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Molecular Mechanisms of IL-2 Gene Regulation Following Costimulation Through LFA-1

Clara Abraham* and Jim Miller2†

The integrin LFA-1 serves as an accessory molecule in T cell activation. In addition to its well-known role as an adhesion molecule, LFA-1 can contribute to T cell activation and up-regulation of IL-2 gene expression. However, the specific mechanisms by which LFA-1 influences T cell activation have not been elucidated. Therefore, we examined the impact of LFA-1:ICAM-1 interactions on transcriptional and posttranscriptional IL-2 gene regulation, using a costimulation-negative cell line transfected with MHC class II alone, or in combination with ICAM-1 or B7-1. IL-2 transcription was assessed utilizing transgenic mice expressing an IL-2 promoter luciferase reporter construct crossed to DO11.10 TCR-transgenic mice, and IL-2 mRNA stability was evaluated by real-time RT-PCR. Comparison of naive and previously activated T cells demonstrates a dramatic increase in IL-2-luciferase transcription in activated T cells that can, in part, be attributed to downstream signaling events. Costimulation through LFA-1 enhances transcription of the transgenic reporter construct across a wide Ag dose range, but does not affect IL-2 mRNA stability. In contrast, CD28 costimulation is clearly mediated through up-regulation of IL-2 transcription and through enhancement of mRNA stability. These results indicate that the primary pathway whereby engagement of LFA-1 through its ligand ICAM-1 up-regulates IL-2 gene expression is through enhanced IL-2 transcription, in the absence of any effect on IL-2 mRNA stabilization. The Journal of Immunology, 2001, 167: 5193–5201.
expression was mediated through enhanced IL-2 transcription, IL-2 mRNA stabilization, or a combination of the two. In this study, we address the role of LFA-1 in IL-2 gene expression by stimulation of CD4⁺ T cells from DO11.10 TCR-transgenic mice with APC generated from cell lines transfected with costimulatory ligands. This system has the advantage of maintaining fluidity on both sides of the membrane during natural receptor-ligand interactions, and of isolating the contributions of individual accessory molecules to T cell activation. Our results indicate that the primary pathway whereby engagement of LFA-1 through its ligand ICAM-1 up-regulates IL-2 gene expression is through enhanced IL-2 transcription, in the absence of any effect on IL-2 mRNA stabilization.

Materials and Methods

Cells

A panel of transfectants in the fibrosarcoma cell line, 6132-PRO (Pro) expressing I-A^d alone (ProAd), or in combination with ICAM-1 (ProAd-ICAm) or B7-1 (ProAd-B7-1) has been previously described (11,14,38,39). In some experiments, Pro cells expressing I-A^d covalently linked to OVA_{223-239} (ProAdOVA), or in combination with ICAM-1 (ProAd-OVA-ICAm) are used (14). Transgenic mice expressing the murine IL-2 promoter region (positions −590 to +40 bp) upstream of the luciferase gene (40) (kindly provided by J. Hanke, Pfizer, Groton, CT) were crossed to DO11.10 TCR-transgenic mice (41). IL-2 promoter/luciferase transgenic mice were screened by PCR with primers to luciferase (forward primer: ATGGAAGCAGCCAAACTAATAGAAAAGCC, reverse primer: TTTGATCTTGGACATTGCAGTTCATTT). The CD4⁺ T cells were purified from lymph nodes of DO11.10 TCR-transgenic mice or IL-2 promoter/luciferase transgenic × DO11.10 TCR-transgenic mice (denoted by IL-2 luc/DO11.10) by negative selection using a mixture of anti-CD8 mAb (M5/114), followed by lysis with rabbit complement (Accurate Chemical, Westbury, NY) and removal of residual Ab-bound cells by incubation with an equal number of sheep anti-rat Ab-coated Dynabeads (Dynal, Oslo, Norway). The efficacy of the CD4⁺ T cell purification was monitored by lack of proliferation to 2.5 μg/ml Con A (Sigma-Aldrich, St. Louis, MO) and by flow cytometry. All cell lines were maintained in DMEM (Life Technologies, Gaithersburg, MD) supplemented with 10% FCS, 2 mM glutamine, 0.1 mM nonessential amino acids, 40 μg/ml gentamicin, and 50 μM 2-ME. G418 (200 μg/ml) and/or MXH (6 μg/ml mycophenolic acid, 250 μg/ml xanthine, and 15 μg/ml hypoxanthine) were added to the culture medium for maintenance of the transfectants. DO11.10 or IL-2 luc/DO11.10 TCR-transgenic activated CD4⁺ T cells were maintained by weekly passage with irradiated BALB/c (The Jackson Laboratory, Bar Harbor, ME) splenocytes, 0.2 μg/ml OVA_{223-239} peptide, and 10 μ/ml human rIL-2 (R&D Systems, Minneapolis, MN) with a maximum of two to three passages in culture, and then used in assays 7–21 days after stimulation.

Flow cytometry

Expression of transfected molecules was determined by flow cytometry using the anti-class II mAb MKD6, the anti-ICAM-1 mAb YN-1.7.1, and the anti-B7-1 mAb 16-10A1. T cells were phenotyped with a biotinylated anti-CD18 mAb, C71/16, M18/2 (BD PharMingen), and 2E6 (American Type Culture Collection). The Abs were immobilized onto 96-well flat-bottom plate by incubation in PBS at 4°C overnight. Plates were rinsed twice with PBS. Ficol-purified T cells (5 × 10⁶) were plated onto Ab-coated plates or cocultured with an equal number of Pro cell transfectants, and cytokine production from supernatants at 24 h was determined by capture ELISA (BD Pharmingen). T cell transfections

An IL-2 promoter reporter construct was produced and generously provided by L. Zuckerman utilizing the pGL2 basic vector (Promega, Madison, WI). A 378-bp SacI/BspHI fragment of the IL-2 promoter was subcloned into pGL2-basic vector. Activated DO11.10 CD4⁺ T cells were Ficol-purified and transfected with the reporter construct by electroporation. The transfected T cells were incubated with 10 μ/ml human rIL-2 (R&D Systems) in DMEM medium for 48 h, and then 2 × 10⁶ T cells were stimulated with an equal number of the Pro cell transfectants in the presence of Ag and analyzed for luciferase activity 16 h later.

Luciferase assay

Naïve (10 × 10⁶) or Ficol-purified activated (2 × 10⁶) IL-2 luc/DO11.10 CD4⁺ T cells were incubated with an equal number of Pro cell transfectants and various concentrations of Ag in six-well flat-bottom plates for 16 h. In other experiments, T cells were stimulated with anti-CD3 mAb in the presence or absence of anti-CD28 mAb or with PMA (10 ng/ml; Sigma-Aldrich) plus ionomycin (0.5 μM; Sigma-Aldrich). Cell extractions were prepared according to the manufacturer’s instructions (Promega) with lysis in cell lysis buffer. Samples were assayed for luciferase activity using a Monolight luminometer (Analytical Luminescence Laboratory, Ann Arbor, MI) (kindly provided by H. Singh, University of Chicago, Chicago, IL).

RNA preparation and real-time RT-PCR

For total IL-2 RNA measurements, Ficol-purified activated DO11.10 CD4⁺ T cells, 1 × 10⁶ T cells were incubated with 1 × 10⁶ Pro cell transfectants with Ag in a six-well flat-bottom plate. In other experiments, 1 × 10⁶ T cells were stimulated with plate-bound anti-CD3 mAb in the presence or absence of anti-CD28 mAb, anti-CD11a, or an Armenian hamster isotype control, anti-TNP mAb, as described above. RNA was isolated utilizing TRizol (Life Technologies), according to the manufacturer’s instructions. Residual DNA was eliminated with DNase I treatment (Promega). RNA was reverse transcribed to cDNA, and the level of IL-2 mRNA was determined by real-time PCR using the predescribed TaqMan probe and primers to IL-2 (Applied Biosystems, Foster City, CA) on the Prism 7700 (Applied Biosystems). The 18s endogenous control (Applied Biosystems) was used to normalize RNA. The ΔΔCt method for relative quantitation was utilized as per Applied Biosystems, and the IL-2 mRNA level in absence of Ag was used as the calibrator. In some cases, RNA stabilization was assessed through addition of 5 μg/ml actinomycin (Sigma-Aldrich) or 0.5 μg/ml cyclosporine (Calbiochem, San Diego, CA) to the T cell assays with harvesting of RNA at designated time points.

Results

Enhanced transcription of the IL-2 reporter transgene in activated vs naïve CD4⁺ T cells

To address the role of LFA-1:ICAM-1 interactions in the regulation of IL-2 gene expression upon T cell activation, we first examined its role in the regulation of IL-2 transcription. We utilized transgenic mice expressing the murine IL-2 promoter region (positions −590 to +40 bp) upstream of the luciferase gene (40) crossed to the DO11.10 TCR-transgenic mouse (denoted as IL-2 luc/DO11.10). To identify the specific role of costimulation, naive IL-2 luc/DO11.10 CD4⁺ T cells were stimulated with transfected cell lines that express class II, I-A^d, alone (ProAd), or in combination with the ligand for LFA-1, ICAM-1 (ProAd-ICAm), or the ligand for CD28, B7-1 (ProAd-B7) (11, 14, 38, 39). In this study, we refer to naïve CD4⁺ T cells as freshly isolated CD4⁺ T cells with the recognition that there is a small percentage of previously activated T cells in this population that will generally contain a rearranged endogenous TCR α-chain (43). Pro cell transfectants induce proliferation and IL-2 secretion in naïve CD4⁺ T cells from

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the IL-2 luc/DO11.10 mice in an analogous pattern, as we have
described with naive CD4 \(^{+}\) T cells from DO11.10 mice (Fig. 1; 
see Refs. 11 and 14). However, transcription from the IL-2-lucif-
erase transgene is detectable only upon stimulation with ProAd-B7 
at maximal doses of Ag (Fig. 2A). Naive CD4 \(^{+}\) IL-2 luc/DO11.10 
T cells do secrete IL-2 (Fig. 1B) upon stimulation with ProAd-
ICAM, albeit at very low levels. These results raise the potential 
caveat that the transgene might not accurately reflect the transcrip-
tional activity of the endogenous IL-2 gene.

To address this concern, we determined whether the IL-2-lucif-
erase response correlated with the induction of endogenous IL-2 
mRNA. Activated T cells are signifi-
cantly more responsive than 
naive T cells to Ag presented by ProAd, ProAd-ICAM, and 
ProAd-B7 cells. IL-2-luciferase transcription and IL-2 mRNA 
from activated T cells were detectable after Ag stimulation by 
ProAd and enhanced when costimulation was provided by either 
LFA-1 (ProAd-ICAM) or CD28 (ProAd-B7) (Fig. 2, A and B). 
Importantly, the pattern of expression of endogenous IL-2 mRNA 
in naive and activated CD4 \(^{+}\) IL-2 luc/DO11.10 T cells (Fig. 2B) 
stimulated under different costimulatory conditions correlates well 
with the pattern seen in IL-2-luciferase expression in the IL-2 luc/
DO11.10 mice (Fig. 2A). As further confirmation that expression 
of the transgenic reporter construct is not modulated by flanking 
sequences at the integration site, we found that transient transfection 
of an independent IL-2 reporter construct into activated 
DO11.10 CD4 \(^{+}\) T cells generated a similar pattern of luciferase 
expression (Fig. 2C). Therefore, the luciferase activity in IL-2 luc/
DO11.10 mice is a good indicator of IL-2 transcriptional activity, 
and the transgenic mice are useful for studying the effect of CD28 
and LFA-1 on IL-2 transcription.

The enhanced strength of signal and costimulation independence 
observed in the activated compared with the naive IL-2 luc/DO11.10 
CD4 \(^{+}\) T cells (Fig. 2, A and B) could be secondary to improved 
adhesion of the activated T cells to APC, or to an inherent increased 
ability of the activated T cells to transduce signals that lead to IL-2 
production. We therefore compared transcriptional activity in naive vs 
activated CD4 \(^{+}\) T cells utilizing methods independent of the adhesion 
characteristics of the T cells. Enhanced transcription of the IL-2-
luciferase transgene in naive vs activated T cells was observed when the
T cells were stimulated with plate-bound anti-CD3/anti-CD28, bypassing cell surface adhesion (Fig. 3A). Of note, however, is that the level of costimulation detected through anti-CD28 is not as great as through natural ligand interactions (Fig. 2A). These results indicate that the difference in response between naive T cells and activated T cells cannot be attributed to a diminished ability of naive T cells to form cell:cell conjugates. Furthermore, this difference was also seen when the T cells were stimulated with PMA/ionomycin, bypassing proximal signaling events (Fig. 3B). Therefore, the increased response in activated T cells appears to be, at least in part, to an increased sensitivity of downstream signaling events or changes in transcriptional regulation.

**Both CD28 and LFA-1 costimulation can enhance IL-2 transcription**

The decreased costimulation dependence for IL-2 secretion and luciferase activity in the activated T cells provides an advantage in our ability to analyze IL-2 transcription in this system. Stimulation of the activated T cells with ProAd alone can be used as the baseline from which to assess additional CD28- or LFA-1-mediated effects on IL-2 expression above and beyond that observed through TCR stimulation alone. To evaluate the effect of LFA-1:ICAM-1 interactions on IL-2 production in activated CD4+ T cells, IL-2 secretion, IL-2 mRNA production, and IL-2-luciferase expression were measured in an Ag dose response. Ag presentation by ProAd does induce expression from the IL-2-luciferase reporter construct, and this level of expression is enhanced by costimulation through LFA-1 across the dose response (Fig. 4A). To verify that the LFA-1-mediated enhanced IL-2-luciferase transcription did not simply reflect a shift in the dose response, we utilized Pro cell transfectants that express I-A^d covalently linked to the OVA(323–339) peptide in the absence (ProAd/OVA) or presence of ICAM-1 (ProAd/OVA-ICAM) (14). These cells present 100-fold more functional class II:peptide complexes than can be presented when ProAd is loaded with maximal concentrations of exogenous OVA peptide (14). ProAd/OVA-ICAM-stimulated CD4+ IL-2 luc/DO11.10 T cells increase the maximum response of IL-2 reporter luciferase activity in comparison to that with ProAd/OVA stimulation (Fig. 4B). Thus, LFA-1 costimulation can enhance IL-2 transcription even at supraoptimal peptide doses. Similar effects of LFA-1 costimulation were seen when expression from the endogenous IL-2 gene was monitored (Fig. 4, C and D). As the relative level of IL-2 transcriptional activity correlated well with the level of IL-2 mRNA and IL-2 secretion, the ability of LFA-1 to promote IL-2 transcription may account for the ability of LFA-1 to costimulate T cell proliferation.

CD28 costimulation can also enhance expression of the IL-2-luciferase construct (Fig. 4A). In general, CD28 was 2- to 3-fold more effective than LFA-1 at enhancing IL-2-luciferase transcription, and 10- to 20-fold more effective at inducing IL-2 secretion. However, in some experiments, LFA-1 and CD28 costimulation induced equivalent levels of IL-2 transcription, but CD28 still induced a substantial increase in IL-2 secretion over that observed with LFA-1. Thus, CD28-mediated enhanced IL-2 transcription alone cannot account for the ability of CD28 to promote IL-2 production. This is most evident in Fig. 4 at high doses of Ag in ProAd-B7-costimulated T cells, where IL-2 transcription plateaus, while IL-2 mRNA and IL-2 secretion continue to increase. The data support the important role of CD28 costimulation in regulating IL-2 production through enhanced mRNA stability (see below).

**FIGURE 3.** Stimulation of naive and activated IL-2 luc/DO11.10 CD4+ T cells is independent of cell-cell interactions. Naive (10 × 10^6) or activated (2 × 10^6 in A; 10 × 10^6 in B) IL-2 luc/DO11.10 CD4+ T cells, as described in Fig. 2, were stimulated with plate-bound anti-CD3 alone or in combination with anti-CD28 (each at 10 μg/ml) (A) or PMA/IO (10 ng/ml) plus ionomycin (0.5 μM) (B). T cells were lysed at 16 h, and luciferase activity was measured with normalization to background activity of T cells alone.

**CD28, but not LFA-1, costimulation induces IL-2 mRNA stability**

The enhanced IL-2 transcription upon LFA-1 costimulation clearly contributes to increased IL-2 mRNA; however, IL-2 gene expression is also regulated posttranscriptionally. As the predominant posttranscriptional regulation of IL-2 expression is through stabilization of IL-2 mRNA, we assessed the effect of LFA-1 stimulation on the t1/2 of IL-2 mRNA. Activated DO11.10 CD4+ T cells were incubated with the Pro cell transfectants in the presence or absence of Ag for 4 h. Actinomycin or cyclosporine was then added to block IL-2 transcription, and levels of IL-2 mRNA were determined at various time points. Actinomycin is traditionally used to inhibit transcription for purposes of studying mRNA stability; however, IL-2 mRNA has been found to be stabilized upon incubation with actinomycin (44). Initially, we performed the IL-2 mRNA stabilization experiments with actinomycin, but we also found artificial stabilization of IL-2 mRNA (data not shown).

Therefore, the majority of experiments were conducted utilizing cyclosporine. Cyclosporine blocks IL-2 transcription by inhibiting calcineurin-dependent nuclear localization of NF-AT (45), and therefore has been used to specifically study IL-2 mRNA stabilization (46). In activated DO11.10 CD4+ T cells, stimulation with ProAd-ICAM did not result in stabilization of IL-2 mRNA compared with TCR stimulation alone. On occasion, ProAd-ICAM-stimulated T cells demonstrated a more rapid decline in IL-2 mRNA t1/2 in comparison with ProAd-stimulated T cells (Fig. 5A). Under these same conditions, stimulation with ProAd-B7 did increase the t1/2 of IL-2 mRNA to 4–6 h (Fig. 5, A and B), corroborating the known ability of CD28 to mediate IL-2 mRNA stabilization (35, 37, 46, 47). These results indicate that LFA-1 costimulation does not mediate stabilization of IL-2 mRNA in DO11.10 CD4+ T cells.
In contrast to our results, stimulation of LFA-1 in combination with anti-TCR stimulation in human T cells has been reported to stabilize the mRNA for a number of proteins, including IL-2 (48, 49). To resolve this discrepancy, we evaluated LFA-1-mediated effects on IL-2 mRNA stability under a number of different activation conditions. The inability of LFA-1 to stabilize IL-2 mRNA in the activated DO11.10 CD4⁺/H11001 T cells was not Ag dose-dependent, as both suboptimal (low Ag dose) and supraoptimal (using ProAd/OVA and ProAd/OVA-ICAM transfectants) TCR signals did not reveal enhanced IL-2 mRNA stability through LFA-1 costimulation (data not shown). The lack of IL-2 mRNA detected in ProAd-stimulated naive DO11.10 CD4⁺ T cells (Fig. 2B) does not allow for detection of LFA-1-mediated IL-2 mRNA stabilization relative to TCR stimulation in this population. Therefore, we evaluated IL-2 mRNA expression in T cells stimulated under conditions that inhibit Th cell differentiation (50). IL-2 mRNA is detected upon stimulation through the TCR alone in these Th precursor (Thp) cells, but IL-2 mRNA stabilization upon additional LFA-1 stimulation was not detected (data not shown). The mRNA of a number of other cytokines, such as IFN-γ, also contain AU-rich elements and are regulated through mRNA stabilization.

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cells resulted in enhanced IL-2 mRNA levels (Fig. 6A) and IL-2 secretion (data not shown) over that of anti-CD3 mAb stimulation alone. However, while anti-CD28 mAb stimulation stabilized IL-2 mRNA, anti-CD11a mAb stimulation did not (Fig. 6B). Of note is that the costimulation provided by Ab cross-linking through either CD28 or LFA-1, as measured by IL-2 mRNA levels, is not enhanced to the same degree as that observed upon natural ligand interactions. Therefore, in the context of a murine system utilizing natural ligands and cell-cell interactions, LFA-1:ICAM-1 interactions allow for enhanced IL-2 mRNA expression and subsequent enhanced IL-2 secretion through up-regulation of IL-2 transcription in the absence of IL-2 mRNA stabilization.

Discussion

The dependence of naive and activated CD4+ T cells on IL-2 for full activation and expansion points to IL-2 gene regulation as an essential juncture in effective T cell activation. Checkpoints of this regulation include transcriptional, posttranscriptional, and translational mechanisms (46, 51, 52). Various signals, including those through TCR stimulation alone, might result in activation of certain transcription factors necessary for IL-2 transcription, but ultimately IL-2 secretion requires the cooperative binding of a number of transcription factors to enable IL-2 transcription (53), along with adequate IL-2 mRNA \( t_{1/2} \). In this study, we have used transgenic mice expressing a reporter construct under the regulation of the IL-2 promoter, and analysis of IL-2 mRNA kinetics, to study the influence of LFA-1 on IL-2 gene regulation. In T cells containing the IL-2-luciferase reporter transgene, LFA-1 costimulates an enhanced level of IL-2-luciferase transcription that can account for the LFA-1-mediated costimulation of endogenous IL-2 expression. Furthermore, the enhanced level of endogenous IL-2 mRNA expression in primary murine T cells upon costimulation through...
LFA-1 is not mediated by stabilization of IL-2 mRNA, as opposed to what is observed upon costimulation through CD28. Therefore, we find that LFA-1:ICAM-1 interactions in activated DO11.10 CD4⁺ T cells influence IL-2 expression through transcriptional mechanisms, in the absence of mRNA stabilization.

The increased sensitivity to Ag dose, decreased costimulation dependence, and enhanced magnitude of effector responses by activated CD4⁺ T cells compared with naive T cells has been well described (7, 54–57). Despite the decreased costimulation dependence in activated IL-2 luc/DO11.10 CD4⁺ T cells, LFA-1:ICAM-1 interactions consistently enhance IL-2 transcription compared with the engagement of the TCR alone. Therefore, as observed in naive CD4⁺ T cells (11, 13, 14), LFA-1 does effectively costimulate activated T cells. The mechanism of these altered characteristics of effector T cells, in comparison with those of naive T cells, is not yet understood. Considerations have included the preferential selection of T cells utilizing a higher affinity TCR, an increased precursor frequency of signaling molecules, or a more efficient arrangement of these molecules, as has been described in the case of the targeting of Lck to the CD8 coreceptor in the case of CD8⁻ T cells (57). Our data demonstrate that the differential magnitude of response in naive vs activated T cells was maintained upon PMA/ionomycin stimulation, suggesting that downstream signals and/or transcriptional regulation play a significant role in this differential response.

The lack of detectable luciferase activity upon stimulation of naive CD4⁺ T cells from IL-2 luc/DO11.10 mice with ProAdICAM was initially surprising because endogenous IL-2 mRNA is readily detectable by RT-PCR (11) and real-time RT-PCR (data not shown) in the same T cells. Although the IL-2-luciferase transgene contains the dominant proximal enhancer sequence, it lacks the locus control region that provides for integration site-independent expression (58). Therefore, expression of this transgene relies on integration into a site that is accessible in T cells. However, low levels of IL-2-luciferase transcription in the naive CD4⁺ T cells appear to be a threshold-related phenomenon, not an artifact of flanking regions at the integration site of the transgene. The increased levels of transcription detected in activated CD4⁺ T cells from IL-2 luc/DO11.10 mice are consistently observed under different costimulation conditions, with anti-CD3/anti-CD28, and with PMA/ionomycin. Furthermore, the level of IL-2-luciferase expression demonstrates analogous regulation to that of endogenous IL-2. Therefore, the IL-2 luc/DO11.10 mice serve as a useful system to better define the kinetics and dose-response characteristics of IL-2 gene expression through LFA-1 stimulation.

The interaction of LFA-1 with ICAM-1 in DO11.10 CD4⁺ T cells mediates the up-regulation of IL-2 mRNA relative to TCR stimulation alone by transcriptional regulation in the absence of IL-2 mRNA stabilization. We have considered two possible mechanisms that might account for the ability of LFA-1 to regulate IL-2 gene transcription. First, LFA-1 itself might initiate a signaling pathway that, along with signals generated from the interaction of the TCR with its ligand, provides for T cell activation. There is much evidence that integrins can transduce important biological signals, and this role has been well documented in nonlymphoid cells (for reviews, see Refs. 17, 59, and 60). A number of these signals, such as Vav (61), JNK (37, 62), and JAB1 (63), can functionally cooperate to enable IL-2 gene expression. However, in T cells, LFA-1 engagement has not been clearly associated with a distinct intracellular signaling pathway. There is evidence that coligation of LFA-1 and TCR can lead to a sustained intracellular calcium response, increased inositol phospholipid hydrolysis, and association with DNAx accessory molecule-1; increased focal adhesion kinase, proline-rich tyrosine kinase 2, JNK, PI3K, and extracellular signal-related kinase-2 activity (18–25); and recruitment of protein kinase C-θ to the immunological synapse.⁴ Differential signals transduced through LFA-1 and CD28 most likely contribute to the difference in IL-2 transcription levels, as well as the differential ability to stabilize IL-2 mRNA. These differences have not been defined, although PI3K activity demonstrates a differential response in CD8⁺ T cells to the two modes of costimulation (21). Whether the enhanced responses upon LFA-1 engagement result from increased signals mediated through the TCR complex or independent signals transduced through LFA-1 and, if so, how these LFA-1-mediated signals are integrated with TCR signaling pathways have not been clearly established.

Second, LFA-1 may function in the structural organization of the adhesion complex between T cells and APC, termed the immunological synapse or supramolecular activation cluster. LFA-1 segregates into the outer perimeter of the adhesion complex, while TCR and engaged MHC-peptide complexes, as well as other molecules, segregate into a small central subdomain of the cell-cell contact region (27, 28). In fact, class II:peptide and LFA-1:ICAM-1 interactions in a lipid bilayer system are sufficient to allow for the formation of the immunological synapse (28). The focal concentration of TCR within the adhesion complex might facilitate the engagement of the TCR on a limited number of MHC:peptide complexes (64) and/or might allow for more efficient lateral interactions that ultimately lead to ligand-induced multimerization of the TCR (65). The level of TCR oligomerization could alter the magnitude and quality of TCR signaling (66–68). Given the association of LFA-1 with the cytosome (69–74) and its enhanced recruitment into lipid rafts (75), LFA-1 may be contributing to the assembly and organization of the various Ag receptors, coreceptors, and adhesion and signaling molecules within immunological synapse (26), which ultimately contributes to T cell activation. These functions of LFA-1 may play a role in the recruitment of the TCR and associated signal-transducing molecules to lipid rafts upon T cell activation (76–80), which, in turn, influence subsequent signaling events, such as a sustained calcium signal (19). Finally, the ability of LFA-1 to reorganize the actin cytoskeleton in the context of TCR engagement has been reported to be a form of anchorage dependence, enabling G₁ to S phase transition with subsequent IL-2 secretion (24).

We have also found that CD28 costimulation can enhance IL-2-luciferase transcription. In previous studies, the ability of CD28 to enhance IL-2 transcription has been somewhat controversial. This pathway has been best established in human T cells, where CD28 costimulation targets a response element (CD28RE) within the proximal IL-2 enhancer that binds AP-1 and NF-κB (30–32, 81, 82). The ability of CD28 to enhance JNK activity and induce AP-1 and NF-κB activation is consistent with its role in up-regulation of IL-2 transcription through the CD28RE (83–87). However, in most studies, transcriptional activity from this site is only detectable when CD28 costimulation is supplemented with PMA. Furthermore, most of the data have been generated in Jurkat T cell tumor lines. In one study of normal human T cells, CD28 costimulation did increase expression of a transfected IL-2-luciferase reporter construct, but mutation of the CD28RE had little effect on transcriptional activity (33). In murine Th1 clones, stable transfection of an IL-2 reporter construct has not supported a role for CD28 costimulation in IL-2 transcription (36, 88). Finally, recent results from transgenic mice expressing green fluorescent protein inserted into the IL-2 locus have shown that anti-CD28 can costimulate anti-CD3-mediated induction of green fluorescent protein expression in freshly isolated T cells.

⁴ C. E. Sedwick, K. Blaine, and J. Miller. Focusing of PKC-θ in the c-SMAC and activation of NF-κB is mediated by CD28 costimulation. Submitted for publication.
cells (89). Our results using the IL-2-luciferase construct and following endogenous IL-2 mRNA expression clearly demonstrate that CD28/B7-1 interactions enhance IL-2 transcription during Ag stimulation of primary murine T cells.

In addition to transcriptional regulation, IL-2 expression is also controlled by posttranscriptional mechanisms, most notably mRNA stabilization. We have found that costimulation through LFA-1 does not stabilize IL-2 mRNA in DO11.10-activated CD4+ T cells. In contrast, two recent reports have indicated that LFA-1 costimulation of human T cells can mediate stabilization of several mRNA molecules, including IL-2 (48, 49). Most of our understanding of the mechanism of IL-2 mRNA stability comes from studies of CD28 costimulation (35, 37, 47, 62). The rapid degradation of unstable mRNA molecules is mediated by AU-rich regions in the 3′ untranslated region (UTR). CD28-mediated stabilization of IL-2 mRNA is dependent on sequences in the 5′ UTR and the coding region as well as the 3′ UTR (36, 37). Within the IL-2 3′ UTR there are several clusters of AU-rich elements, and JNK-mediated stabilization requires the first cluster of four AUUUA pentameric regions (37). In contrast, in the case of urokinase plasminogen-activating receptor mRNA, LFA-1 activation is able to modulate the effects of a destabilizing nonanumeric AU-rich sequence in the 3′ UTR (48). Finally, two RNA-binding proteins, nucleolin and YB-1, bind to the 5′ UTR in human IL-2 to mediate JNK-induced stabilization (62). However, it is not clear whether IL-2 mRNA stability is regulated the same in murine and human T cells. Although CD28-mediated IL-2 mRNA stability is thought to be transmitted through JNK in human Jurkat T cells (37), JNK is not required for CD28-mediated costimulation of T cell activation and IL-2 production in mice (90). Although we cannot exclude technical differences, such as the nature of the stimulatory Abs or the responding T cells used, these results raise the possibility that differences in the signaling pathways and RNA-binding proteins, as well as structural difference in the IL-2 gene, may contribute to the differences in regulation of human vs murine IL-2 mRNA.

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References


44. Bachmann, M. F., A. Gallimore, S. Linkert, V. Cerundolo, A. Lanzavecchia, J. S. Andersen, G. Gaietta, K. Ju


66. The Journal of Immunology 5201


