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IL-2 and IL-15 Regulate CD154 Expression on Activated CD4 T Cells[1]

Søren Skov,[2]*† Mark Bonyhadi,* Niels Ødum,† and Jeffrey A. Ledbetter*

The cellular and humoral immune system is critically dependent upon CD40-CD154 (CD40 ligand) interactions between CD40 expressed on B cells, macrophages, and dendritic cells, and CD154 expressed primarily on CD4 T cells. Previous studies have shown that CD154 is transiently expressed on CD4 T cells after T cell receptor engagement in vitro. However, we found that stimulation of PBLs with maximal CD28 costimulation, using beads coupled to Abs against CD3 and CD28, led to a very prolonged expression of CD154 on CD4 cells (>4 days) that was dependent upon autocrine IL-2 production. Previously activated CD4 T cells could respond to IL-2, or the related cytokine IL-15, by de novo CD154 production and expression without requiring an additional signal from CD3 and CD28. These results provide evidence that CD28 costimulation of CD4 T cells, through autocrine IL-2 production, maintains high levels of CD154 expression. This has significant impact on our understanding of the acquired immune response and may provide insight concerning the mechanisms underlying several immunological diseases. *The Journal of Immunology,* **2000, 164: 3500–3505.**

The current model of a T cell-mediated response suggests that T cells are primed in the T cell zone of secondary lymphoid organs, primarily by dendritic cells. The initial interaction requires cell to cell contact between Ag-loaded MHC molecules on APC and the TCR-CD3 complex on T cells. Engagement of the TCR-CD3 complex induces CD154 expression predominantly on CD4 T cells that in turn activates the APC through CD40 engagement, leading to strong activation of their Ag presentation efficacy (1). This is caused partly by up-regulation of CD80 and CD86 expression on the APC, both of which are ligands for the important CD28 costimulatory molecule on T cells. However, CD40 engagement of APC also leads to prolonged surface expression of MHC-Ag complexes, expression of ligands for 4-1BB and OX-40, that are potent costimulatory molecules expressed on activated T cells. Furthermore, CD40 engagement leads to secretion of various cytokines (including IL-12, IL-15, TNF- α, IL-1, IL-6, and IL-8) and chemokines (including RANTES, macrophage-inflammatory protein-1α, macrophage-inflammatory protein-1β, and mannose chemoattractant protein-1), which have important effects on both APC and T cell activation and maturation (1, 2). Humans with a genetic defect in the CD154 gene and knockout mice lacking either CD40 or CD154 have emphasized the crucial role of CD40-CD154 interactions for generation of a thymus-dependent humoral immune response and major parts of the cellular immune response (3). Not surprisingly, subsequent studies have demonstrated a central role of CD40-CD154 interactions for generation of protective T cell-mediated tumor immunity, conversion of tolerant tumor-specific CD4 and CD8 cells, efficient clearance of viral infections, and generation of CD8 effector CTLs (4–10). Thus, the CD40-CD154 interaction is vital for the initiation and duration of the delicate process where T cells and APCs undergo mutually beneficial interactions leading to activation of the acquired immune system.

Materials and Methods

Cells, culture, and activation

PBMC were obtained from healthy human volunteer donors using standard procedures. Phagocytic cells were depleted by incubation with uncoated Dynal beads, two beads per cell for 2 h at 37°C, followed by magnetic depletion (monocytes and macrophages efficiently phagocytose the beads). Cells were stimulated with Dynal beads coupled to anti-CD3 (OKT-3) and anti-CD28 (9.3) Abs (11), three beads per cell. Pure CD4 T cells were obtained by reacting cells with Abs against human CD8 (10 μg/ml), CD14 (1 μg/ml), CD16 (1 μg/ml), and CD20 (10 μg/ml) followed by two rounds of magnetic depletion with sheep anti-mouse-coated Dynal beads, six beads per cell. Neutralizing anti-human IL-2, IL-4, and IFN-γ Abs were used at 10 μg/ml and obtained from PharMingen (San Diego, CA). IL-2 was obtained from Boehringer Mannheim (Indianapolis, IN).

Flow cytometry

Cells were labeled with PE-coupled anti-human CD154 Ab (Becton Dickinson, Mountain View, CA) or PE-coupled IgG1 isotype control Ab and FITC-coupled anti-human CD4 (Immunotech, Westbrook, ME), washed, and resuspended in 1% paraformaldehyde. For intracellular CD154 staining, cells were first surface labeled as described above, but with a nonconjugated anti-human CD154 Ab or control Ab. Cells were then fixed and permeabilized using a Becton Dickinson intracellular staining kit and labeled with PE-conjugated anti-human CD154 Ab or isotype control Ab. Data acquisition and flow cytometric analysis were performed on a Becton Dickinson FACSCalibur using CellQuest software.

RT-PCR

RNA was purified from 10^6 cells using Trizol. RT-PCR was run with a CD154 primer set: (CTGCAAGGTGACACTGTTC;CACAGCATGATCG AAACATAC) or (GGTGATTCTAGACACAGCATGATCGAAACATA CAAC;GGTGATTCTAGAAGGTGACACTGTTCAGAGTTTGAG) and a GADPH primer set: (CGCTGAGTACGTCGTGGAGTCCAC;GACAT CAAGAAGGTGGTGAAGCAG) using Titan one-step RT-PCR (Boehringer Mannheim) with 35 PCR cycles: 94°C for 30 s, 56°C for 60 s, and 68°C for 180 s.

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Coculture experiment

Purified CD4 T cells were stained with 5-(and 6)-carboxyfluorescein diacetate succinimidyl ester (CFDA-SE)[3] (Molecular Probes, Eugene, OR) for 10 min in PBS, at 25°C, washed, and fixed in 0.5% paraformaldehyde for 15 min at 25°C. After extensive washing, cells were cultured along with the Daudi B cell lymphoma line at a 1:1 ratio for 24 h. Cells were subsequently stained with PE-coupled anti-human CD54 (Coulter, Palo Alto, CA). CD54 expression on Daudi cells was measured by excluding CFDA-SE-positive cells. CD40-Ig fusion protein was used at 10 μg/ml.

Results

CD3/CD28 activation of CD4 cells induce prolonged CD154 expression through a calcineurin-independent pathway

Expression of CD154 on CD4 T cells has mainly been studied after TCR or ionomycin/PMA stimulation, which has revealed a very transient expression of CD154 (<24 h) that is dependent on calcineurin/NF-AT activation (12–14). To study CD154 expression under more physiological conditions, we used Abs against CD3 and CD28 coupled to beads (3x28 beads) as a form of surrogate APC. These beads are more potent than soluble or plate-bound Abs, probably due to their topological resemblance to cells, facilitating polarized interactions; an important factor in cell activation that has recently emerged from several excellent studies (15–17).

Fig. 1*A* shows primary/naive human PBLs stimulated with 3x28 beads. As expected, there was a rapid induction of surface CD154 expression on CD4 T cells. The level of surface CD154 was comparable or slightly higher than previously reported with plate-bound anti-TCR Abs with or without anti-CD28 costimulation (13, 18, 19). Effector cells, generated by 12–14-day expansion of cultures stimulated with 3x28 beads at day 0, also rapidly induced CD154 expression on CD4 cells upon restimulation with 3x28 beads; however, the level of induction was substantially higher than in naive cells (Fig. 1*A*). CD154 expression on both activated naive and effector CD4 cells did not decline as expected and was still expressed at high levels 4 days after the initial stimulation (Fig. 1*A*). Fig. 1*B* shows a schematic representation of CD154 expression on CD4 cells from PBL cultures stimulated with 3x28 beads at day 0 and restimulated at day 14. A similar CD154 profile was seen when purified CD4 cells were stimulated (data not shown), suggesting that CD8 T cells, B cells, or NK cells are not positively involved in the regulation of CD154 surface expression. It has previously been shown that large numbers of CD40-positive cells can induce down-modulation of CD154 on CD4 cells (20, 21); however, we did not see this effect in our PBL cultures, possibly due to the low amount of B cells present (3–11%).

A potential explanation for the prolonged CD154 expression could be that it is caused by repeated engagement between CD4 cells and 3x28 beads. However, after 2–4 days of stimulation, CD154 expression on both naive and effector CD4 cells was very resistant to cyclosporin A (CsA) treatment. In contrast, CsA efficiently inhibited CD154 expression when present before the 3x28 bead stimulation (Fig. 1*C*). Similar results were seen with the non-CsA-related calcineurin inhibitor FK-506 (data not shown). This suggests that a signal pathway distinct from CD3/CD28-induced NF-AT activation is, at least in part, responsible for the sustained CD154 expression.

IL-2 and IL-15 induce CD154 expression on previously activated CD4 cells

One of the main responses to CD28 costimulation of T cells is autocrine production of IL-2; intriguingly, TCR engagement without CD28 costimulation does not activate T cells to produce IL-2 (22). The 3x28 bead-stimulated T cells produce up to 150 U/ml of free IL-2 as measured by ELISA (data not shown).

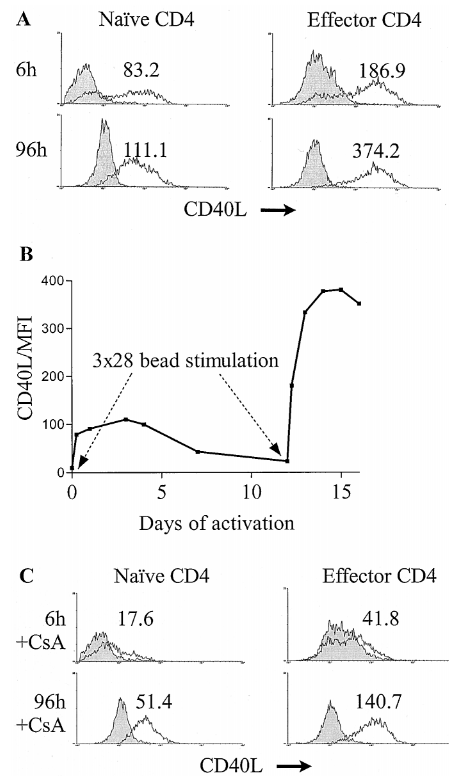

FIGURE 1. CD154 surface expression on CD4 cells after 3x28 bead stimulation. *A*, Naive (*left*) or effector (*right*) PBL were stimulated for 6 h or 96 h with 3x28 beads (three beads per cell). CD154 surface expression on CD4 cells: isotype control staining (shaded), CD154 staining (full line), mean fluorescence intensity (MFI) for CD154 staining is included. *B*, Graphic representation of CD154 expression on CD4 cells after 3x28 bead stimulation of PBL on day 0 and restimulation on day 12. The CD154 level on CD4 cells, as MFI corrected for isotype control Ab staining. Similar results, although with different intensities, were seen with PBL from eight different donors with no exceptions. *C*, Effect of CsA on CD154 expression. Cells were stimulated as described in *A*, but in the presence of 1 μg/ml CsA either 30 min before or 72 h after 3x28 bead stimulation. The experiment was repeated three times with similar results.

To investigate the influence of IL-2 on the sustained CD154 expression, naive or effector cell populations were cultured for 3 days with 3x28 beads to induce CD154 expression, and cells were then washed and cultured an additional 24 h with 1) 3x28 beads and 100 U/ml IL-2; 2) 3x28 beads and a blocking anti-IL-2 Ab; 3) without the 3x28 beads but with 100 U/ml IL-2; and 4) without the 3x28 beads and with the blocking anti-IL-2 Ab. Fig. 2*A* shows the results of this experiment. Combined removal of the 3x28 beads and blocking of IL-2 completely inhibited CD154 expression on activated naive CD4 cells. Furthermore, the massive CD154 expression on effector cells was radically reduced; however, not to baseline levels. Interestingly, blocking of IL-2 in the presence of beads inhibited CD154 expression on naive cells by ~55% and on effector cells by ~70%. The effect was specific, as blocking Abs against IL-4 or IFN-γ had no influence (data not shown). In the absence of 3x28 bead stimulation, IL-2 induced approximately half of the CD154 expression on activated naive CD4 cells and induced CD154 expression on the majority of activated effector CD4 cells. Furthermore, 60–90% of the IL-2-induced CD154 expression was maintained for 2 days in the absence of 3x28 stimulation (data not

[3] Abbreviations used in this paper: CFDA-SE, 5-(and 6)-carboxyfluorescein diacetate succinimidyl ester; CsA, cyclosporin A; CD40L, CD40 ligand; MFI, mean fluorescence intensity.

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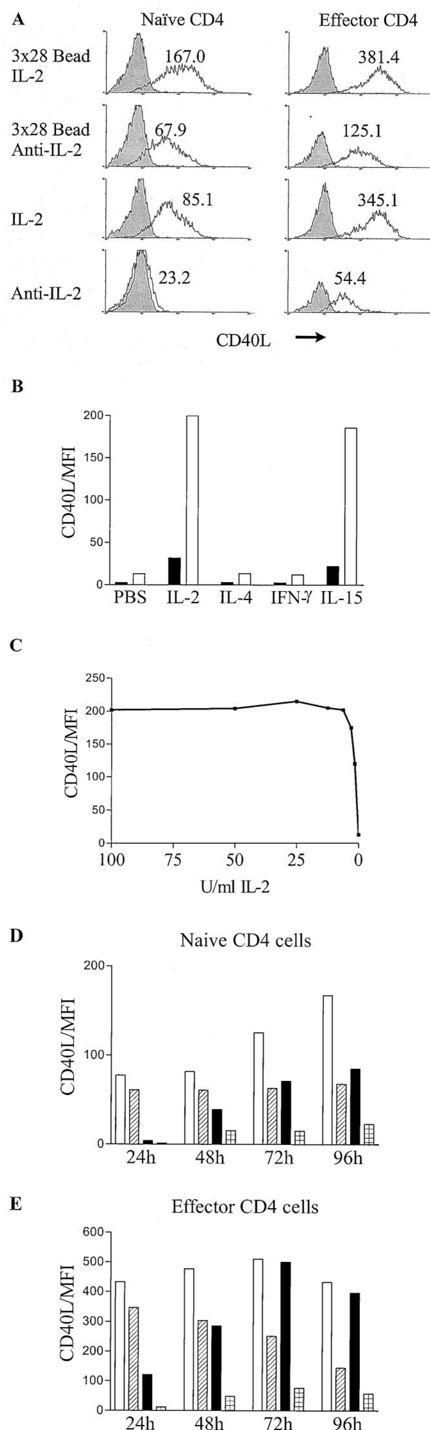

FIGURE 2. Influence of IL-2 on CD154 expression. *A*, Naive (*left*) or effector (*right*) PBL were stimulated for 3 days with 3x28 beads and then cultured for 24 h with I) 3x28 beads (three beads per cell) and 100 U/ml IL-2; II) 3x28 beads and 10 μg/ml blocking anti-IL-2 Ab; III) 100 U/ml IL-2; and IV) 10 μg/ml blocking anti-IL-2 Ab. CD154 surface expression on CD4 cells: isotype control staining (shaded), CD154 staining (full line), MFI for CD154 staining is included. The experiment was repeated five times with similar results. *B*, Naive (■) or effector PBL (□) were stimulated for 3 days with 3x28 beads and then cultured for 24 h without 3x28 beads and IL-2 (as described above for *A,* 4) and then restimulated with PBS, 100 U/ml IL-2, 50 ng/ml IL-4, 1000 U/ml IFN-γ, or 100 ng/ml IL-15 for 24 h. CD154 level on CD4 cells, as MFI corrected for isotype control Ab staining. The experiment was repeated three times with similar results. *C*, Titration of amount of IL-2 needed for CD154 reinduction. Various amounts of IL-2 was used to reinduce CD154 expression on effector PBL as described in *B*. CD154 level on CD4 cells, as MFI corrected for isotype control Ab staining. The experiment was repeated once with a similar result. *D*, The relative importance of IL-2 and/or 3x28 bead for CD154 expression on naive PBL after various lengths of activation. Cells were stimulated the last 24 h with 3x28 beads and IL-2 (□), 3x28 beads and anti-IL-2 Ab (▨), IL-2 (■), or anti-IL-2 Ab (⊞) as described in *A* from days 0 to 1, 1 to 2, 2 to 3, or 3 to 4 (for 2–4 days of culture, cells were stimulated with 3x28 beads up to the last 24 h). CD154 level on CD4 cells, as MFI corrected for isotype control Ab staining. The experiment was repeated three times with similar results. *E*, Same experiment as in *D*, but with effector PBL. The experiment was repeated three times with similar results.

Table I. *CD25 and CD122 expression on naive and effector CD4 cells after stimulation with 3x28 beads*[a]

Day	Naive Cells CD25	Naive Cells CD122	Effector Cells CD25	Effector Cells CD122
0	6	3	177	4
1	861	17	823	7
2	821	16	784	8
3	844	9	790	9
4	799	8	723	16

[a] CD25 and CD122 expression are shown as MFI, before (control) or after 1–4 days of stimulation.

shown). To rule out that withdrawal of IL-2 for 24 h was directly toxic for the cells, we took the activated naive or effector CD4 cells previously cultured without 3x28 beads and IL-2 and restimulated them with 100 U/ml IL-2 for 24 h. This could readily reinduce CD154 expression, an effect not seen with 50 ng/ml IL-4 or 1000 U/ml IFN-γ (Fig. 2*B*). Beyond emphasizing the functional integrity of the IL-2-deprived cells, this also demonstrates that IL-2 by itself could induce, and not only sustain, CD154 expression on activated CD4 T cells. Reactivation with IL-2 did not change the constitutive high HLA-DR expression or induced expression of 4-1BB or CCR5 (data not shown), demonstrating that IL-2 restimulation did not unspecifically alter expression of other surface molecules. Subsequent titration showed that 5–10 U/ml of IL-2 was necessary for maximal CD154 reinduction on naive or effector cells and that 1–2 U/ml induced half the maximal CD154 expression (Fig. 2*C* and data not shown).

Interestingly, 100 ng/ml of IL-15 could reinduce CD40L expression in the absence of IL-2 (Fig. 2*B*) and could also sustain CD40L in the absence of 3x28 beads and IL-2 (data not shown). IL-2 and IL-15 share many biological activities and their receptors use the same β- and γ-chain, but have individual α-chains (23). In contrast to IL-2, IL-15 is mainly produced by activated professional APCs including monocytes, macrophages, and dendritic cells; it is therefore tempting to speculate that IL-15 produced during T cell-APC interaction might also participate in the regulation of CD40L expression in vivo.

It is well known that resting CD4 cells have virtually no CD25 (IL-2R α-chain) and low CD122 (IL-2R β-chain) expression. Furthermore, our in vitro generated effector CD4 cells had low, but significant, CD25 expression and low IL-2R β-chain before restimulation (Table I). To investigate the relative importance of 3x28 beads and IL-2 at different intervals after activation, we incubated cells in the presence or absence of 3x28 beads and/or IL-2 (as described in Fig. 2*A*) from days 0 to1, 1 to 2, 2 to 3, and 3 to 4. As seen in Fig. 2, *D* and *E*, IL-2, without prior 3x28 bead stimulation, had no effect on CD154 expression on naive cells and only a limited effect on effector cells. In line with this, the 3x28

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bead activation accounted for nearly all CD154 expression after 24 h, as the CD154 level was only very weakly affected by withdrawal of IL-2. However, gradually the 3x28 bead response weakened and IL-2 took over, most strikingly seen in effector CD4 cells where IL-2 induced 90% of the CD154 expression after 3–4 days of activation. Subsequent analysis showed that induction of IL-2 receptor α- and β-chain expression correlated with the ability of IL-2 to induce CD154 expression (Table I). However, there were no significant differences between IL-2 receptor levels on activated naive and effector CD4 cells, suggesting that the notable higher CD154 expression on effector cells may be due to enhanced responsiveness.

IL-2 induces de novo production and expression of CD154

To investigate how IL-2 regulates CD154 expression, we reinduced CD154 expression on effector CD4 cells previously rested for 24 h as described in Fig. 2*B*, but in the presence of various inhibitors. CD154 expression was measured after 6 h of 3x28 bead or IL-2 stimulation to limit the toxic effects of the inhibitors. Fig. 3*A* shows that pretreatment with actinomycin D, cyclohexamide, or brefeldin A, inhibitors of transcription, translation, and transport through the Golgi complex, respectively, blocked IL-2-induced reexpression of CD154 on previously activated effector CD4 cells, clearly demonstrating that IL-2 induces CD154 expression by de novo synthesis. Further analysis showed that rapamycin, an inhibitor of the IL-2-induced p70s6 kinase, significantly, but not dramatically, inhibited IL-2-induced CD154 expression (Fig. 3*A*). Rapamycin had only a limited effect on 3x28 bead-induced CD154 expression (when a blocking anti-IL-2 Ab was included to neutralize the effect of newly produced IL-2). As expected, CsA completely inhibited 3x28 bead-induced CD154 expression; however, IL-2-induced CD154 expression was also affected to some degree (Fig. 3*A*). Current knowledge in the field suggests that CsA does not inhibit IL-2-induced signal transduction. Thus, the most likely explanation would be that a basal level of calcineurin activity is involved in IL-2-induced CD154 expression. In conclusion, these results show that IL-2 induces de novo CD154 production by a distinct and until now unrecognized signal pathway.

Fig. 3*B* shows the CD154 mRNA level, assessed by RT-PCR, of 3-day 3x28 bead-activated naive or effector CD4 cells cultured in the presence or absence of 3x28 beads and/or IL-2 for 24 h. Generally, the CD154 mRNA level corresponded to the surface CD154 expression. However, although it is difficult to directly compare, the CD154 mRNA level did not seem to be reduced to the same extent as the surface expression in CD4 cells incubated for 24 h without 3x28 beads and IL-2. An explanation could be that the residual mRNA is either nonfunctional or requires complementation from other signals/factors to be translated, or that it is actively translated to CD154 protein that is sequestered within the cell. A previous study has shown that memory cells can store CD154 in intracellular vesicles (24). To investigate this in greater detail, we stained activated naive and effector CD4 cells for intracellular CD154 when incubated in the presence or absence of 3x28 beads and/or IL-2 for 24 h. Cells were reacted with a nonconjugated anti-CD154 Ab before intracellular staining to block surface CD154 staining. Fig. 3*C* shows that IL-2 and, to a lesser degree, 3x28 bead stimulation induced pronounced intracellular CD154 protein levels in effector CD4 cells. However, very low levels of preformed CD154 was observed after 24 h without IL-2 and 3x28 bead stimulation, indicating that the residual CD154 mRNA is not actively transcribed. This again suggests that IL-2-induced re-expression of CD154 on the cell surface is dependent on transcription and translation and is not simply a result of translocation from preformed intracellular stores.

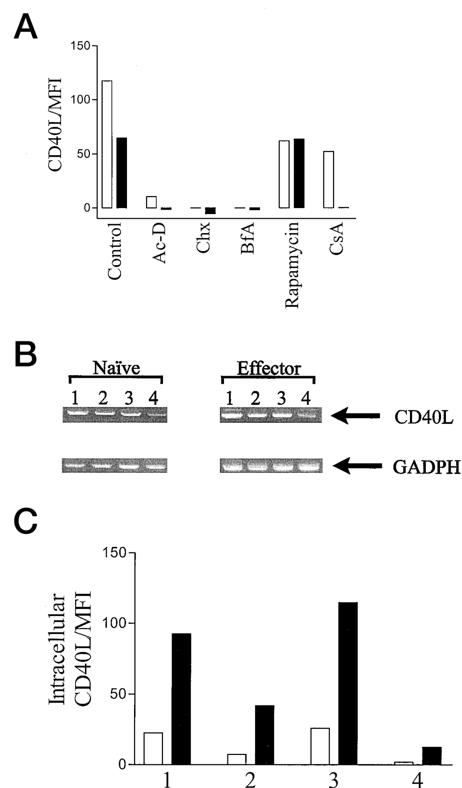

FIGURE 3. Mechanism for IL-2-induced CD154 expression. *A*, Effector PBL were stimulated for 3 days with 3x28 beads and then cultured for 24 h without 3x28 beads and IL-2 (as described above for Fig. 2*A*, 4) and then restimulated with 3x28 beads (three beads per cell; ■) or 100 U/ml IL-2 (□) for 6 h. A blocking anti-IL-2 Ab (10 μg/ml) was included with the 3x28 bead-stimulated cells to prevent stimulation from induced IL-2 production. Cells were preincubated in the presence or absence of actinomycin D (100 ng/ml), cyclohexamide (100 μM), brefeldin A (5 μg/ml), rapamycin (100 nM), or CsA (1 μg/ml) for 60 min before stimulation. CD154 level on effector CD4 cells, as MFI corrected for isotype control Ab staining. The experiment was repeated three times with similar results. *B*, RT-PCR measurement of CD154 mRNA in CD4 cells. Naive or effector PBL were stimulated for 3 days with 3x28 beads. CD4 cells were then purified by negative selection and cultured for 24 h with 1) 3x28 beads (three beads per cell) and 100 U/ml IL-2; 2) 3x28 beads and 10 μg/ml blocking anti-IL-2 Ab; 3) 100 U/ml IL-2; and 4) 10 μg/ml blocking anti-IL-2 Ab. Subsequently, RNA was purified from 10^6 cells. RT-PCR using primers specific for CD154 or GADPH. The experiment was repeated twice with similar results. *C*, Intracellular CD154 staining of naive (□) or effector (■) CD4 cells. Cells were stimulated with 3x28 beads and IL-2 (1), 3x28 beads and anti-IL-2 Ab (2), IL-2 (3), or anti-IL-2 Ab (4) as described in *B*. Intracellular CD154 expression in CD4 cells (surface CD154 was preblocked by a reaction with a nonlabeled anti-CD154 Ab), intracellular CD154 expression is shown as MFI corrected for intracellular isotype control Ab staining. The experiment was repeated twice with similar results.

CD154 induced by IL-2 is functionally active

To verify the functional capability of IL-2-induced CD154 on CD4 cells, a 24-h coculture experiment with Daudi B lymphoma cells was done. Daudi cells markedly up-regulate CD54 (ICAM-1) expression in response to CD40 engagement. Effector CD4 cells were purified by negative selection, stained with the fluorescent cell dye CFDA-SE, and fixed in 0.5% paraformaldehyde before coculture. As seen in Fig. 4, activated effector CD4 cells previously cultured for 24 h in the presence of 100 U/ml IL-2 alone strongly up-regulated CD54 expression on the Daudi cells, which

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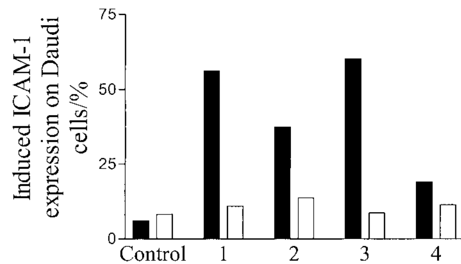

FIGURE 4. Coculture between Daudi B lymphoma cells and effector CD4 cells. Effector PBL were restimulated for 3 days with 3x28 beads. CD4 cells were then purified by negative selection and cultured for 24 h with 1) 3x28 beads (three beads per cell) and 100 U/ml IL-2; 2) 3x28 beads and 10 μg/ml blocking anti-IL-2 Ab; 3) 100 U/ml IL-2; and 4) 10 μg/ml blocking anti-IL-2 Ab. CD4 cells were stained with CFDA-SE and fixed in 0.5% paraformaldehyde. Daudi cells were cultured with (1–4) or without (control) the various CD4 cells at a 1:1 ratio for 24 h. Percentage of Daudi cell expression CD54 in the absence (□) or presence (■) of 10 μg/ml CD40-Ig. The experiment was repeated twice with similar results.

could be blocked by inclusion of a CD40-Ig fusion protein. Conversely, activated effector CD4 cells previously cultured without IL-2 or 3x28 beads for 24 h induced very weak CD54 expression on the Daudi cells. Essentially, the same results were observed with activated naive CD4 cells, but their weaker CD154 expression corresponded to a weaker induction of CD54 expression (data not shown). Control experiments showed that unstimulated naive CD4 cells had no significant effect on CD54 expression; in contrast anti-CD40 Abs coupled to beads induced robust CD54 expression on the Daudi cells (data not shown). These results demonstrate that the CD154 induced by IL-2 is functionally active.

Discussion

CD40 engagement of APC is essential for generation of effective humoral and cellular immunity. Of particular interest, recent data have demonstrated that CD40-activated APC can convert tolerogenic tumor-specific CD4 and CD8 cells to effective immunogeneic cells (9, 10). Furthermore, CD40-activated APC acquires the capacity to stimulate CD8 cells in the absence of CD4 cells, suggesting that the essential CD4 help during CTL generation is mediated through CD154 expressed on activated CD4 cells (6–8). Thus, we now have a substantial knowledge concerning the importance of CD40 activation of APC. However, since most studies have used Abs reactive against CD40 for activation, an in depth knowledge of the regulation of the endogenously receptor for CD40, CD154, is lacking. Since CD40 is generally constitutively expressed on APC, regulation of CD154 expression might be crucial for modulation of the immune response.

In this study, we demonstrate that efficient Ag activation and CD28 costimulation of CD4 cells has a dual function in connection with CD154 regulation: 1) induction of transient CD154 expression by NF-AT-dependent signals, and 2) production of IL-2 and up-regulation of its high affinity receptor, which maintains CD154 expression by IL-2-dependent signals. IL-15 produced by activated APC might furthermore enhance CD154 expression. This will produce a highly regulated feedback mechanism and underscores the interdependence of CD28 and CD40 signal pathways during T cell-APC interaction.

These findings might be of possible clinical interest, since the serum level of IL-15 is elevated in patients with rheumatoid arthritis, a disease characterized by elevated CD40L expression on CD4 cells (25, 26). Furthermore, a previous study has shown a strong correlation between diminished CD154 and IL-2 mRNA levels in a large fraction of patients with common variable immunodeficiency (27). Given the present study, it is likely that the diminished CD154 is caused by the lack of IL-2, in particular since these patients have a normal CD154 gene.

With regard to regulation of CD154 expression on activated CD4 T cells in vivo, future studies with knockout mice lacking either CD25, IL-2, or IL-15Rα may give insights into the relative contribution and importance of IL-2 and IL-15, although interpretation of data obtained in knockout models may be complicated by the multitude of functions performed by IL-2 and IL-15.

One of the hallmarks of anergic T cells is their inability to produce IL-2 even after appropriate costimulation. Furthermore, anergic T cells have been shown to express significantly reduced levels of CD154 after activation (28), which is not surprising given the data in the current report. We would like to propose that anergic CD4 cells lack the ability to activate APC, at least in part, due to poor CD154 expression. Since CD40-activated APC have the ability to activate otherwise anergic/tolerant T cells, this establishes a sort of "catch 22," which potentially is of fundamental importance for regulation of anergy/tolerance vs immunity.

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