Requirement of high-affinity IL-2–IL-2R interaction for T cell anergy induction

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Abstract

Incomplete T cell antigen receptor-mediated signaling induces an unresponsive state known as anergy. Previously, we had shown that anergy can be induced in antigen-primed but not naive T cells. In this report, we found that in vitro primed T cells from IL-2Rα-deficient mice were resistant to anergy induction in contrast to comparably treated wild-type T cells. This resistance persisted even after proliferation of IL-2Rα chain-deficient CD4 T cells with high-dose IL-2–IL-2Rβγ chains interaction. Thus, antigen activation, and/or progression through cell cycle are not sufficient to induce anergy susceptibility in T cells. The high-affinity IL-2–IL-2R interaction appears to play a critical role in this process.

Introduction

T cells play a central role in the defense of the host, possessing a wide array of effector functions that influence the other members of the immune system. Left unchecked, T cell responses can lead to clinically relevant conditions such as autoimmune diseases and rejection in the setting of organ transplantation. Thus, improving our understanding of how their responses are altered is important to gain insight in the regulation of this system. Investigations in the late 1980s revealed that a partial TCR-mediated signal could induce an unresponsive state rendering the T cell incapable of proliferating or producing IL-2 to subsequent antigen challenge (1, 2). This means of inducing ‘anergy’, as it was called was reversible as the exogenous administration of IL-2 could re-establish competence following proliferation. Further studies have since identified several means of eliciting this unresponsive state. These include treatments of the T cell with (i) chemically fixed antigen-presenting cells (APCs) plus antigen, (ii) calcium ionophores such as ionomycin and (iii) class II and antigen incorporated into planar membranes (1, 3). These modes of partial signaling all lack a critical ‘second signal’ now known to be due to the engagement of accessory molecules on the T cell surface with the APC particularly that involves CD28 with B7-1 or 2 (4, 5). Despite these advances, identifying the precise means by which anergy is induced has remained elusive.

Our laboratory embarked upon studies examining the regulation of anergy after our initial observation that not all cells were susceptible to anergy induction (6). When naive T cells were subjected to either fixed APC or ionomycin, these cells remained responsive to antigen challenge with normal APC, producing IL-2 in comparable amounts to untreated cells. This was in contrast to previously activated T cells that were rendered unresponsive by such treatment. Thus, naive cells are resistant to anergy induction but could be rendered susceptible by in vitro activation.

Further investigations using a transgenic model system with T cells bearing the TCR specific for amino acids 323–339 of the ovalbumin peptide (OVA) (DO11.10) demonstrated that naive cells could acquire susceptibility to anergy in vivo following immunization by antigen (6). Thus, activation of T cells with antigen, either in vitro or in vivo, is a necessary step to acquire susceptibility to anergy induction.

In the present study, we utilized a mouse line deficient in the α chain of the IL-2R bred on a mouse line bearing the DO11.10 TCR. This allowed us to examine the role of IL-2 and its interaction with its high-affinity receptor on anergy induction both in vitro and in vivo. Our findings indicate that signaling through the high-affinity IL-2R is critical in converting an anergy-resistant cell (naive) to a susceptible one. Thus, our results suggest that IL-2 plays an important role in anergy induction.
role in both the activation and down-regulation of the immune system.

Methods

Mice

IL-2Rα chain-deficient mice (homozygous for deficient gene) (7) were obtained from the Jackson Laboratory and were bred at the Washington University School of Medicine and RIKEN Yokohama Institute in a specific pathogen-free facility. IL-2Rα chain disruption was identified by PCR analysis of tail DNA as previously described (8). The DO11.10 TCR transgenic mice were described previously (9). DO11.10 TCR mice were bred at the Washington University School of Medicine and RIKEN (7) were obtained from the Jackson Laboratory and were bred at the Washington University School of Medicine and RIKEN Yokohama Institute in a specific pathogen-free facility. IL-2Rα chain-deficient mice (homozygous for deficient gene) were described previously (8). The DO11.10 TCR transgenic mice were designated DO11.10 IL-2rα+/−, while those mice bearing the transgene TCR and heterozygous for the α chain of the IL-2R were designated DO11.10 IL-2rα+/− and were considered to have normal IL-2R expression.

Flow cytometry

Cell-surface immunofluorescence analysis of T cell populations was performed as described previously (10). In brief, cells were incubated with the antibody at saturating concentrations at 4°C for 30 min, washed and further incubated with appropriate secondary reagents for an additional 30 min. Control samples were prepared in the same manner, but without primary antibody or with an isotype-matched control. Samples were analyzed by a FACS-Calibur analyzer (Becton Dickinson, Mountain View, CA, USA) using the CellQuest program. For sorting, cells were stained by the same manner and sorted using FACSVantage cell sorter (Becton Dickinson). mAbs used include KJ1-26, specific for an idiotype expressed on the transgene-derived TCR, DO11.10 (9), anti-CD62L and -CD4 (Pharmingen, San Diego, CA, USA).

To assess cell cycle progression in vitro, antigen-activated T cells from DO11.10 mice were labeled with chloromethylfluorescein diacetate succinimidyl ester (CFSE) (Molecular Probes, Eugene, OR, USA) (11). Antigen-activated splenic T cells were suspended at 10 × 10^6 ml^{-1} in DMEM with no FCS, and the CFSE was added to a final concentration of 1 μM. The cells were incubated for 10 min at 37°C, at which time the labeling was terminated by the addition of cold DMEM containing 5% calf serum, and washing the cells three times. Cells were then incubated in culture at low density at 1 × 10^5 cells ml^{-1} in either media alone, low concentrations of IL-2 (100 units ml^{-1}) or high concentrations of IL-2 (5 × 10^5 units ml^{-1}), and incubated at 37°C for 7 days. The cells were then collected, stained with KJ1-26 linked to PE and T cells expressing the TCR transgene were analyzed by flow cytometry for CFSE level.

Stimulation of T cells and lymphokine assays

Cell proliferation assay was performed by isolating spleen cells and incubating them at a concentration of 2.5 × 10^6 cells ml^{-1} in a final volume of 200 μl DMEM with 5% FCS in 96-well flat bottom plates (Costar, Cambridge, MA, USA). Conditions consisted of either media alone (control) or different dose of antigen (OVA) for 72 h with proliferation measured by 6-h pulse with 1 μCi titrated thymidine per well.

In vitro primed T cells were prepared by stimulating total spleen cells (2.5 × 10^5 ml^{-1}) or sorted CD4^+ transgenic TCR-positive T cell (5 × 10^2 ml^{-1}) and irradiated (2000 rad) BALB/c spleen cells (2 × 10^6 ml^{-1}) with 1 mg ml^{-1} of OVA in a final volume of 2 ml DMEM with 5% FCS in 24-well plates (Corning, Corning, MI, USA).

IL-2-dependent T cell proliferation was measured by culturing T cells (1 × 10^5 ml^{-1}) in a final volume of 200 μl of DMEM with 5% FCS containing IL-2 in 96-well flat bottom plates. The cells were incubated at 37°C for 48 h, and proliferation was measured as described above.

IL-2 activity was assayed using the IL-2-dependent CTLL line (12). Briefly, supernatants obtained from the final stimulation of the anergy assay (see below) were serially diluted and incubated with CTLL (1 × 10^4 ml^{-1}) in a final volume of 200 μl DMEM with 5% FCS in 96-well flat bottom plates. Cultures were incubated for 24 h and proliferation was measured as described above. Units are defined as reciprocal dilution of culture supernatants which gave 50% of maximum activity.

Anergy induction assay

Anergy induction with ionomycin was performed as previously described (3). Cells (5 × 10^5 ml^{-1}) were incubated with ionomycin (Calbiochem, La Jolla, CA, USA) at a final concentration of 1 μM for 20 h in a final volume of 2 ml of DMEM with 5% FCS, in 24-well plates. Cells were harvested, washed with medium three times and recovered cells (1 × 10^6 ml^{-1}) were stimulated with either normal 2000 rad irradiated BALB/c splenic APC (2.5 × 10^6 ml^{-1}) alone or APC with OVA (1 mg ml^{-1}) or phorbol myristate acetate (PMA) (10 ng ml^{-1}) (Sigma, St Louis, MO, USA) and ionomycin (1 μM) in a final volume of 200 μl DMEM with 5% FCS in 96-well round bottom plates (Costar). After 24 h of culture, supernatants were harvested and presence of IL-2 was measured.

Preparation of fixed APC was according to previously described conditions (1). Briefly, 2000 rad irradiated BALB/c splenocytes (5 × 10^7 ml^{-1}) were incubated for 1 h on ice in 0.4 ml of 0.9% NaCl containing 75 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) (Calbiochem). The cells were washed extensively in serum-free medium to stop the coupling reaction. T cells (1 × 10^6 ml^{-1}) were incubated with EDCI-treated APC (5 × 10^6 ml^{-1}) in the presence of 50 μM OVA (323-339) peptide in 24-well plates in a total volume of 2 ml of DMEM with 5% FCS for 16-20 h. Recovered cells were tested in the same manner for anergy susceptibility. All anergy induction experiments were repeated two to three times with similar results.

In vivo priming and isolation of activated T cells

DO11.10 IL-2rα−/− mice were immunized in the footpad with OVA (100 μg in 100 μl) in CFA. Lymph node cells from OVA-primed mice (7 days after immunization) were stained with biotinylated KJ1-26 and MEL-14, followed by incubation with PE-avidin and goat anti-rat FITC secondary reagents. The cells from the draining lymph nodes were isolated 7 days after immunization. T cells bearing the transgenic TCR with low expression of CD62L were isolated as in vivo primed
T cells (13, 14) using FACSVantage cell sorter as previously described (6). The CD4 T cell bearing transgenic TCR from non-immunized mice were used as non-primed T cells. The resulting populations were >98% pure. Cells were then tested for anergy susceptibility as described above.

Results

Lack of tolerance induction in IL-2 receptor chain-negative DO11.10 CD4 T cells

In order to dissociate T cell activation by antigen from IL-2-driven proliferation in the development of anergy susceptibility, we chose the mouse line deficient in the α chain of the IL-2R (IL-2Rα−/−) (7). Since we defined anergy as the inability to generate IL-2 in response to antigen stimulation (1), we needed to maintain the ability to measure IL-2 production for these studies. The IL-2Rα−/− mouse retains its ability to produce IL-2 in response to antigen stimulation thus fulfilling this requirement (7). IL-2Rα−/− mice were bred with the DO11.10 TCR transgenic to generate DO11.10 TCR transgenic, IL-2Rα−/−/mouse line (DO11.10 IL-2Rα−/−). This enabled us to examine the role of anergy induction in the context of antigen-specific responses.

Young DO11.10 IL-2Rα−/− mice (4–5 weeks old) do not have lymphoproliferation as non-TCR transgenic IL-2Rα−/− mice. They are, in fact, comparable to mice (DO11.10 IL-2Rα+/−) bearing the wild-type IL-2Rα as described previously. However, it should be noted that T cells with activated phenotype increased in old DO11.10 IL-2Rα−/− mice similar to a previous report (8). Thus, we used mice at 4 weeks of age for the experiments.

T cells from DO11.10 IL-2Rα−/− mice were stimulated with antigen in vitro for 7 days and recovered cells were tested for their susceptibility to anergy induction with ionomycin. These T cells responded to antigen re-challenge and produced IL-2 at levels comparable to T cells not treated with ionomycin (Table 1). In contrast, activated T cells from normal DO11.10 mice failed to produce IL-2 after such treatment. Thus, it seems that T cells from DO11.10 IL-2Rα−/− mice are resistant to anergy induction even after in vitro activation with antigen.

T cells from DO11.10 IL-2Rα−/− mice exhibited significant differences in proliferation upon stimulation with antigen from wild-type DO11.10 mice. T cells from DO11.10 IL-2Rα−/− showed very little proliferation, while those from mice with functional IL-2Rα chain proliferated vigorously under the same condition (Fig. 1A). The DO11.10 IL-2Rα−/− mice produce IL-2 upon stimulation with antigen but respond only to high dose of IL-2 due to the expression of the low-affinity β and γ chains after antigen stimulation (7, 15) (Fig. 1B). Thus, our results clearly demonstrated that antigen activation of the T cell alone is not sufficient to induce an anergy-susceptible state in naive T cells. However, a requirement of antigen-driven, and presumably IL-2-dependent, proliferation of T cells for the phenotypic change remained a viable possibility.

Dissociation of resistance to anergy induction and in vitro proliferation

In order to assess whether proliferation of T cells in vitro was an important step for rendering a naive cell susceptible to anergy induction, it was necessary to establish conditions where all of the activated cells proceeded through cell cycle regardless of their affinity to IL-2. We labeled the cells with CFSE to determine the percentage of cells progressing through cell cycle following expansion with IL-2 (11). DO11.10 IL-2Rα−/− T cells were stimulated with antigen for 2 days, washed, labeled with CFSE and then cultured in vitro in two different concentrations of IL-2. The cells were then analyzed after 7 days in culture as this was the time used for the anergy induction assays. As shown in Fig. 2, DO11.10 IL-2Rα+/− T cells proceed through cell cycle at both low and high concentrations of IL-2. In contrast, DO11.10 IL-2Rα−/− T cells could not proliferate at low concentrations of IL-2 but proceeded through cell cycle to a similar degree as IL-2-sufficient T cells at higher concentrations of the cytokine. Thus, despite lacking the high-affinity receptor, antigen-activated DO11.10 IL-2Rα−/− T cells are capable of proliferating with high doses of exogenous IL-2 to a degree comparable to mice bearing the wild-type receptor.

Having achieved conditions that allowed the DO11.10 IL-2Rα−/− T cells to undergo cell cycles in vitro, we then examined whether these cells were susceptible to anergy induction. T cells stimulated with antigen and grown in IL-2 were treated with ionomycin (1 μM). After 20 h, the cells were washed and stimulated with antigen and APC, PMA and ionomycin or nothing. DO11.10 IL-2Rα−/− T cells, grown in low-dose IL-2 and treated with ionomycin, became unresponsive to subsequent stimulation with antigen and APC and produced no IL-2 (Table 2). These anergic cells are still viable as they still make IL-2 in response to PMA and ionomycin, a pharmacological stimulus to T cells. In contrast, untreated cells are fully capable of responding to antigen and APC and produce significant amounts of IL-2 upon challenge. DO11.10 IL-2Rα−/− T cells grown in high doses of IL-2 produce much less IL-2 in response to antigen and APC similar to the phenomenon reported previously (16). However, these cells

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Spleen cells from DO11.10 IL-2Rα+/− and DO11.10 IL-2Rα−/− mice were stimulated with antigen (OVA 1 mg ml−1) for 7 days as described in the Methods. T cells were then incubated with media alone (none), PMA (10 ng ml−1) and ionomycin (1 μM) (P + I), or OVA (1 mg ml−1) and irradiated APC (OVA) as a 2nd stimulus for 24 h and the supernatants were subsequently assayed for IL-2 activity using the IL-2 indicator line CTLL. IL-2 activity is expressed in units per milliliter.
CD4 T cell anergy and IL-2

Fig. 1. Proliferation of T cells from DO11.10 IL-2Rα+/– and DO11.10 IL-2Rα mice. (A) Spleen cells from DO11.10 IL-2Rα+/– (closed circle) and DO11.10 IL-2Rα+/– (closed triangle) were cultured at a concentration of \(2.5 \times 10^5\) cells ml\(^{-1}\) with different amounts of OVA in microculture plates. Proliferation was assessed after 72 h of incubation with 6-h pulse by tritiated thymidine. Data reflect mean of triplicate samples. (B) Spleen cells from DO11.10 IL-2Rα+/– (closed circle) and DO11.10 IL-2Rα+/– (closed triangle) mice were stimulated for 2 days with antigen (OVA, 1 mg ml\(^{-1}\)) at a density of \(2.5 \times 10^6\) cells ml\(^{-1}\). The cells were then washed and cultured in 96-well flat bottom plates at a low density of \(1 \times 10^5\) cells ml\(^{-1}\) and varying amounts of IL-2 (units ml\(^{-1}\)). Proliferation was assessed after 48 h of culture with 6-h pulse with tritiated thymidine.

Fig. 2. Cell cycle progression of T cells from DO11.10 IL-2Rα+/– and DO11.10 IL-2Rα+/– mice in response to exogenous IL-2. Spleen cells from DO11.10 IL-2Rα+/– and DO11.10 IL-2Rα+/– mice were stimulated with antigen (OVA 1 mg ml\(^{-1}\)) for 48 h, washed free of antigen and labeled with CFSE (1 μM for 10 min at 37°C). Labeled cells were cultured at low density (\(1 \times 10^5\) cells ml\(^{-1}\)) for 7 days in either nothing (control), low dose (100 units ml\(^{-1}\)) or high dose (5 \(\times 10^5\) units ml\(^{-1}\)) of IL-2. Green fluorescence intensity (CFSE incorporation) was assessed in cells expressing transgenic TCR using the anti-clonotypic antibody KJ1-26.

still remain susceptible to anergy induction as ionomycin treatment still renders them unresponsive to subsequent challenge by antigen and APC.

In contrast, when DO11.10 IL-2Rα+/– T cells were tested for their anergy susceptibility in the same manner, a substantially different result was observed. Despite antigen activation and growth with high-dose IL-2, these cells remain resistant to anergy induction analogous to our previously described studies of naive T cells. This suggests that interaction of IL-2 with its high-affinity receptor plays an important role in the down-regulation of IL-2 production in T cells which cannot be compensated with high-dose IL-2 administration in IL-2Rα+/– T cells.

Identical results were observed when chemically fixed APCs were used to induce anergy instead of ionomycin (Table 3). In vitro cultured DO11.10 T cells were cultured with fixed APC and antigen for 20 h, washed and tested for their capacity to produce IL-2 upon re-stimulation with antigen and irradiated APC. Like ionomycin, antigen presented by fixed APC induced anergy in in vitro cultured DO11.10 T cells making them incapable of producing IL-2 with subsequent stimulation with antigen and functional APC. Naive DO11.10 T cells are resistant to anergy induction under the same conditions as they maintain the ability to produce IL-2 in response to antigen and APC. In contrast, exposure of in vitro activated DO11.10 IL-2Rα+/– T cells to antigen presented by fixed APC had no effect on their capacity to produce IL-2 upon re-stimulation with antigen and intact APC. Thus, these cells are still resistant to anergy induction and are thus similar to naive T cells. Taken together, these results clearly indicate that acquiring susceptibility to anergy induction is independent from IL-2-driven proliferation. Furthermore, the interaction between IL-2 and the high-affinity αβγ IL-2R but not the low-affinity βγR is an important step in this phenotypic change of the T cell.

Lack of anergy susceptibility in IL-2Rα+/– mice in vivo
Our previous investigations had demonstrated that priming of T cells in vivo with antigen rendered naive cells susceptible to anergy induction (6). To assess the role of the high-affinity IL-2–IL-2R interaction for this change in T cell phenotype in vivo, we primed DO11.10 IL-2Rα+/– mice with OVA in CFA. After 7 days, draining lymph nodes were harvested and activated T cells were identified as those bearing the transgenic TCR using the clonotypic antibody KJ1-26, and expressing low levels of CD62 using the antibody MEL-14 and were isolated by fluorescent-activated cell sorting. These in vivo activated T cells from DO11.10 IL-2Rα+/– were tested for their susceptibility to anergy induction using ionomycin and compared
CD4-positive transgene-positive T cells from DO11.10 IL-2r+/- and DO11.10 IL-2r-/- mice were stimulated with antigen (OVA 1 mg ml^-1) in the presence of low (100 units ml^-1) or high (500 000 units ml^-1) concentrations of IL-2 for 7 days. T cells were tested for susceptibility to anergy induction as described in Table 1.

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<td></td>
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Non-stimulated T cells from DO11.10 IL-2r+/- mice and in vitro activated spleen T cells from DO11.10 IL-2r+/- and DO11.10 IL-2r-/- mice (obtained by the methods described in Table 1) were incubated with media alone (none) or OVA peptide-pulsed EDCI-treated splenic APC as described in the Methods (1st stimulus). Recovered cells were tested for susceptibility to anergy induction as described in Table 1.

Discussion

Previous studies by our laboratory had demonstrated that naive T cells are resistant to anergy induction elicited by partial TCR signaling but could be made susceptible by either in vitro activation with antigen or antigen priming in vivo (6). In this study, we have established a system that can dissociate T cell activation by antigen from IL-2-mediated proliferation to assess each of their roles in the acquisition of susceptibility to anergy induction. This was achieved using the IL-2Rα-chain-deficient mouse line which eliminated the formation of and signaling through the high-affinity IL-2R (7).

In vitro activated T cells from DO11.10 IL-2r-/- mice cultured with OVA are totally resistant to anergy induction with ionomycin compared with wild-type DO11.10 T cells cultured under the same conditions. Thus, activation with antigen alone was not sufficient to induce susceptibility to anergy induction. However, there is a fundamental difference between these two cultured T cell populations. As demonstrated previously (7), T cells lacking the IL-2Rα chain failed to proliferate upon stimulation with antigen, while T cells with an intact receptor proliferated vigorously under the same conditions. Thus, the differences in their susceptibility to anergy in the two T cell populations could have reflected differences in proliferation in vitro.

We overcame this obstacle with the administration of high concentrations of IL-2 to the antigen-activated IL-2r-/- T cells that only bear the lower affinity to IL-2R. These culture conditions, in agreement with previous results (7), resulted in a strong proliferative response in these cells as reflected in assays measuring thymidine incorporation and CFSE labeling. An assessment using CFSE staining also showed that after 7 days in culture in high-dose IL-2, majority of the recovered T cells have progressed through cell cycle. It should be noted that these cells showed very heterogeneous decrease of CFSE in the initial stage of the culture (data not shown) but accumulate as a relatively homogeneous staining populations at the end of the culture period (Fig. 2). As a consequence, these culture conditions allowed us to examine comparable T cell populations from the IL-2r-/- and wild-type T cells to assess their susceptibility to anergy induction.

Using conditions that we and others have shown can induce anergy in vitro (1, 3, 6), the data in this report demonstrate that even after extensive proliferation in vitro, DO11.10 IL-2r-/- T cells remain resistant to anergy induction. These results indicate that activation of T cells with antigen and IL-2-mediated proliferation via the low-affinity βγ IL-2R is not sufficient to induce an anergy susceptibility. This also indicates that signaling through the high-affinity IL-2R composed of αβγ chains is necessary to induce susceptibility to anergy in T cells.

These observations raise questions regarding the role of the α chain of the IL-2R in the development of anergy and...
Table 4. In vivo immunized IL-2R−/− cells are resistant to anergy induction

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</table>

Naive and in vivo primed T cells from DO11.10 IL-2R−/− mice and in vivo primed T cells from DO11.10 IL-2R−/− mice were sorted based on their surface phenotype as described in the Methods. Cells were tested for susceptibility to anergy induction as described in Table 1.

tolerance in vivo. IL-2, initially identified and characterized as a growth factor for T cells in vitro (17), plays an important, yet paradoxical role in the regulation of the immune response. This became evident with the phenotype observed in mice that are deficient in either IL-2 production (IL-2−/−) (18) or signaling via the IL-2R (either α and β chains deficient) (7, 19). Such mice develop a severe lymphoproliferative condition with marked enlargement of the lymph nodes and spleen. In addition, severe autoimmune disease is commonly observed in these mice including autoimmune hemolytic anemia which ultimately contributes to an early death. Thus, despite IL-2’s prominent role as a T cell growth factor in vitro (17), these mouse models indicate that the absence of IL-2 production and/or signaling paradoxically result in unregulated lymphoproliferation and autoimmune disease suggesting that the IL-2 is important in the down-regulation of T cell responses. In our system, IL-2Rα−/− T cells expressing the DO11.10 TCR are not activated, and failed to demonstrate the lymphoproliferative disease which is a prominent feature of IL-2Rα−/− mice lacking a transgenic TCR. This would indicate that, in the absence of TCR transgene expression, a significant number of T cells in the IL-2Rα−/− mice would be continuously stimulated by either self or environmental antigens. Thus, the activation of self-reactive T cells in non-TCR transgenic mouse and a lack of regulatory T cell functions (20, 21) are responsible for the lymphoproliferation observed in mice deficient in IL-2 or in the IL-2Rs.

The IL-2R is a tightly regulated molecule expressed primarily on T cells emphasizing its important role in T cell regulation. The high-affinity receptor consists of α, β and γ chains (15). The γ chain of the receptor is shared by other cytokine receptors including IL-4, IL-7, IL-9 and IL-15 (22), while the β and γ chains are shared by IL-15 (23). Despite these shared components, studies of knockout mice have demonstrated the distinct features of each of these receptor components. Disruption of the γ chain leads to profound abnormalities that are manifested by severe combined immunodeficiency in humans with a profound deficiency in T cell function (22). β Chain-deficient mice like the IL-2x chain-deficient mice develop autoimmune disease and lymphoproliferation emphasizing the role of IL-2 in the regulation of these processes (19). However, β chain-deficient mice are also deficient in NK function and are devoid of memory CD8 and intestinal epithelial lymphocytes (IEL) which is not seen in the IL-2Rγ-deficient mice (17, 19). Subsequent studies examining mice with disruption of the α chain of the IL-15R confirm that the deficiencies in NK, CD8 and IEL cells are properties conferred by IL-15 signaling (24). Thus, the disruption of the α chain for both the IL-2R and IL-15R result in distinct and profound effects on the immune system.

Previous studies examining the role of the α chain of both the IL-2R and IL-15R have failed to demonstrate that they play a significant role in cytokine signaling (25, 26). Studies have utilized cell lines transfected with altered forms of the α chain of the IL-2R and have examined proliferation following receptor–ligand interaction. Although expression of the α chain increases the affinity of the receptor for its ligand (15), modification of its cytoplasmic tail leaving ligand binding unperturbed results in no effect on proliferation when compared with cells bearing an intact α chain (25, 27). Thus, in established cell lines the IL-2x chain’s role appears to be to primarily increase the receptor’s affinity for the ligand with little apparent influence on intracellular signaling. There are, however, limitations to these studies as they relate to our observations on anergy susceptibility. All of these studies were performed in established cell lines and not naive cells. Proliferation was the primary outcome measured and no previous studies have examined the role of other possible functions of the α chain such as inducing anergy susceptibility. As demonstrated in this study, proliferation can be easily elicited in antigen-activated T cells deficient in the IL-2Rα chain with high doses of IL-2, yet our studies indicate that proliferation and progression through cell cycle are insufficient to render a cell susceptible to anergy induction. Taken together, it is apparent that signal through αβγR and βγR has different effects on CD4 T cells for the certain functions, such as induction of anergy susceptibility. Additional studies are needed to further characterize this newly assigned function of the IL-2Rα chain.

In summary, our studies indicate that the α chain of the IL-2R plays an important role in the acquisition of susceptibility to anergy induction. In the absence of the α chain, the T cell is resistant to anergy induction and this cannot be overcome by stimulation through the low-affinity IL-2R with increased levels of ligand sufficient to drive proliferation. Our data suggest that T cell activation with antigen with resultant expression and signaling through the high-affinity IL-2R is a necessary event to facilitate changes in the cell to make it susceptible to anergy induction. In the absence of this signal, stimuli that induce this state of T cell unresponsiveness are compromised. This may account in part for the lymphoproliferation and autoimmunity observed in the IL-2Rα−/− deficient mice. Further characterization of this system is necessary to gain further insight on the regulation of this process.
Abbreviations

APC  antigen-presenting cell
CFSE  chloromethylfluorescein diacetate succinimidyl ester
EDCI  1-ethyl-3(3-dimethylaminopropyl) carbodiimide
IEL  intestinal epithelial lymphocytes
OVA  ovalbumin peptide
PMA  phorbol myristate acetate

References