-regulated more rapidly by H-2$^{b/d}$ than H-2^{b} DN cells following Ag stimulation (15). To address whether Fyn has a role in these processes, we stimulated H-2$^{b/d}$ DN cells with or without Fyn with the antigenic ligand and assayed by FACS their ability to up-regulate these two activation markers. As seen in Fig. 3 (*top*), the H-2$^{b/d}$ Fyn$^{-/-}$ DN cells were able to up-regulate CD25 to maximal levels with p2Ca/L^{d} at 1 μM, whereas the H-2$^{b/d}$ DN required 10 μM p2Ca/L^{d} to maximally express CD25. Interestingly, with the low affinity p2Ca/K^{b} ligand, the H-2$^{b/d}$ Fyn$^{-/-}$ DN achieved maximal up-regulation with the 1 μM p2Ca dose, but the H-2$^{b/d}$ DN were unable to maximally up-regulate CD25 even with the 10 μM p2Ca dose. These observations suggest that the H-2$^{b/d}$ Fyn$^{-/-}$ DN cells have an even lower threshold for CD25 up-regulation than the H-2$^{b/d}$ Fyn$^{+/+}$ DN cells. In contrast to CD25 up-regulation, CD69 up-regulation did not appear to be influenced by the expression of Fyn, with rapid increases in surface expression being observed in both the H-2$^{b/d}$ and H-2$^{b/d}$ Fyn$^{-/-}$ samples (Fig. 3, *bottom*). These observations suggest that Fyn may have some specific negative effect on aspects of TCR signaling related to the up-regulation of CD25 but not CD69. They also provide an explanation for the higher proliferative responses of Ag-stimulated H-2$^{b/d}$ Fyn$^{-/-}$ DN cells as compared with the H-2$^{b/d}$ Fyn$^{+/+}$ DN cells (Fig. 1). This increased proficiency of expressing high affinity IL-2 receptors by H-2$^{b/d}$ Fyn$^{-/-}$ DN cells may support their greater proliferative response by allowing them to more effectively use the small amounts of IL-2 they produce (14). To further investigate the importance of the rapid up-regulation of CD25 in the enhanced proliferation of the H-2$^{b/d}$ Fyn$^{-/-}$ DN cells we repeated the proliferation assays described in Fig. 1, but with the addition of blocking Abs for either IL-2 or CD25 (Fig. 4). With the addition of anti-CD25 Abs, the proliferation of the H-2$^{b/d}$ Fyn$^{-/-}$ DN cells was reduced by half. The addition of anti-IL-2 Abs completely eliminated the improvement in the H-2$^{b/d}$ Fyn$^{-/-}$ DN cells. These results suggest that the enhanced proliferation of the H-2$^{b/d}$ Fyn$^{-/-}$ DN cells is due to an enhanced ability to respond to IL-2, and that this is at least partially mediated by CD25.

Elevated expression of IL-2Rβ on H-2$^{b/d}$ DN cells and their enhanced survival in culture

We have previously shown that the H-2$^{b/d}$ DN cells have an activated/memory phenotype in terms of expression of some surface Ags, notably CD44 (14). Here we examined the expression of IL-2Rβ, another cell surface Ag up-regulated on memory cells (32). Examinations of IL-2Rβ expression on ex vivo cells revealed elevated levels on the H-2$^{b/d}$ DN relative to H-2^{b} DN (Fig. 5*A*). Increased expression was also detected on the H-2$^{b/d}$ Fyn$^{-/-}$ DN cells. The high level of IL-2Rβ expression by immediately ex vivo H-2$^{b/d}$ DN cells decreased to near basal levels after 18 h of culture without stimulation (Fig. 5*B*). Interestingly, in H-2$^{b/d}$ DN cells stimulated with L^{d}/p2Ca (Fig. 5*B*) (or K^{b}/p2Ca, data not shown), the expression of IL-2Rβ did not decrease to base level after 18 h of culture. This observation suggests that TCR signaling can at least partially block this decrease. By contrast, Ag stimulation of H-2$^{b/d}$ DN Fyn$^{-/-}$ cells did not prevent the down-regulation of

Downloaded from http://journals.aai.org/jimmunol/article-pdf/166/3/1540/1138831/1540.pdf by guest on 01 March 2024

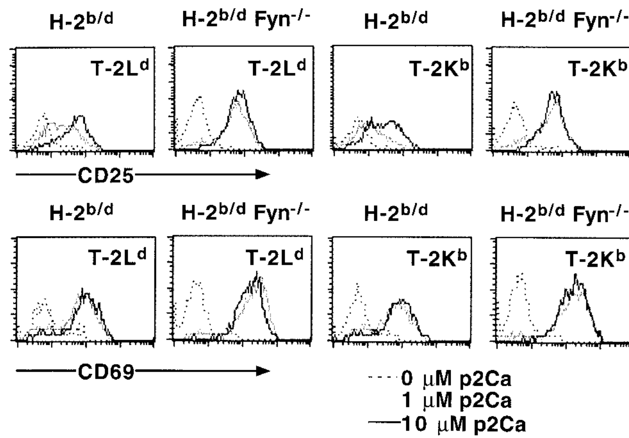


FIGURE 3. Efficient up-regulation of CD25 on Ag-stimulated H-$2^{b/d}$ $Fyn^{-/-}$ DN cells. Purified DN cells were stimulated with mitomycin C-treated T2-L^d or T2-K^b cells, with the indicated concentrations of p2Ca peptide. After 18 h of culture the cells were washed and stained with anti-CD25 (*top*) and anti-CD69 (*bottom*), as well as the 1B2 mAb (specific for the 2C TCR). The expression of CD25 and CD69 on $1B2^+$ cells are as indicated. Data from one representative experiment of three are shown.

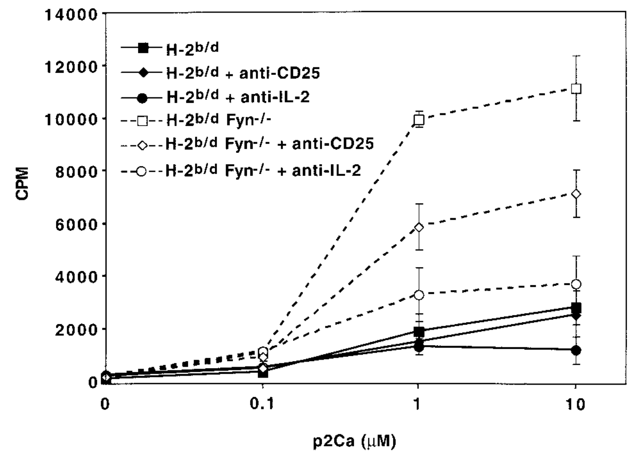


FIGURE 4. Enhanced responsiveness to IL-2 accounts for the recovery of the proliferation defect in H-$2^{b/d}$ $Fyn^{-/-}$ DN cells. H-$2^{b/d}$ and H-$2^{b/d}$ $Fyn^{-/-}$ DN cells were stimulated with T2-L^d cells as in Fig. 1, but with no addition, anti-CD25 (10 μg/ml), or anti-IL-2 (10 μg/ml).

IL-2Rβ to base levels in these cells. This finding suggests that Fyn is necessary in preventing the decrease in IL-2Rβ expression of Ag-stimulated H-$2^{b/d}$ DN cells.

As the H-$2^{b/d}$ DN cells demonstrated increased expression of IL-2Rβ, we sought to attach a functional significance to this finding. IL-2Rβ has been shown to play an important role in promoting the survival of $CD8^+$ memory cells (33) and has been demonstrated to associate with Fyn and Lck (34, 35). Activation of both of these kinases are important events for the activation of PI3K via IL-2Rβ (36–38). PI3K activation from IL-2R$\beta\gamma_c$ is believed to play a critical role in supporting survival (39). Without the addition of any exogenous growth factors, H-$2^{b/d}$ DN T cells displayed enhanced survival compared with H-2^bDN cells (Fig. 6, *top*). This enhanced survival is Fyn dependent, considering H-$2^{b/d}$ $Fyn^{-/-}$ DN cells did not have a survival advantage compared with H-2^b $Fyn^{-/-}$ DN cells (Fig. 6, *bottom*). Another indication that Fyn may confer a survival advantage on H-$2^{b/d}$DN cells is that we consistently recover about twice as many DN cells from H-$2^{b/d}$2C mice than from H-$2^{b/d}$ $Fyn^{-/-}$ mice, suggesting that the enhanced survival of anergized DN T cells in vivo is also dependent on Fyn.

IL-15 or IL-2 interacts with IL-2Rβ to promote the survival of H-$2^{b/d}$ DN cells

Next, we determined whether the increase in IL-2Rβ expression is reflected in increased responsiveness to IL-2 and IL-15, two cytokines known to signal through the IL-2Rβγc complex (31). With the addition of IL-2 we observed an improvement of cell survival by both wild-type and Fyn-deficient H-2^b and H-$2^{b/d}$ DN cell types (Fig. 7*A*). Therefore, the effects of IL-2 seem to be independent of the level of IL-2Rβ expression by these cells. IL-15 also confers survival benefits on all four cell types but with one notable difference (Fig. 7*B*). DN cells from H-$2^{b/d}$ mice were able to respond better to low concentrations of IL-15 as compared with H-$2^{b/d}$ Fyn-deficient cells. After 3 days of culture in medium containing 1 ng/ml of IL-15, the percentage of survival in the H-$2^{b/d}$ DN group was 55% compared with 29% for the H-$2^{b/d}$ $Fyn^{-/-}$ DN group (Fig. 7*B*). After 2 days of culture, the percentages of survival for these two groups of cells were 69% and 50%, respectively (values are averages from triplicate cultures). Additionally, H-$2^{b/d}$ DN cells were more responsive to IL-15 as determined by cell size after culture in IL-15-containing medium (Fig. 8). The cell size of H-$2^{b/d}$ DN cells cultured in 10 ng/ml of IL-15 for 2 or 3 days blasted larger than similarly cultured H-$2^{b/d}$ $Fyn^{-/-}$ DN cells. This effect was specific for IL-15 as the cell size of these two groups of cells increased to a similar extent when cultured in 10 ng/ml of IL-2 (Fig. 8).

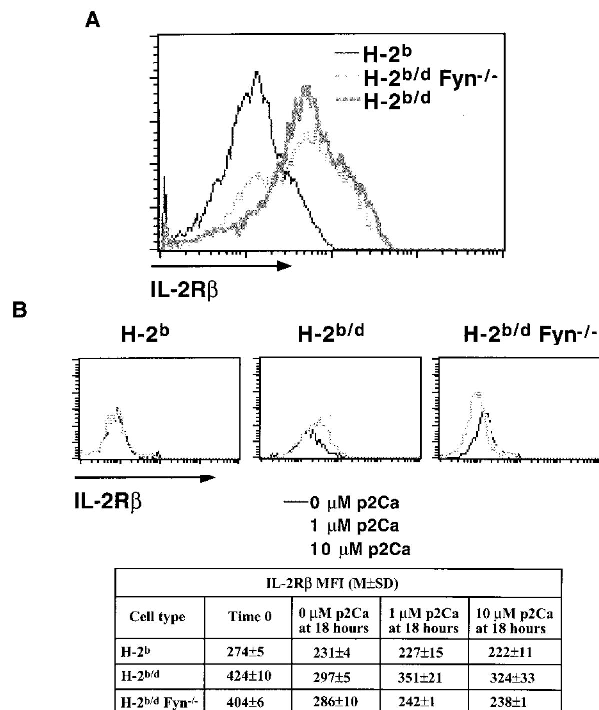
To determine whether IL-2Rβ/IL-15 and/or IL-2Rβ/IL-2 interactions contributed to the survival of H-$2^{b/d}$ DN cells, these cells were cultured in vitro in the presence of blocking anti-IL-2Rβ or anti-CD25 Abs. The presence of anti-IL-2Rβ, but not anti-CD25, reduced the survival of the H-$2^{b/d}$ DN cells (Fig. 9*A*). Furthermore, the enhanced survival in response to exogenously added IL-2 or IL-15 was specifically blocked by the anti-IL-2Rβ, but not the anti-CD25 Ab (Fig. 9*B*). These results suggest that the enhanced survival of the H-$2^{b/d}$ DN cells is mediated by interaction of either IL-15 or IL-2 with IL-2Rβ.

Discussion

In this study we addressed questions regarding the importance of Fyn in an in vivo model of T cell anergy. Anergized T cells lacking Fyn exhibited an amelioration of their proliferative capacity and exhibited an enhanced ability to up-regulate CD25. In addition, cultured anergic DN cells displayed a survival advantage over $Fyn^{-/-}$ anergic DN cells. The increased survival of anergic DN cells correlated with elevated IL-2Rβ expression and an enhanced ability to respond to IL-15. As this survival advantage was lost in Fyn-deficient anergic cells, and because Fyn associates with IL-2Rβ, a potential explanation for these observations is that Fyn is required for efficient signaling through IL-2Rβ.

We have previously shown that anergic DN cells express elevated levels of Fyn (15). The enhanced proliferative ability of the H-$2^{b/d}$ $Fyn^{-/-}$ DN cells suggests that the elevated level of Fyn in anergic cells may negatively regulate T cell activation. Fyn has been shown to associate with the CD3 ζ-chains in anergic T cells (13). It has also been demonstrated that Fyn and c-Cbl constitutively associate in anergic T cells (40). c-Cbl has also been shown to be an inhibitor of ZAP-70 kinase activity (41), and we have demonstrated previously that ZAP-70 phosphorylation is significantly decreased in H-$2^{b/d}$ DN cells upon TCR stimulation (15). Consistent with this observation, we have seen LAT phosphorylation to be reduced in H-$2^{b/d}$ DN cells upon TCR stimulation (15). The role Fyn plays in in vivo induced T cell anergy may be to

Downloaded from http://journals.aai.org/jimmunol/article-pdf/166/3/1540/1138831/1540.pdf by guest on 01 March 2024



IL-2Rβ MFI (M±SD)				
Cell type	Time 0	0 μM p2Ca at 18 hours	1 μM p2Ca at 18 hours	10 μM p2Ca at 18 hours
H-2b	274±5	231±4	227±15	222±11
H-2b/d	424±10	297±5	351±21	324±33
H-2b/d Fyn-/-	404±6	286±10	242±1	238±1

FIGURE 5. Immediately ex vivo H-$2^{b/d}$ DN have elevated IL-2Rβ expression. *A*, purified DN cells from the indicated mouse lines were stained with anti-IL-2Rβ mAb and assayed by FACS. *B*, DN cells were stimulated with mitomycin C-treated T2-L^d cells plus the indicated concentration of p2Ca peptide and assayed at 18 h for surface expression of IL-2Rβ. The level of IL-2Rβ expression by the various cell types is expressed as mean fluorescence intensity (MFI) values and the means are averages of triplicate cultures. Data from one representative experiment of three are shown.

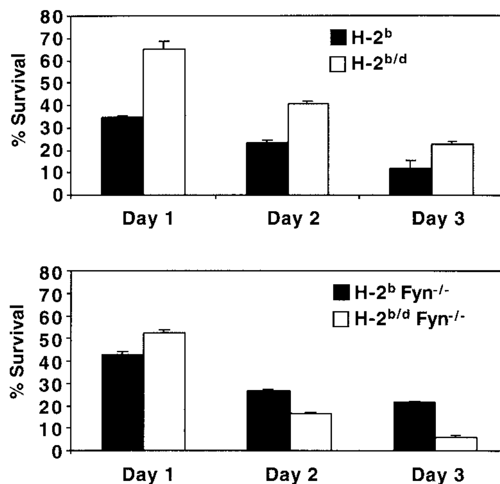


FIGURE 6. Enhanced survival of cultured H-$2^{b/d}$ DN cells is dependent on Fyn. DN cells from $Fyn^{+/+}$ (*top*) and $Fyn^{-/-}$ (*bottom*) H-2^b and H-$2^{b/d}$ mice were cultured without stimulation and their survival was assessed by 7-AAD staining and FACS analysis. The error bars represent SD values of triplicate cultures. Data from one representative experiment of three are shown.

bring c-Cbl to the CD3 ζ-chains and provide a constitutive negative regulation of TCR signaling (40). According to this model, LAT phosphorylation is expected to be increased in anergic $Fyn^{-/-}$ DN cells after TCR stimulation. However, our data are inconsistent with this hypothesis as we failed to detect increases in LAT phosphorylation upon TCR stimulation of H-$2^{b/d}$ $Fyn^{-/-}$ cells (our unpublished observations). One explanation for this discrepancy regarding LAT phosphorylation is that the cell types used for the analysis of T cell anergy are of diverse origin. We have used DN cells that express a transgenic TCR for our studies, whereas others have used anergic alloantigen-specific human T cells for their studies (40). Furthermore, these DN cells may be of the $\gamma\delta$ T cell lineage as a result of the early expression of the transgenic $\alpha\beta$ TCR during ontogeny (42). It is possible that Fyn may perform distinct functions in different T cell lineages and, in this way, contributes to different observations.

An alternative explanation for the enhanced proliferative response of $Fyn^{-/-}$ anergic DN cells to Ag stimulation is that the $Fyn^{-/-}$ anergic cells have not been anergized to the same extent as their Fyn-expressing counterparts due to less efficient TCR signaling. This hypothesis is supported by our observation that Fyn is required for optimal Ag-induced proliferative responses of non-anergic DN cells (Fig. 1). Our observation that DN cells from male mice expressing a low affinity transgenic male-specific TCR are not anergic (16) also supports the hypothesis that the strength of the autoreactive stimulus is important for the induction of the anergic state. Therefore, it is possible that the lack of Fyn may reduce the magnitude of autoreactive signaling, thereby reducing the severity of the anergic state of the $Fyn^{-/-}$ DN cells. However, it is clear that the $Fyn^{-/-}$ DN cells receive anergizing stimulus because H-$2^{b/d}$ $Fyn^{-/-}$ DN cells are defective in producing IL-2 in response to Ag stimulation. Furthermore, they possess a lower activation threshold for the induction of CD25 when compared with anergized $Fyn^{+/+}$ DN cells.

CD25 expression is induced either by Ags or cytokines and is controlled by at least three positive regulatory regions within the 5′ regulatory region of the IL-2Rα gene (43, 44). Regions I and II are for mitogen-induced IL-2Rα expression, and region III is essential for IL-2-induced IL-2Rα expression. The deletion of Fyn may affect the signaling pathways responsible for mitogen-stimulated up-regulation of CD25. The removal of Fyn-mediated negative regulation allows for more efficient up-regulation of CD25 (Fig. 3, *top*), hence the observed increases in proliferative capacity (Fig. 1, *B* and *C*). The fact that up-regulation of CD69 is not influenced by the presence or absence of Fyn (Fig. 3, *bottom*) suggests that signaling to up-regulate this molecule is not subjected to the same regulation.

The enhanced survival of the anergic H-$2^{b/d}$ DN cells in culture correlated with elevated expression of IL-2Rβ on these cells. Other studies have shown that memory $CD8^+$ T cells express elevated levels of IL-2Rβ, and their enhanced survival in vivo is likely mediated by IL-15 and not IL-2 (33). IL-15 is ubiquitously expressed in mouse tissues including placenta, skeletal muscles, kidney, lung, heart, fibroblasts, epithelial cells, and monocytes (45). Subsequently, we have detected expression of IL-15 in H-2^b and H-$2^{b/d}$ DN by RT-PCR (our unpublished observations). We propose that because the H-$2^{b/d}$ DN cells express elevated levels of IL-2Rβ, they may be responding to IL-15 in an auto- or paracrine manner and that this may explain their enhanced survival. We have provided evidence that the enhancement of survival by IL-15 is mediated by IL-2Rβ (Fig. 9). Although ex vivo H-$2^{b/d}$ $Fyn^{-/-}$ DN cells exhibit elevated IL-2Rβ expression, they did not exhibit a survival advantage in culture, implicating a role for Fyn in this increased longevity (Fig. 5). Furthermore, we noted that H-$2^{b/d}$ $Fyn^{-/-}$ DN cells were less responsive to IL-15 in cell survival and blastogenesis assays as compared with H-$2^{b/d}$ DN cells. These observations suggest that IL-15/IL-2Rβ signaling may be Fyn dependent and that this function of Fyn is not compensated for by Lck in $Fyn^{-/-}$ cells.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/166/3/1540/1138831/1540.pdf by guest on 01 March 2024

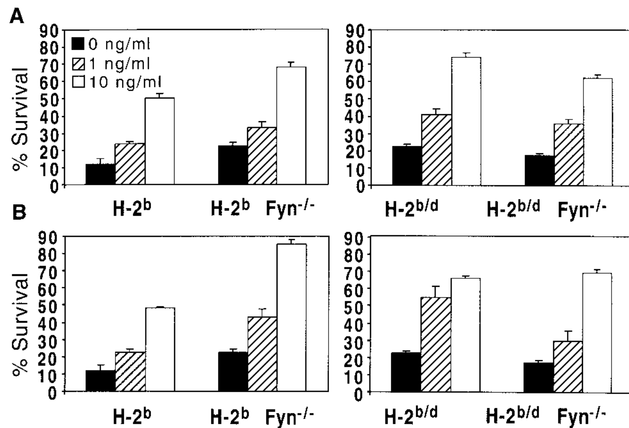


FIGURE 7. IL-2 and IL-15 enhance the survival of cultured DN cells. DN cells were cultured with 0, 1, or 10 ng/ml IL-2 (*A*) or IL-15 (*B*). After 72 h, cells were washed and stained with 7-AAD and assayed by FACS. The error bars represent SD values of triplicate cultures. Data from one representative experiment of five are shown.

In our in vitro assays, IL-2 can perform the same function as IL-15 in promoting the survival of anergic DN cells. The effects of IL-2 are also mediated by IL-2Rβ. As we observed enhanced survival of both wild-type and Fyn$^{-/-}$ H-2^{b} or H-2$^{b/d}$ DN cells in response to IL-2, it is clear that the enhanced survival promoted by IL-2 is not Fyn dependent. It is conceivable that Lck may compensate for the lack of Fyn under these conditions. Anergic DN cells have been shown to be defective in the production of IL-2, a cytokine primarily made by activated T cells (Ref. 14 and Fig. 2). Therefore, it is likely that IL-2 is generally not available to anergic DN cells under physiological conditions. By contrast, IL-15 is ubiquitously expressed (45) and, therefore, readily accessible to the DN cells under physiological conditions. We have shown that the anergic DN cells express high levels of the IL-2Rβ receptor, allowing them to respond to low concentrations of IL-15 in a Fyn-dependent manner. Furthermore, the higher recovery of anergic DN cells from Fyn$^{+/+}$ mice also supports the hypothesis that Fyn is important for the survival of anergic DN cells under physiological conditions. In summary, we have provided data that support the hypothesis that Fyn plays a dual role in T cell anergy: one in negatively regulating aspects of TCR signaling and the other in promoting cell survival.

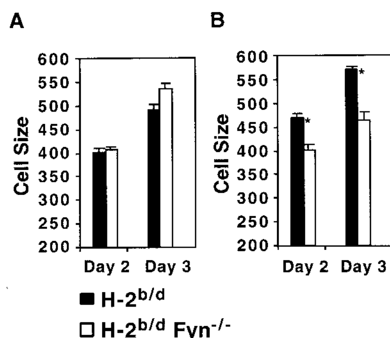


FIGURE 8. Blastogenic response of H-2$^{b/d}$ DN cells to IL-15 impaired by Fyn$^{-/-}$ mutation. DN cells from the indicated mouse line were cultured with 10 ng/ml IL-2 (*A*) or 10 ng/ml IL-15 (*B*). After the indicated time, cell size was determined by forward scatter using FACS analysis. Annotation with * indicates the H-2$^{b/d}$ Fyn$^{-/-}$ samples were determined to be statistically different from the corresponding H-2$^{b/d}$ sample (see *Materials and Methods* for details). The error bars represent SD values of triplicate cultures. Data from one representative experiment of three are shown.

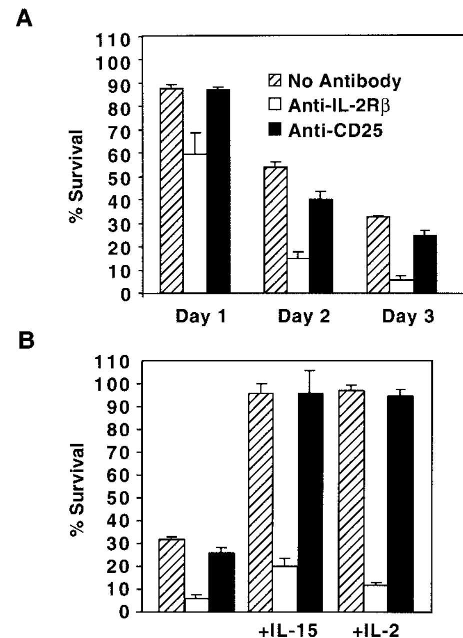


FIGURE 9. Enhanced survival of H-2$^{b/d}$ DN cells is blocked by anti-IL-2Rβ Ab. H-2$^{b/d}$ DN cells were cultured with no stimulation in the presence of the indicated Abs (*A*). Cells were also cultured with 10 ng/ml IL-2 or 10 ng/ml IL-15 and with the indicated Abs for 72 h (*B*). At the indicated time, cells were washed and stained with 7-AAD and assayed by FACS. The error bars represent SD values of triplicate cultures. Data from one representative experiment of two are shown.

Acknowledgments

We thank Edward Kim for excellent technical assistance. We also thank Dr. Dennis Loh for providing breeders for the 2C TCR-transgenic mice, Dr. Peter Cresswell for the T2-K^{b} and T2-L^{d} cell lines, Dr. Herman Eisen for the 1B2 hybridoma, and Dr. Richard Locksley for the pPQRS plasmid.

References

1. White, J., A. Herman, A. M. Pullen, R. Kubo, J. W. Kappler, and P. Marrack. 1989. The V β-specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice. *Cell 56:27.*
2. Kawabe, Y., and A. Ochi. 1991. Programmed cell death and extrathymic reduction of Vβ8^{+} CD4^{+} T cells in mice tolerant to *Staphylococcus aureus* enterotoxin B. *Nature 349:245.*
3. Carlow, D. A., S. J. Teh, N. S. van Oers, R. G. Miller, and H. S. Teh. 1992. Peripheral tolerance through clonal deletion of mature CD4^{-}CD8^{+} T cells. *Int. Immunol. 4:599.*
4. Rammensee, H. G., R. Kroschewski, and B. Frangoulis. 1989. Clonal anergy induced in mature Vβ6^{+} T lymphocytes on immunizing Mls-1b mice with Mls-1a expressing cells. *Nature 339:541.*
5. Miller, J. F., and G. Morahan. 1992. Peripheral T cell tolerance. *Annu. Rev. Immunol. 10:51.*
6. Ohashi, P. S., S. Oehen, K. Buerki, H. Pircher, C. T. Ohashi, B. Odermatt, B. Malissen, R. M. Zinkernagel, and H. Hengartner. 1991. Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell 65:305.*
7. Mueller, D. L., M. K. Jenkins, and R. H. Schwartz. 1989. Clonal expansion versus functional clonal inactivation: a costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. *Annu. Rev. Immunol. 7:445.*
8. Schwartz, R. H. 1996. Models of T cell anergy: is there a common molecular mechanism? *J. Exp. Med. 184:1.*
9. Migita, K., K. Eguchi, Y. Kawabe, T. Tsukada, Y. Ichinose, S. Nagataki, and A. Ochi. 1995. Defective TCR-mediated signaling in anergic T cells. *J. Immunol. 155:5083.*
10. Gajewski, T. F., P. Fields, and F. W. Fitch. 1995. Induction of the increased Fyn kinase activity in anergic T helper type 1 clones requires calcium and protein synthesis and is sensitive to cyclosporin A. *Eur. J. Immunol. 25:1836.*
11. Gajewski, T. F., D. Qian, P. Fields, and F. W. Fitch. 1994. Anergic T-lymphocyte clones have altered inositol phosphate, calcium, and tyrosine kinase signaling pathways. *Proc. Natl. Acad. Sci. USA 91:38.*
12. Denny, M. F., B. Patai, and D. B. Straus. 2000. Differential T-cell antigen receptor signaling mediated by the Src family kinases Lck and Fyn. *Mol. Cell. Biol. 20:1426.*

Downloaded from http://journals.aai.org/jimmunol/article-pdf/166/3/1540/1138831/1540.pdf by guest on 01 March 2024

13. Boussiotis, V. A., D. L. Barber, B. J. Lee, J. G. Gribben, G. J. Freeman, and L. M. Nadler. 1996. Differential association of protein tyrosine kinases with T cell receptor is linked to the induction of anergy and its prevention by B7-family mediated costimulation. *J. Exp. Med. 184:365.*
14. Caveno, J., Y. Zhang, B. Motyka, S. J. Teh, and H. S. Teh. 1999. Functional similarity and differences between selection-independent $CD4^- CD8^- \alpha\beta$ T cells and positively selected CD8 T cells expressing the same TCR and the induction of anergy in $CD4^-CD8^- \alpha\beta$ T cells in antigen-expressing mice. *J. Immunol. 163:1222.*
15. Utting, O., S. J. Teh, and H. S. Teh. 2000. A population of in vivo anergized T cells with a lower activation threshold for the induction of CD25 exhibit differential requirements in mobilization of intracellular calcium and mitogen-activated protein kinase activation. *J. Immunol. 164:2881.*
16. Sha, W. C., C. A. Nelson, R. D. Newberry, D. M. Kranz, J. H. Russell, and D. Y. Loh. 1988. Positive and negative selection of an antigen receptor on T cells in transgenic mice. *Nature 336:73.*
17. Sha, W. C., C. A. Nelson, R. D. Newberry, D. M. Kranz, J. H. Russell, and D. Y. Loh. 1988. Selective expression of an antigen receptor on CD8-bearing T lymphocytes in transgenic mice. *Nature 335:271.*
18. Udaka, K., T. J. Tsomides, and H. N. Eisen. 1992. A naturally occurring peptide recognized by alloreactive $CD8^+$ cytotoxic T lymphocytes in association with a class I MHC protein. *Cell 69:989.*
19. Udaka, K., T. J. Tsomides, P. Walden, N. Fukusen, and H. N. Eisen. 1993. A ubiquitous protein is the source of naturally occurring peptides that are recognized by a $CD8^+$ T-cell clone. *Proc. Natl. Acad. Sci. USA 90:11272.*
20. Sha, W., C. Nelson, R. Newberry, J. Pullen, L. Pease, J. Russell, and D. Loh. 1990. Positive selection of transgenic receptor-bearing thymocytes by K^b antigen is altered by K^b mutations that involve peptide binding. *Proc. Natl. Acad. Sci. USA 87:6186.*
21. Sykulev, Y., A. Brunmark, M. Jackson, R. J. Cohen, P. A. Peterson, and H. N. Eisen. 1994. Kinetics and affinity of reactions between an antigen-specific T cell receptor and peptide-MHC complexes. *Immunity 1:15.*
22. von Boehmer, H., J. Kirberg, and B. Rocha. 1991. An unusual lineage of α/β T cells that contains autoreactive cells. *J. Exp. Med. 174:1001.*
23. Russell, J. H., P. Meleedy-Rey, D. E. McCulley, W. C. Sha, C. A. Nelson, and D. Y. Loh. 1990. Evidence for CD8-independent T cell maturation in transgenic mice. *J. Immunol. 144:3318.*
24. Teh, H. S., H. Kishi, B. Scott, and H. Von Boehmer. 1989. Deletion of autospecific T cells in T cell receptor (TCR) transgenic mice spares cells with normal TCR levels and low levels of CD8 molecules. *J. Exp. Med. 169:795.*
25. Appleby, M., J. A. Gross, M. P. Cooke, S. D. Levin, X. Qian, and R. M. Perlmutter. 1992. Defective T cell receptor signaling in mice lacking the thymic isoform of $p59^{fyn}$. *Cell 70:751.*
26. Kranz, D. M., D. H. Sherman, M. V. Sitkovsky, M. S. Pasternack, and H. N. Eisen. 1984. Immunoprecipitation of cell surface structures of cloned cytotoxic T lymphocytes by clone-specific antisera. *Proc. Natl. Acad. Sci. USA 81:573.*
27. Alexander, J., J. A. Payne, R. Murray, J. A. Frelinger, and P. Creswell. 1989. Differential transport requirements of HLA and H-2 class I glycoproteins. *Immunogenetics 29:380.*
28. Reiner, S. L., S. Zheng, D. B. Corry, and R. M. Locksley. 1994. Constructing polycompetitor cDNAs for quantitative PCR. *J. Immunol. Methods 175:275.*
29. Utting, O., S. J. Teh, and H. S. Teh. 1998. T cells expressing receptors of different affinity for antigen ligands reveal a unique role for $p59^{fyn}$ in T cell development and optimal stimulation of T cells by antigen. *J. Immunol. 160:5410.*
30. DiSanto, J. P. 1997. Cytokines: shared receptors, distinct functions. *Curr. Biol. 7:424.*
31. Ma, A., D. L. Boone, and J. P. Lodolce. 2000. The pleiotropic functions of interleukin 15: not so interleukin 2-like after all. *J. Exp. Med. 191:753.*
32. Cho, B. K., C. Wang, S. Sugawa, H. N. Eisen, and J. Chen. 1999. Functional differences between memory and naive CD8 T cells. *Proc. Natl. Acad. Sci. USA 96:2976.*
33. Ku, C. C., M. Murakami, A. Sakamoto, J. Kappler, and P. Marrack. 2000. Control of homeostasis of $CD8^+$ memory T cells by opposing cytokines. *Science 288: 675.*
34. Kobayashi, N., T. Kono, M. Hatakeyama, Y. Minami, T. Miyazaki, R. M. Perlmutter, and T. Taniguchi. 1993. Functional coupling of the src-family protein tyrosine kinases $p59^{fyn}$ and $p53/56S^{lyn}$ with the interleukin 2 receptor: implications for redundancy and pleiotropism in cytokine signal transduction. *Proc. Natl. Acad. Sci. USA 90:4201.*
35. Hatakeyama, M., T. Kono, N. Kobayashi, A. Kawahara, S. D. Levin, R. M. Perlmutter, and T. Taniguchi. 1991. Interaction of the IL-2 receptor with the src-family kinase $p56^{lck}$: identification of novel intermolecular association. *Science 252:1523.*
36. Augustine, J. A., S. L. Sutor, and R. T. Abraham. 1991. Interleukin 2- and polyomavirus middle T antigen-induced modification of phosphatidylinositol 3-kinase activity in activated T lymphocytes. *Mol. Cell. Biol. 11:4431.*
37. Carrera, A., L. Rodriguez-Borlado, C. Martinez-Alonso, and I. Merida. 1994. T cell receptor-associated α-phosphatidylinositol 3-kinase becomes activated by T cell receptor cross-linking and requires $pp56^{lck}$. *J. Biol. Chem. 269:19435.*
38. Taichman, R., I. Merida, T. Torigoe, G. N. Gaulton, and J. C. Reed. 1993. Evidence that protein tyrosine kinase $p56^{Lck}$ regulates the activity of phosphatidylinositol-3′-kinase in interleukin-2-dependent T-cells. *J. Biol. Chem. 268:20031.*
39. Gonzalez-Garcia, A., I. Merida, A. C. Martinez, and A. C. Carrera. 1997. Intermediate affinity interleukin-2 receptor mediates survival via a phosphatidylinositol 3-kinase-dependent pathway. *J. Biol. Chem. 272:10220.*
40. Boussiotis, V. A., G. J. Freeman, A. Berezovkaya, D. L. Barber, and L. M. Nadler. 1997. Maintenance of human T cell anergy: blocking of IL-2 gene transcription by activated Rap 1. *Science 278:124.*
41. Murphy, M. A., R. G. Schnall, D. J. Venter, L. Barnett, I. Bertoncello, C. B. Thien, W. Y. Langdon, and D. D. Bowtell. 1998. Tissue hyperplasia and enhanced T-cell signalling via ZAP-70 in c-Cbl-deficient mice. *Mol. Cell. Biol. 18:4872.*
42. Bruno, L., H. J. Fehling, and H. von Boehmer. 1996. The $\alpha\beta$ T cell receptor can replace the $\gamma\delta$ receptor in the development of $\gamma\delta$ lineage cells. *Immunity 5:343.*
43. John, S., C. M. Robbins, and W. J. Leonard. 1996. An IL-2 response element in the human IL-2 receptor α chain promoter is a composite element that binds Stat5, Elf-1, HMG-I(Y) and a GATA family protein. *EMBO J. 15:5627.*
44. Sperisen, P., S. M. Wang, E. Soldaini, M. Pla, C. Rusterholz, P. Bucher, P. Corthesy, P. Reichenbach, and M. Nabholz. 1995. Mouse interleukin-2 receptor α gene expression: interleukin-1 and interleukin-2 control transcription via distinct *cis*-acting elements. *J. Biol. Chem. 270:10743.*
45. Grabstein, K. H., J. Eisenman, K. Shanebeck, C. Rauch, S. Srinivasan, V. Fung, C. Beers, J. Richardson, M. A. Schoenborn, M. Ahdieh, et al. 1994. Cloning of a T cell growth factor that interacts with the β chain of the interleukin-2 receptor. *Science 264:965.*

Downloaded from http://journals.aai.org/jimmunol/article-pdf/166/3/1540/1138831/1540.pdf by guest on 01 March 2024