Differences Between Responses of Naive and Activated T Cells to Anergy Induction

Robert J. Hayashi, Dennis Y. Loh, Osami Kanagawa and Fanping Wang

*J Immunol* 1998; 160:33-38; ;
http://www.jimmunol.org/content/160/1/33

**References** This article cites 33 articles, 25 of which you can access for free at:
http://www.jimmunol.org/content/160/1/33.full#ref-list-1

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

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

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Differences Between Responses of Naive and Activated T Cells to Anergy Induction

Robert J. Hayashi, Dennis Y. Loh, Osami Kanagawa, and Fanping Wang

T cell unresponsiveness to Ag stimulation can be induced by several means. The precise mechanism by which this process occurs remains poorly understood. Preincubating T cells with either EDCI-fixed APC or ionomycin is a proven means of inducing T cell anergy with reduced IL-2 production in response to Ag stimulation. Using T cells from mice expressing the TCR transgene DO11.10, which is specific for a peptide (323–339) derived from hen egg OVA, we demonstrate that naive cells obtained directly from the host are resistant to the anergy induction by either fixed APC or ionomycin. TCR transgenic mice also deficient in the recombination-activating gene-2 (RAG-2\(^{-/-}\)), preventing the formation of T cells with endogenous TCRs, were immunized with OVA, and in vivo activated T cells with low expression of CD62 were isolated. These primed cells possess the same sensitivity to ionomycin-induced anergy as in vitro activated cell lines. This unresponsive state most profoundly affects Ag-induced IL-2 production, with IFN-\(\gamma\) and IL-3 affected to a lesser degree and no effect observed on IL-4 production. Thus, T cells in vivo can be distinguished phenotypically by their susceptibility to anergic stimuli. Anergy so induced affects selected T cell functions. The Journal of Immunology, 1998, 160: 33–38.

Abbreviations used in this paper: RAG-2, recombination-activating gene-2; PKC, protein kinase C.
Materials and Methods

Mice

The DO11.10 TCR transgenic mice were described previously (21) and bred at the Washington University School of Medicine (St. Louis, MO) in a pathogen-free facility with monitored surveillance and serologic testing of sentinel mice every 3 mo. The RAG-2+/− mice were a gift from Dr. Y. Shinkai (22) and were used to establish DO11.10 TCR RAG-2+/−, H-2b mice. DO11.10 TCR RAG-2+/− mice were identified as those mice possessing T cells bearing the transgene-derived receptor using the Ab KJ1-26 and lacking cells expressing B cell surface markers as determined by FACS analysis of peripheral blood. Male and female mice used in the experiments discussed below were between 6 and 8 wk of age.

Flow cytometry

Cell surface immunofluorescence analysis of T cell populations was performed as described previously (10). In brief, 1 × 106 cells were incubated with the Ab at saturating concentrations at 4°C for 30 min, washed, and further incubated with FITC-, phycoerythrin-, or tricolor-conjugated appropriate secondary reagents for an additional 30 min. Control samples were prepared in the same manner, but without primary Ab or with an isotype-matched control. Samples were analyzed by a FACSscan analyzer (Becton Dickinson, Mountain View, CA) using the CellQuest program. The mAbs used include KJ1-26, specific for an Id expressed on the transgenic-derived TCR; DO11.10 (21); and MEL-14 (anti-CD62, Pharmingen, San Diego, CA). Lymph node cells from OVA-primed mice were stained with KJ1-26 and biotinylated MEL-14, with a goat anti-mouse FITC secondary Ab (Caltag, San Francisco, CA) for KJ1-26, and with phycoerythrin-avidin secondary staining for MEL-14. CD62 low KL1-26-posi-
tive cells were separated by FACSVantage (Becton Dickinson). The resulting populations were >98% pure.

In vitro cultures

In vitro cultured T cell lines were established by culturing 2 × 105 spleen cells from transgenic mice in DMEM with 5% FCS and OVA at a final concentration of 1 mg/ml for 10 days.

Induction of anergy with fixed APC

Preparation of fixed APC was performed under previously described conditions (1). Briefly, BALB/c spleenocytes were irradiated at a dose of 2600 rad and were incubated for 1 h on ice in 0.44 ml of 0.9% NaCl containing 75 mM EDCI (Calbiochem, La Jolla). The cells were washed extensively in a serum-free medium to stop the coupling reaction. Normal APC consisted of BALB/c spleenocytes irradiated at a dose of 2600 rad. T cells (2 × 105 cells) were incubated with EDCI-treated or normal APC (10 × 106 cells) in the presence of 50 μg of OVA23–323 peptide in 24-well plates (Corning, Corn-
ing, NY) in a total volume of 2 ml of DMEM with 5% FCS for 16 to 20 h. Cells were harvested and washed with medium three times, and 2 × 105 recovered cells were stimulated with normal APC (5 × 103) alone or with APC plus OVA (1 mg/ml) or PMA (10 ng/ml; Sigma Chemical Co., St. Louis, MO) and ionomycin (1 μM; Calbiochem, La Jolla, CA) in DMEM with 5% FCS a final volume of 200 μl in 96-well round-bottom plates (Costar, Cambridge, MA). After 24 h of culture, supernatants were harvested, and the presence of cytokine was measured.

Induction of anergy with ionomycin

Cells (1 × 106) were incubated with ionomycin (Calbiochem) at a final concentration of 1 μM for 20 h in DMEM with 5% FCS in a volume of 2 ml. Recovered cells were stimulated in the same manner as fixed APC-stimulated T cells for cytokine production.

Proliferation and lymphokine assays

IL-2-dependent T cell proliferation was measured by cultured T cells (2 × 105) in a final volume of 200 μl of DMEM with 5% FCS containing IL-2 at a final concentration of 10 U/ml in 96-well flat-bottom plates (Costar). The cells were incubated at 37°C for 24 h and harvested following a 6-h incubation with tritiated thymidine (New England Nuclear, Boston, MA) at 1 μCi/well. IL-2 activity was assayed using the IL-2-dependent CTLL line (23). IL-4 was measured using the IL-4-dependent cell line, 6/4, developed in this laboratory. IL-3 was assayed using the IL-3-dependent line FDC-P3 (24). IFN-γ activity was determined using the inhibition assay with the indicator line WEHI-279 (25). Units are defined as reciprocal dilution of culture supernatants that gave 50% of the maximum activity.

Results

Naive T cells are resistant to anergy induction by EDCI-fixed APC

Treatment of T cell clones with stimuli such as anti-CD3 Abs or Ag presented by chemically fixed APC results in the generation of an anergic state. T cells in this state are not capable of producing IL-2 upon challenge with Ag and functional APC. The effects of such treatments on freshly isolated naive T cells have not been clearly demonstrated. To facilitate this analysis, we used T cells from mice possessing a transgene-derived TCR (DO11.10) that is specific for OVA23–323 peptide presented by I-Ab. Most of this mouse line’s peripheral T cells possess the transgenic TCR, providing a naive T cell population whose function can be analyzed. Freshly isolated, naive T cells from DO11.10 transgenic mice and cell lines generated from in vitro activated T cells from this same mouse strain were incubated with fixed APC plus peptide for 16 to 20 h. The cells were washed and rested for 3 days. Surviving T cells were counted and stimulated with Ag and APC. The supernatants derived from these cultures were then assayed for IL-2 production. Surprisingly, naive T cells treated with fixed APC were capable of producing amounts of IL-2 comparable to those produced by untreated T cells (Fig. 1A). In contrast, in vitro cultured cell lines became anergic with exposure to Ag presented by fixed APC, with a failure to produce IL-2 upon rechallenge with Ag and functional APC. This experiment was performed three times, and in each instance, IL-2 production, in response to Ag stimulation, was maintained in the naive T cell population, while IL-2 production was virtually undetectable in the in vitro activated cell line after ionomycin treatment. This unresponsive state induced in the T cell lines was not due to decreased cell viability, as they were still able to proliferate when the treated cells were incubated with exogenous IL-2 (Fig. 1B). Naive, untreated T cells that did not express significant amounts of the IL-2R, as expected, did not proliferate in response to IL-2 exposure. However, naive T cells treated with Ag and fixed APC did respond to IL-2 exposure (Fig. 1B). This indicates that the Ag presented by the fixed APC induced a change in the naive cells, making them responsive to the cytokine. Thus, naive T cells appear to be fundamentally different from T cell clones in their responses to Ag presented by fixed APC and their susceptibility to become anergic by such means.

Differences in response to ionomycin treatments of naive T cells and T cell lines

Previous studies have demonstrated that anergy induction via chemically fixed APCs involves a rise in intracellular Ca2+ (8). Furthermore, this anergic state can be reproduced by raising intracellular Ca2+ levels in susceptible T cell clones with ionophores such as ionomycin. However, used in the context of additional stimuli, Ca2+ influx can result in distinctly different responses in the cell. This is illustrated in Table I. In vitro cultured cell lines derived from TCR transgenic mice were stimulated with PMA, ionomycin, and the combination of PMA and ionomycin. Alone, PMA and ionomycin were insufficient to elicit significant IL-2 production, while together they elicited a vigorous response. However, ionomycin and PMA had drastically different effects on the cell when the treated cells were subsequently challenged with Ag and appropriate APC. The ionomycin-treated T cell line failed to generate measurable IL-2 in response to Ag stimulation. PMA, in contrast, had no significant effect on the capacity of T cells to respond to the secondary stimulation. Thus, although both PMA and ionomycin failed to elicit any IL-2 production from the T cells when used alone, ionomycin, but not PMA, induced a unique change in the state of the cell to make it unresponsive to antigenic
stimulation. This effect, however, was dependent upon the state of the cell, as naive freshly isolated T cells maintained their ability to produce IL-2 with Ag stimulation even after ionomycin treatment (Table I). Naive cells, however, still appeared to be influenced by ionomycin, as the magnitude of the secondary response was greater than that of the response of the untreated population. This indicates that ionomycin induces a distinctly different state in the naive cell from that in the in vitro cultured T cell. Ionomycin was used in all subsequent experiments to further characterize the difference between naive and activated T cells. This method is cell density independent and thus excludes the possibility that the resistance of the naive cells to the fixed APC treatments is due to differences in engagement at the TCR level. The differences observed in this experimental system are more likely due to inherent differences in the T cell populations in their susceptibility to anergy induction.

In vivo primed T cells are analogous to T cell lines in their susceptibility to anergy induction

Most studies examining the effects of anergy-inducing stimuli have used T cell lines maintained in culture for at least several days. Since freshly isolated T cells are resistant to anergy induction, one explanation is that the maintenance of cells in culture renders them susceptible. Alternatively, if ionomycin sensitivity is a property acquired with T cell activation, primed T cells isolated directly from the immunized host should possess the same sensitivity to ionomycin as that observed in the T cell line. To address the susceptibility of in vivo primed T cells to anergy induction, it was necessary to acquire a means by which uniform cell populations could be isolated from the host in both primed and unprimed states. Ideally, this would entail the identification of a cell population with a single TCR and would exclude those cells that may possess alternative receptors. To accommodate these needs, DO11.10 mice were bred with mice who were homozygous for the disruption of the RAG-2 gene (RAG-2<sup>−/−</sup>) (22). This prevents the rearrangement of endogenous receptor genes and assures that all the T cells in the periphery possess a single, uniform TCR. These mice were used in the subsequent analysis.

DO 11.10 TCR RAG-2<sup>−/−</sup> transgenic mice were immunized in the footpad with OVA (100 μg) in CFA. The T cells from the draining lymph nodes were isolated 7 days after immunization. The in vivo primed T cell population was analyzed by surface immunofluorescence and compared with cells from nonimmunized mice. Lymph node cells from naive mice were stained with an anti-Ig Ab (KJ1-26) and counterstained with an anti-CD62 Ab.
As shown in Figure 2, the vast majority of TCR-positive cells in the naive mouse express high levels of CD62 (L-selectin). Upon priming with Ag, the number of cells in draining lymph node as well as in spleen increased significantly (Table II), and the expansion of Ag-reactive T cells accounted for this increase as the cells expressed the transgene-derived receptor, as measured by the Ab KJ1-26 (Fig. 2). A significant fraction of T cells from immunized mice were now low for CD62 expression, consistent with the findings of other studies characterizing the surface phenotype of activated T cells (26, 27).

To determine whether the in vivo activated population had acquired sensitivity to ionomycin-induced anergy, the low CD62 population was purified using FACS, so that $98\%$ of the cells were low for expression of CD62 yet still expressed the transgene-derived TCR. This purified T cell population was compared with freshly isolated naive mice, which were stained with the same Abs as the in vivo primed cells, and the in vitro cultured T cell line, all derived from the DO11.10 RAG-2$^{-/-}$ mouse line for their sensitivity to ionomycin. The three cell populations were incubated with ionomycin for 16 to 20 h, washed, and rechallenged with Ag and APC. Consistent with the previous studies, the in vitro cultured T cell line was rendered unresponsive with ionomycin treatment, while the naive T cell remained reactive after the same treatment (Table III). These cells were still viable and capable of IL-2 production.

Table II. Comparison of total cell number in the lymph node and spleen in naive and primed DO11.10 Rag-2$^{-/-}$ mice

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Lymph Node</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>0.15 ± 0.04</td>
<td>19 ± 10</td>
</tr>
<tr>
<td>Primed</td>
<td>1.80 ± 0.4</td>
<td>59 ± 20</td>
</tr>
</tbody>
</table>

*Total cell numbers obtained from the spleen and lymph nodes of DO11.10 Rag-2$^{-/-}$ mice primed with 100 µg of OVA in CFA in the footpad and harvested 7 days later are compared with age-matched naive mice. Numbers reflect mean ± SD of four mice in each group.

Table III. Similar sensitivities of in vivo-primed and in vitro-stimulated cell lines to ionomycin-induced anergy

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>1st Stimuli</th>
<th>2nd Stimuli</th>
<th>IL-2 (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>None</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>P + I</td>
<td>Ova</td>
<td>1,300</td>
</tr>
<tr>
<td></td>
<td>Ova</td>
<td></td>
<td>140</td>
</tr>
<tr>
<td>Ionomycin</td>
<td>None</td>
<td>P + I</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ova</td>
<td></td>
<td>1,400</td>
</tr>
<tr>
<td>Mel 14$^{-}$</td>
<td>None</td>
<td>Pma + Ion</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ova</td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>Ionomycin</td>
<td>None</td>
<td>P + I</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ova</td>
<td></td>
<td>3,300</td>
</tr>
<tr>
<td>Mel 14$^{-}$</td>
<td>None</td>
<td>Pma + Ion</td>
<td>10,000</td>
</tr>
<tr>
<td></td>
<td>Ova</td>
<td></td>
<td>660</td>
</tr>
</tbody>
</table>

*T cells from DO11.10 Rag-2$^{-/-}$ mice derived from naive T cells obtained from an unprimed animal (Naive), stimulation in vitro with Ag and APC for 7 days (Cell Line), or KJ1-26 positive, MEL-14 low cells obtained by fluorescent cell sorting of LN cells primed in vivo with OVA in CFA for 7 days (Mel 14$^{-}$) were each incubated with media alone (None), or ionomycin (1 µM) as the primary stimulus for 20 h. Each group was then washed and incubated with media alone (None), PMA and ionomycin (P + I), or OVA at 1 mg/ml with APC (Ova) as a secondary stimulus for 24 h, and the supernatants were subsequently assayed for IL-2 activity as outlined in Materials and Methods. The above results are representative of three separate experiments.
production upon stimulation with PMA and ionomycin. Thus, the unresponsive state elicited by ionomycin was not due to a toxic effect on the cells. When the in vivo primed, low CD62 T cells were exposed to ionomycin and tested for their Ag reactivity, their capacity to produce IL-2 in response to Ag was greatly reduced compared with the that of the same T cell population not exposed to ionomycin. This experiment was performed three times, and in each case, ionomycin treatments of the in vivo primed, low CD62 population reduced the amount of IL-2 production in response to Ag stimulation by at least 1 order of magnitude. This effect was simply not due to the effects of the Abs used to isolate the cells, since similar treatments of the naïve population failed to affect their resistance to ionomycin. Thus, in vivo activated T cells are sensitive to ionomycin-induced anergy induction in a fashion analogous to that of the in vitro activated T cell line.

The susceptibility to anergy induction is confined to IL-2 expression

Further analysis was performed to determine whether other cytokine production pathways were affected by ionomycin in the same fashion as IL-2. In vitro cultured T cells were incubated with ionomycin for 16 h and stimulated with Ag and APC. Culture supernatants were tested for IL-2, IL-3, IL-4, and IFN-γ. Similar to the previous assay, ionomycin-treated cells produced no IL-2 upon restimulation with Ag and APC. However, IL-4 production remained comparable to that of untreated T cells. In contrast, the amounts of IFN-γ and IL-3 produced by ionomycin-treated cells were significantly lower than those produced by nontreated cells, but were still readily detectable (Table IV). This experiment was performed three times. In each instance, IL-4 production following Ag stimulation did not decrease in response to ionomycin treatment, while the effects of ionomycin on IFN-γ and IL-3 expression failed to reduce the response more than 3- to 10-fold, with detectable activity remaining. This is in contrast to IL-2 production, for which ionomycin treatments resulted in no measurable biologic activity in the in vitro cell line after Ag stimulation. Thus, each of these cytokines must be regulated in a different fashion than IL-2, and anergy induction seems to be specific for IL-2 and IL-2-dependent proliferation of T cells in vitro.

Discussion

We have demonstrated that the naïve T cells are distinctly different from preactivated T cells in their susceptibility to anergy induction. In vitro cultured T cells can be rendered anergic by either Ag presented by fixed APC or by exposure to ionomycin. These T cells fail to produce IL-2 or proliferate upon rechallenge with Ag presented by functional APC. The treated cells are still viable, as they proliferate in response to exogenous IL-2 and produce IL-2 in response to PMA and ionomycin. In contrast, naïve T cells are resistant to such anergy induction and maintain their ability to generate IL-2. This resistance is not due to a lack of signaling of the T cells with these stimuli, since naïve T cells exposed to such stimuli exhibit an increased reactivity to IL-2 and greater cytokine-producing capacity than nontreated naïve T cells. This functional change indicates that events elicited in the naïve cell by the anergy-inducing stimuli are distinct from those induced by in vitro activated T cells.

The data presented above indicate that naïve cells can be converted into ionomycin-susceptible cells with conventional immunization. This was demonstrated in TCR transgenic mice on a RAG-2−/− background in which all the T cells expressed only one receptor. After priming, this uniform T cell population expanded in significant numbers in response to the Ag. A large fraction of cells expressed low levels of CD62 (L-selectin), but a significant number of T cells remained high in CD62 expression. Since the low CD62 population alone cannot account for the entire increase in the number of T cells, some T cells were stimulated by Ag and maintained high CD62 expression. We observed similar heterogeneity for IL-2R and CD44 (pgp-1) expression (data not shown). It is not clear at present whether this heterogeneity represents a distinct T cell activation state. However, it is likely that this heterogeneity represents different stages of the T cell as it undergoes Ag-driven proliferation. Alternatively, the increase in the total number of high CD62 cells could be due to the migration of T cells into the lymphoid tissues that have not yet encountered Ag and lowered their expression of CD62. Thus, to restrict our analysis to an Ag-primed population, we have used exclusively low CD62 T cells and have demonstrated that the conversion from a naïve to a primed T cell renders the cell susceptible to ionomycin-induced anergy. The relationship among surface phenotypic changes, cell cycle regulation, and susceptibility to anergy induction in primed mice requires further investigation to help elucidate a biochemical basis for the anergy susceptibility.

Other investigators have noted previously that naïve cells appeared to be uninfluenced by the effects of fixed APC treatments (28). These studies were unable to clearly demonstrate differences between primed and naïve cells in IL-2 production following exposure to fixed APCs and concluded that the means by which anergy is induced may only affect certain T cell subtypes. Our assay systems, however, were able to detect clear differences between naïve and primed T cells in their susceptibility to anergy induction. By using cell sorting for a low CD62 population to isolate in vivo primed T cells and by allowing the in vitro activated cell line to incubate for a sufficient amount of time in culture, we were able to analyze more uniform populations that were devoid of cells that may be at different stages of activation and thus may possess different susceptibilities to anergic stimuli. In addition, the use of ionomycin as the means of inducing anergy ensured that the T cells assayed were uniformly treated so that the response assessed was not dependent upon the degree of engagement of the cell’s receptor with a fixed APC, which may fail to induce anergy. Our data suggest that selecting for cells expressing low CD62 expression is a means of identifying a T cell population that is sensitive to the effects of anergic stimuli.

Ionomycin treatments have different effects on different cytokine production pathways. IL-2 production is dramatically reduced with subsequent Ag stimulation, while IL-4, in contrast, appears to be

Table IV. Ionomycin’s anergy induction primarily affects IL-2 expression in in vitro-cultured T cell lines with little influence on other cytokines

<table>
<thead>
<tr>
<th>Cytokine Assay</th>
<th>1st Stimuli</th>
<th>2nd Stimuli</th>
<th>IL-2</th>
<th>IL-3</th>
<th>IL-4</th>
<th>IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion</td>
<td>None</td>
<td>0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion</td>
<td>P + 1</td>
<td>5000 660 33 140</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion</td>
<td>Ova</td>
<td>100 2000 10 33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ion</td>
<td>Ova</td>
<td>0 660 33 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* In vitro-cultured T cell lines from DO11.10 Rag-2−/− were incubated with media alone, (None) or ionomycin (Ion) as a primary stimulus for 20 h, then washed and incubated with media alone (None), PMA and ionomycin (P + 1), or OVA plus APC (Ova) as a secondary stimulus for 24 h and assayed for cytokine activity.

* Results from cytokine assays for IL-2, IL-3, IL-4, and IFN-γ are expressed in U/ml. The above results are representative of three separate experiments.
unaffected. Ionomycin reduced the responses of IFN-γ and IL-3 production to Ag, but its effects on these two cytokines were incomplete. The T cell lines used in these experiments were only stimulated for 7 days and were thus heterogeneous. Cells within this mixture predominantly consist of CD4 Th cells expressing Th1 (IFN-γ) or Th2 (IL-4) cytokines at various stages of differentiation. Since IL-2 can be generated by cells that produce either Th1 or Th2 (29) cytokines, ionomycin must have influences on more than one population, since no significant IL-2 activity can be measured following such treatments.

One possibility accounting for the different effects of ionomycin on the expression of different cytokines is that ionomycin may render activated T cells unresponsive to Ag-induced protein kinase C (PKC) activation. Previous investigations have demonstrated the requirement for PKC activation for IL-2 expression, while other cytokines, such as IL-4, lack this dependence (30–32) This lack of kinase activation is not irreversible, since PMA and ionomycin can still induce IL-2 from anergized T cells. It is unclear whether a cytokine’s dependence on PKC activation directly determines its sensitivity to anergic stimuli. The specific biochemical events required for anergy induction remain to be defined and thus may involve additional pathways distinct from PKC activation and Ca²⁺ influx (33) Despite this possibility, our investigations have demonstrated that T cells can possess different susceptibilities to anergy induction, and the use of T cells that resist anergy induction would provide a useful tool for further investigation of the biochemical nature of this process.

Our findings presented in this report do not address the critical steps of tolerance induction in vivo or whether prior activation is required to induce tolerance in vivo. Furthermore, the physiologic role of anergy defined by a lack of IL-2-producing capacity in vivo is not clearly understood. The observations identify two distinct states for the T cell: the naive, anergy-resistant state and the primed, anergy-susceptible state. Further characterization of the two states and the molecular event that maintains each state will provide a better understanding of immune regulation.

References


