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Immunology

T-lymphocyte interleukin 2-dependent tyrosine protein kinase signal transduction involves the activation of p56^{lck}

(interleukin 2 receptor/tyrosine phosphorylation)

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ABSTRACT **Addition of interleukin 2 (IL-2) to IL-2-dependent T cells results in tyrosine protein kinase signal transduction events even though the IL-2 receptor α and β chains lack intrinsic enzymatic activity. Here we report that addition of IL-2 to IL-2-dependent human T cells transiently stimulates the specific activity of p56^{lck}, a member of the *src* family of nonreceptor tyrosine protein kinases expressed at high levels in T lymphocytes. The ability of IL-2 to induce p56^{lck} activation was found to be independent of the capacity of p56^{lck} to associate with either CD4 or CD8. Following IL-2 treatment, p56^{lck} was found to undergo serine/threonine phosphorylation modifications that resulted in altered mobility of the lck gene product on polyacrylamide gels. These observations raise the possibility that p56^{lck} participates in IL-2-mediated signal transduction events in T cells.**

Antigen-specific activation of mature peripheral blood T cells stimulates the synthesis and secretion of interleukin 2 (IL-2)—a cytokine critical for T-cell proliferation (1). To exert its proliferative effect on T cells, IL-2 must interact with specific cell surface receptors responsible for signal transduction culminating in the entry of the cells into S phase (2, 3). The high-affinity IL-2 receptor (IL-2R) is composed of at least two distinct subunits. The human IL-2Rα chain is a 55-kDa glycoprotein containing a 219-amino acid external domain, a 19-amino acid transmembrane domain, and a 13-amino acid cytoplasmic domain (4, 5). The IL-2Rβ chain is an ≈75-kDa protein possessing a 214-amino acid external domain, a 25-amino acid transmembrane domain, and a 286-amino acid cytoplasmic domain (6). Recent studies suggest that in addition to the α and β chains, several other proteins appear to participate in the formation of a multiprotein receptor structure and may be important for IL-2-dependent signal transduction events (7–11).

Although it is clear that addition of IL-2 to T cells expressing high-affinity IL-2R results in the transmission of a proliferative stimulus, the biochemical nature of the receptor's initial signaling mechanism has not been elucidated. However, increasing evidence suggests that tyrosine protein kinases (TPKs) and serine/threonine protein kinases may be involved in the IL-2-dependent proliferative signals (12–21). As none of the proteins comprising the IL-2R complex has been shown to possess detectable kinase activity, it is likely that enzymes distinct from the identified IL-2R components play roles in signal transduction.

We have evaluated whether one of the most abundant T-cell TPKs, p56^{lck}, is involved in signal transduction following the interaction of IL-2 with the IL-2R. Our results demonstrate that p56^{lck} TPK activity is rapidly stimulated following IL-2 addition and suggest that p56^{lck} undergoes subsequent IL-2-dependent serine/threonine phosphorylation alterations. These results are consistent with the hypothesis that TPKs such as p56^{lck} and as yet unidentified serine/threonine protein kinase(s) play a role in IL-2-induced signal transduction.

MATERIALS AND METHODS

Cells. The following IL-2-dependent human T-cell clones/lines were used: G619-3-53 (clone 53) is a previously described major histocompatibility complex (MHC) class II-specific CD4$^+$CD8$^-$ clone derived from a normal donor (22). Q302 is an alloreactive uniformly CD4$^-$CD8$^+$ cytotoxic line (derived from the same donor as clone 53), which is MHC class I (A2) specific. 975-V-421 (421) is a uniformly CD4$^-$CD8$^-$ TCR/CD3$^+$ line derived from the same donor as clone 53 following stimulation with murine C57/B6 spleen cells. The cells were grown following the appropriate antigenic stimulation in RPMI medium containing 20% screened fetal calf serum and recombinant IL-2. The cells were incubated in the absence of exogenous IL-2 for at least 12 hr prior to experimental use. Peripheral CD4$^+$CD8$^-$ human T cells were prepared as described (23).

Biochemical Assays. Immune complex protein kinase and immunoblot assays were conducted as described (24). Preparation of antibodies directed against *src* TPK family members has been described (25). All antibodies were obtained from rabbits immunized with synthetic peptides corresponding to amino acid sequences in the unique domain of the individual *src* family members (25). Antibodies to human CD4 were obtained following immunization of rabbits with a TrpE fusion protein containing the extracellular domain of CD4 (26).

RESULTS

TPK Signaling Accompanies Addition of IL-2. Alterations in cellular protein phosphorylation on tyrosine residues in response to the addition of IL-2 were evaluated by antiphosphotyrosine (APT) immunoblotting. Within 30 sec following addition of IL-2 to the CD4$^+$ G619-3-53 clone (clone 53) several T-cell proteins become reactive with APT antibodies (Fig. 1). Time-dependent changes in the reactivity of the various molecular mass protein species and alterations in the pattern of reactive proteins were evident for at least 30 min following IL-2 addition. The results of these experiments are consistent with the idea that IL-2 association with the high-affinity IL-2R results in the tyrosine phosphorylation of a

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Abbreviations: APT, antiphosphotyrosine; IL-2, interleukin 2; IL-2R, IL-2 receptor; TPK, tyrosine protein kinase.

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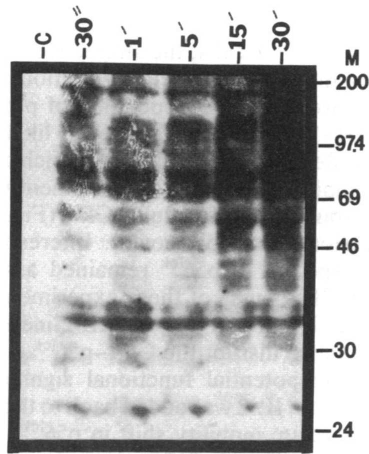
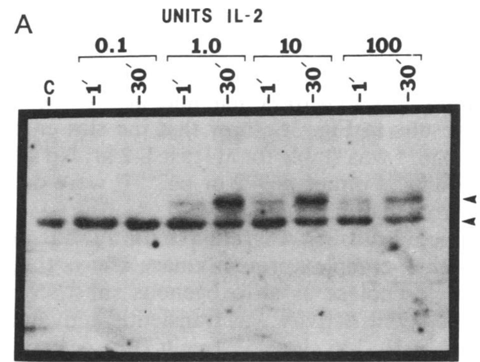


FIG. 1. Time course of APT-reactive proteins following addition of IL-2; APT immunoblot of clone 53 total cell lysates (50 μg of protein per lane) following addition of IL-2 (10 units per 1×10^6 cells). The positions of prestained molecular mass markers (M) (Amersham) are indicated (in kDa). C, control.

variety of T-cell proteins. Similar results were obtained with the other human T-cell clones (data not shown).

Expression of the *src* TPK Family in Human T Cells. The results of immune-complex protein kinase assays from $CD4^+$ or $CD8^+$ T-cell lysates and lysates derived from $CD4^+$ peripheral human T cells demonstrated the presence of only three members of the *src* family. As shown in Fig. 2, the human T-cell lines as well as the $CD4^+$ peripheral T cells were found to express detectable $p56^{lck}$, $p60^{fyn}$, and $p62^{c\text{-}yes}$. Of the *src* kinases expressed, $p56^{lck}$ was generally found to be more abundant than either $p60^{fyn}$ or $p62^{c\text{-}yes}$ based upon protein kinase activity. These results are consistent with previous results examining the expression pattern of the *src* family in heterologous populations of peripheral human T cells (25).

IL-2 Induces Alteration in the Gel Mobility of $p56^{lck}$. Addition of IL-2 to clone 53 T cells resulted in diminished SDS/polyacrylamide gel mobility of a portion of $p56^{lck}$ (Fig. 3*A*). The observed changes in $p56^{lck}$ mobility were found to be dependent upon the concentration of IL-2 added to the cells as well as dependent upon time following the addition of IL-2. The slower migrating lck-related band could be shifted to a species migrating with authentic $p56^{lck}$ following treatment with alkaline phosphatase (data not shown), suggesting



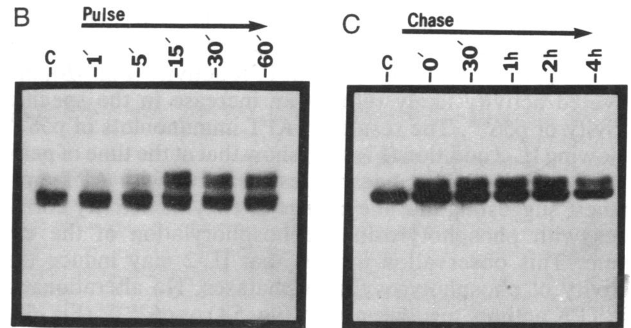


FIG. 3. Altered gel mobility of $p56^{lck}$ following addition of IL-2. (*A*) lck immunoblot of clone 53 total cell lysates (50 μg of protein per lane) at 1 min and 30 min following addition of the indicated amounts of IL-2 per 1×10^6 cells. The positions of $p56^{lck}$ and $p59\text{-}p60^{lck}$ are indicated by the arrowheads. (*B*) lck immunoblot of clone 53 total cell lysates (100 μg of protein per lane) prepared at the times indicated following IL-2 addition. (*C*) Clone 53 cells incubated for 30 min with IL-2 as noted above. The cells were washed and incubated for the indicated times in IL-2-free medium prior to cell lysis and lck immunoblot analysis.

that the alteration in protein mobility resulted from IL-2-dependent serine/threonine-induced kinase activity. Interestingly, the slower migrating form of $p56^{lck}$, which possessed an electrophoretic mobility of $\approx$59–60 kDa, was found to comigrate with the altered mobility species of $p56^{lck}$ induced following treatment of T cells with activators of protein kinase C (e.g., phorbol esters) previously described (ref. 24, data not shown). However, down-modulation of endogenous protein kinase C by chronic phorbol ester treatment or treatment of the cells with inhibitors of protein kinase

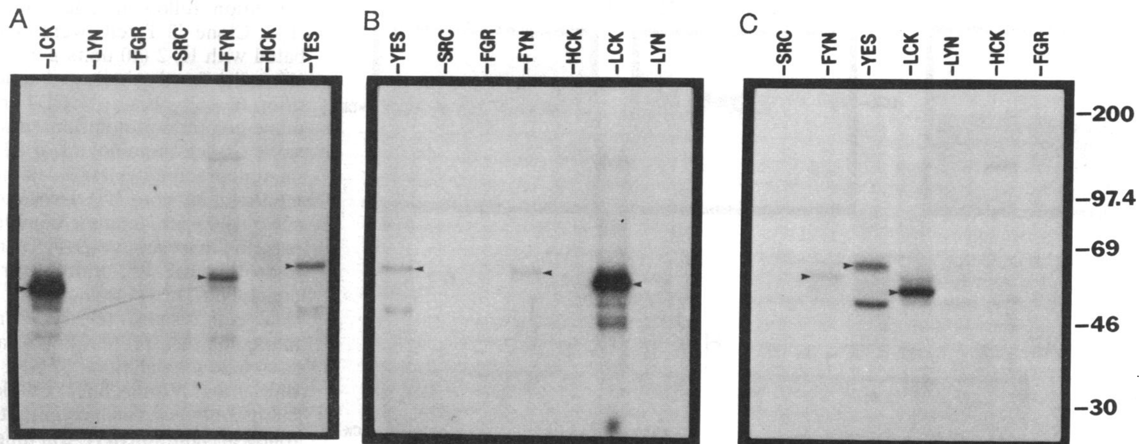


FIG. 2. *src* family expression in human T cells. T-cell lysates (100 μg of protein per reaction) were immunoprecipitated with the specified antibodies directed against individual members of the *src* family of TPK and immune-complex protein kinase assays were conducted. The arrowheads indicate the positions of the detected *src* family members. The positions of prestained molecular mass markers are indicated (in kDa). (*A*) Clone 53 lysate. (*B*) Q302 lysate. (*C*) $CD4^+CD8^-$ peripheral human T-cell lysate.

C such as H7 (27, 28) did not block the IL-2-induced mobility change of $p56^{lck}$ (data not shown). Alterations in the mobility of $p56^{lck}$ that could be detected as soon as 1 min following IL-2 addition appeared to be maximal at ≈15–30 min (Fig. 3*B*). The results in Fig. 3*C* show that the slower migrating species of $p56^{lck}$ was stable for at least 1–2 hr. No alterations in the mobility of either $p60^{fyn}$ or $p62^{c\text{-}yes}$ were detected in these experiments (data not shown).

IL-2 Induces Transient Alteration in the Specific Activity of $p56^{lck}$. Immune-complex protein kinase assays that utilized rabbit muscle enolase as an exogenous substrate revealed that $p56^{lck}$ enzyme activity was transiently activated following addition of IL-2 to clone 53 T cells (Fig. 4 *A* and *D*). The increase in $p56^{lck}$ activity was evident whether CD4-associated $p56^{lck}$ (Fig. 4*A*) or total cellular $p56^{lck}$ (Fig. 4*D*) was assayed. As the abundance of $p56^{lck}$ was not altered at the time of the detected increase in TPK activity (Fig. 4*B*), the elevated activity likely reflects an increase in the specific activity of $p56^{lck}$. The results of APT immunoblots of $p56^{lck}$ following IL-2 addition (Fig. 4*E*) show that at the time of peak TPK activation $p56^{lck}$ became less reactive with APT antibodies, suggesting that the change in enzyme activity correlates with phosphotyrosine dephosphorylation of the enzyme. This observation implies that IL-2 may induce the activity of phosphotyrosyl phosphatases. No alterations in the TPK activity of either $p60^{fyn}$ (Fig. 5*A*) or $p62^{c\text{-}yes}$ (Fig. 5*B*) were detected following addition of IL-2. Similar results were obtained with the $CD8^+$ T-cell line and with $CD4^+$ peripheral T cells (data not shown). The results of other experiments using the $CD4^-CD8^-$ T-cell line 421 (Fig. 5*C*) indicate that elevated $p56^{lck}$ activity following IL-2 treatment is not dependent upon the expression of either CD4 or CD8.

As previously noted, the mobility of a portion of $p56^{lck}$ was altered following addition of IL-2. The slower migrating form of $p56^{lck}$ was observed in the protein kinase assays (Figs. 4 *A* and *D* and 5*C*) as well as in lck and APT immunoblot assays. In the clone 53 T cells, the altered form of $p56^{lck}$ was more prominent in the CD4-associated fraction of $p56^{lck}$ (Fig. 4*B*) when compared with the population of $p56^{lck}$ not associated with CD4 (Fig. 4*C*). However, it is likely this observation simply reflects the normally high stoichiometry of the CD4–$p56^{lck}$ complex in these cells. Treatment with IL-2 did not affect the abundance of cellular CD4 (Fig. 4*F*) or CD4 surface expression (data not shown). Interestingly, the altered mobility species of $p56^{lck}$ remained associated with CD4 throughout the course of these experiments, indicating that phosphorylation of $p56^{lck}$ by the IL-2-induced kinase(s) was not sufficient to disrupt the CD4–$p56^{lck}$ complex.

To address the potential functional significance of the $p56^{lck}$ modifications, IL-2 was added back to the T cells at the time where maximum mobility shift in $p56^{lck}$ was observed (30 min) and the activity of $p56^{lck}$ was evaluated. The results of this experiment (Fig. 4 *G* and *H*) show that following IL-2 readdition, $p56^{lck}$ TPK activity was not increased even though the amount of IL-2 bound to the cells and the surface expression of IL-2 α and β chains at this time were the same as in naive cells (data not shown).

DISCUSSION

The results presented in this report indicate that $p56^{lck}$ is activated by IL-2 stimulation of T cells. These observations raise the possibility that $p56^{lck}$ represents one of the T-cell TPKs that aid in the mediation of IL-2 signal transduction. The data support the idea that $p56^{lck}$ may play a dual role in T-cell signal transduction events: (*i*) by complex formation with either CD4 or CD8, thereby participating in T-cell antigen-dependent activation events, and (*ii*) a role, independent of CD4 and CD8 interactions, in IL-2-dependent proliferative pathways.

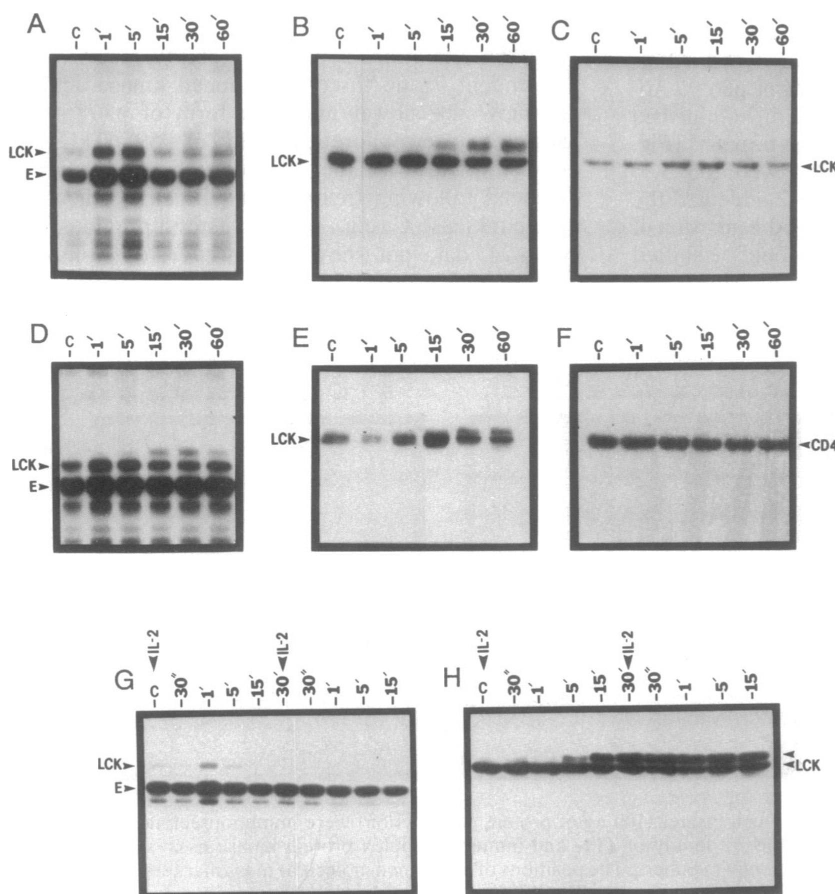


FIG. 4. Time course of $p56^{lck}$ activation following addition of IL-2. Clone 53 T cells were incubated with IL-2 (10 units per 1×10^6 cells) for the indicated times prior to cell lysis. (*A*) CD4 immune-complex protein kinase assays. (*B*) lck immunoblot of CD4 immunoprecipitates. (*C*) lck immunoblot of non-CD4-associated $p56^{lck}$. (*D*) lck immune-complex protein kinase assay. (*E*) APT immunoblot of lck immunoprecipitates. (*F*) CD4 immunoblot of total cell lysates. (*G*) CD4 immune-complex protein kinase assays following addition of IL-2 at time 0 and 30 min later. (*H*) lck immunoblot of total cell lysates following addition of IL-2 at time 0 and 30 min later. The positions of $p56^{lck}$ (LCK), CD4, and the exogenous protein kinase substrate rabbit muscle enolase (E) are indicated.

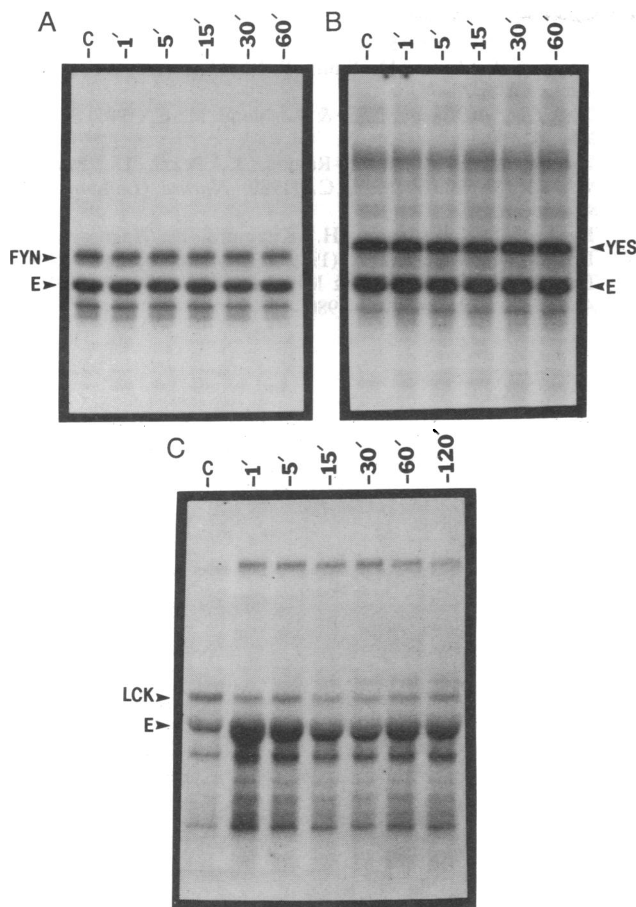


FIG. 5. $p60^{fyn}$ (*A*) and $p62^{c\text{-}yes}$ (*B*) immune-complex protein kinase assays of cell lysates prepared at different times following addition of IL-2 to clone 53 T cells (10 units per 1 × 10^6 cells). (*C*) $p56^{lck}$ immune-complex protein kinase assays of cell lysates prepared at the indicated times following addition of IL-2 to 421 $CD4^-CD8^-$ T cells (10 units/1 × 10^6 cells). The positions of $p60^{fyn}$ (FYN), $p62^{c\text{-}yes}$ (YES), $p56^{lck}$ (LCK), and rabbit muscle enolase (E) are indicated.

The data shown demonstrate that IL-2 is capable of inducing an early, transient change in specific activity of $p56^{lck}$. The mechanism through which $p56^{lck}$ is activated in these cells has not been determined, although the diminished reactivity of $p56^{lck}$ with APT antibodies suggests that IL-2-dependent phosphotyrosyl phosphatases may play a role in this process. Following enzymatic activation, $p56^{lck}$ was observed to undergo IL-2-dependent modifications by what appear to be serine/threonine protein kinases and it is the phosphorylation of $p56^{lck}$ that results in the altered mobility of the enzyme. Similar alterations in $p56^{lck}$ mobility have been observed following crosslinking of the T-cell receptor and as a consequence of treatment of T cells with activators of protein kinase C (24). An interesting contrast in the modifications induced by IL-2 compared with those induced by protein kinase C was the absence of CD4–$p56^{lck}$ complex dissociation following IL-2 treatment and the lack of surface CD4 down-modulation. As inhibition of protein kinase C abundance and/or function did not block the IL-2-induced alterations in $p56^{lck}$ gel mobility, our results suggest that T-cell kinases distinct from protein kinase C mediate these modifications. Though the significance of the IL-2-induced modification of $p56^{lck}$ is not known, our failure to reactivate $p56^{lck}$ with subsequent IL-2 treatment suggests that the modifications may play a role in regulating $p56^{lck}$ activity. Interestingly, we have observed in several IL-2-dependent murine T-cell lines that addition of mitogenic doses of IL-2 does not detectably alter $p56^{lck}$ gel mobility even though stimulation of protein kinase C produces the predicted $p56^{lck}$ gel shift. Thus, the presumed activation of nonprotein kinase C kinases observed in the human T cells is apparently not an obligate event in all IL-2-induced proliferative responses.

Although the data reported here exclusively evaluated IL-2-dependent signaling in human T cells, we have obtained similar results in other cells. In the IL-2-dependent murine T-cell lines CTLL-2 and HT-2 rapid activation of $p56^{lck}$ was also found to accompany IL-2 addition. Similar findings have also been observed with normal human large granular lymphocytes (LGLs) or natural killer (NK) cells and with several IL-2-dependent adult T-cell leukemia/lymphoma cell lines. Since some of these cells preferentially express the β chain of the IL-2R, our observations are consistent with those of previous reports (29–32) indicating that it is the β chain that appears to play the major role in interacting with cellular signal transduction components. As the normal expression pattern of the *lck* gene appears to be limited primarily to T cells and LGL/NK cells (25)—both IL-2-responsive cell types (1, 31)—our findings suggest that $p56^{lck}$ may be performing similar proliferation-related functions in both cell types. This observation may explain the presence of $p56^{lck}$ in LGL/NK cells that lack either CD4 or CD8 expression (25, 33) and suggest a potential function for $p56^{lck}$ in $CD4^-CD8^-$ T cells.

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