CCL2 Inhibits the Apoptosis Program Induced by Growth Factor Deprivation, Rescuing Functional T Cells[1]

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The precise mechanisms involved in the switch between the clonal expansion and contraction phases of a CD8$^+$ T cell response remain to be fully elucidated. One of the mechanisms implicated in the contraction phase is cytokine deprivation, which triggers apoptosis in these cells. CCR2 chemokine receptor is up-regulated following IL-2 deprivation, and its ligand CCL2 plays an essential role preventing apoptosis induced by IL-2 withdrawal not only in CTLL2 cells, but also in mouse Ag-activated primary CD8$^+$ T cells because it rescued functional CD8$^+$ T cells from deprivation induced apoptosis, promoting proliferation in response to subsequent addition of IL-2 or to secondary antigenic challenges. Thus, up-regulation of the CCR2 upon growth factor withdrawal together with the protective effects of CCL2, represent a double-edged survival strategy, protecting cells from apoptosis and enabling them to migrate toward sites where Ag and/or growth factors are available. *The Journal of Immunology,* 2007, 179: 7352–7357.

Despite the continuous generation of T lymphocytes in the body, their total number is tightly regulated, implying that T cells disappear at about the same rate as they are being produced. During the course of an in vivo acute viral infection, the appropriate Ag presentation by APCs leads to a rapid clonal expansion of CD8$^+$ T cells (by ~4–5 log, ~1 wk), generating a population of effector cells where up to 50% respond to that single infectious agent (1). After infection clearance, there is a clonal contraction (by ~1–2 log, 8–30 days), leaving a smaller population of virus-specific memory cells (~5% of the virus-specific CD8$^+$ T cells present at the response's peak), which are maintained for years (1, 2).

Recent data have shown that after stimulation, Ag-specific T cells continue to divide in a "programmed" Ag-independent manner (3–6). Interestingly, the population of epitope-specific CD8$^+$ T cells changes exponentially during both the clonal expansion and contraction phases (1, 7), where apoptosis plays a prominent role maintaining the system homeostasis.

Apoptotic cell death plays a critical role not only during T cell development, but also for the homeostatic control of peripheral lymphoid organs and infected tissues, limiting the extent and duration of immune responses and providing a safeguard against immunopathology (8–10). Three physiological mechanisms are known to trigger apoptosis of Ag-stimulated T cells. The first one is induced by TCR stimulation of naive T cells in the absence of costimulatory signals. Such T cell death can be inhibited in vivo by inflammation and in vitro by cytokines; bacterial products promote T cell survival by a mechanism involving Bcl-3 (11). The second is induced by repeated TCR stimulation of activated T cells (activation induced cell death), increasing Fas ligand expression, which induces apoptosis of neighboring Fas$^+$ T cells; its role on mature T and B cell homeostasis is shown by Fas and Fas ligand mutants, which trigger progressive lymphadenopathies (12, 13). Finally, during the clonal contraction phase of acute primary infections, inflammation wanes, causing a reduction in cytokine expression and triggering growth factor withdrawal-induced apoptosis. This apoptotic pathway is independent of Fas-mediated signaling (14) and strictly dependent on de novo transcription and translation (15). It has been suggested that Bcl-2 protein family members are implicated in this type of apoptosis, requiring the proapoptotic BH3-only member Bim (16).

To gain insight on the mechanisms involved in the switch between clonal expansion and clonal contraction phases of CD8$^+$ T cell responses, the analysis of apoptosis induced by growth factor deprivation was undertaken, uncovering the up-regulation of the chemokine receptor CCR2 upon growth factor deprivation. Its specific ligand CCL2 significantly inhibited the apoptosis induced by IL-2 withdrawal in IL-2-dependent CTL cells, CTLL2 as well as in Ag-activated primary T cells. This inhibition led to an increase in the frequency of cells able to proliferate in response to either exogenous IL-2 or to a secondary antigenic stimulation. These data allow hypothesizing that in cytokine-deprived activated CD8$^+$ T cells, CCL2 signaling can modulate the "choice" between survival and apoptosis; enabling them to migrate toward sites where Ag or growth factors are available.

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Materials and Methods

Cell lines, culture conditions, and reagents

CTLL2 (no. TIB214; American Type Culture Collection) (17) and B6.1 (18) are mouse CTL cell clones strictly dependent on exogenous IL-2 for growth. In CTLL2 cells IL-2 deprivation induces apoptosis, where as this treatment leads to a reversible proliferation arrest in B6.1 cells (19, 20). Cells were cultured in IMDM containing 10% heat-inactivated FCS, 10 mM HEPES (pH 7.0), 0.05 mM 2-ME, 2 mM glutamine and saturating

concentrations of mouse recombinant IL-2 (1% ×63 mIL-2 supernatant). Murine recombinant chemokines (PeproTech) were reconstituted at 0.1 mg/ml in water and used at 200 ng/ml CCL2, 100 ng/ml CCL5, and 10 ng/ml CXCL9, unless otherwise indicated. Goat anti-mouse CCR2 (CKR2b; Santa Cruz Biotechnology) revealed with a secondary rabbit anti-goat-FITC (Biomedia), and a PE-conjugated anti-mouse CCR5 Ab (BD Pharmingen), were used at 0.2 μg of Ab/10^6 cells for FACS analysis, as described (21). CCR2 and CCR5 analyses were conducted by gating the life cell population, which was negative for propidium iodide (PI)[4] and Annexin V (PI$^-$Annexin V$^-$).

Primary CD8$^+$ T cells and APCs

F5-TCR$^{+/+}$ mice on a Rag1$^{-/-}$ background (22) were maintained in homozygosis on a C57BL/6 background. Animals were housed and bred in our animal facility, and in all experiments were treated in accordance to the European Union and National Guide Lines and the Helsinki Declaration. The F5-TCR (Vα_4Vβ_{11}) is positively selected on the H-2^b haplotype; the mature T cells are MHC class I-restricted CD8$^+$ V$\alpha_4$$^+V\beta_{11}$$^+$, able to recognize the influenza virus A nucleoprotein peptide (366–374) in the context of H2-D^b (23). As a source of APCs, lymph node or spleen cells from Rag2$^{-/-}$ animals were purified, x-ray-irradiated (1.4 Gy) and erythrocytes removed by ammonium chloride lysis.

Primary CD8$^+$ T cell activation

Single cell suspensions from lymph nodes of F5-TCR$^{+/+}$ mice (1000 cells/well) were activated with 240 pM antigenic peptide NH$_2$-ASNENMDAM COOH (Isogen Bioscience) in the presence of mouse recombinant IL-2 and irradiated APCs (100 cells/well). Under these conditions, cell numbers reached 7×10^5–1.5×10^6 cells/ml by day 6.

IL-2 deprivation

For IL-2 deprivation, cells that did not receive fresh IL-2 for the last 48 h were used. Cells were washed twice in fresh medium (300 $\times$ g, 10 min), incubated for 30 min at 37°C, and washed once again (300 $\times$ g, 10 min) to completely remove IL-2 from the medium and bound to IL-2R.

Cytoplasmic RNA and quantitative RT-PCR

Cytoplasmic RNA from CTLL2 cells was prepared using the Nonidet P-40 lysis method (24). Quantitative RT-PCR from each sample was performed using the Transcript or First Strand cDNA Synthesis kit (Roche), by random hexamer priming at 50°C for 1 h, using 1 μg of RNA/sample, following the manufacturer's instructions. After synthesis of the cDNA First Strand, quantitative PCR was performed on an ABI Prism 7900 (Applied Biosystems), using the FastStart Taqman Probe Master (Rox; Roche), and with sets of primers and Universal ProbeLibrary probes (Roche) designed online with ProbeFinder v.2.20 (Roche). Probes specific for CCR2 are primers (forward) 5′-CAGGGCTCTATCACATTGGTT, (reverse) 5′-TCAATTGTCAGGAGGATAATGAAA, and Universal ProbeLibrary probe 31, which gives a 61 nt amplicon. Probes specific for 18 S rRNA are primer (forward) 5′-AAATCAGTTATGGTTCCTTTGGTC, (reverse) 5′-GCTCTAGAATTACCACAGTTATCCAA, and Universal ProbeLibrary probe 55, which was used as a loading control. Each sample was amplified with 1 cycle at 95°C for 10 min (to activate the polymerase) and 40 cycles at 95°C for 15 s and 60°C for 1 min as described (25).

Cell cycle analysis

To analyze the DNA profiles, cells were permeabilized, stained with 20 μg/ml PI, and after RNase A digestion (30 min, 37°C), analyzed by FACS analysis (XL; Coulter) to determine the fraction of viable (G$_0$/G$_1$, S, and M phase-specific DNA content) and nonviable (sub G$_0$/G$_1$ DNA content) cells (26).

BrdU staining

Exponentially growing CTLL2 cells were deprived of IL-2 and pulsed with 20 μM BrdU (product no. B-5002; Sigma-Aldrich) for 30 min at 37°C. Cells were permeabilized for 30 min at room temperature in 1 ml of solution containing 200 μg of pepsin (Sigma-Aldrich) in 2 M HCl (pepsin solution). After permeabilization, cells were washed three times in PBS at 300 $\times$ g for 10 min at room temperature. The pellet was then resuspended in 0.3 ml of PBS supplemented with 0.5% Tween 20 and 0.5% FCS, containing 15 μl of anti-BrdU-FITC Ab (product no. 347583; BD Biosciences) for 1 h at room temperature; subsequently,

cells were resuspended in PBS and stained with PI. For FACS analysis, the whole cell population was analyzed, after exclusion of cell doublets.

Limiting dilution analysis

Ag-activated primary CD8$^+$ T cells (7–15 days upon activation) were deprived of IL-2. Viable cells were FACS sorted and directly collected in 100 μl of IMDM, either in the absence or presence of recombinant IL-2 (100 U/well) or CCL2 (200 ng/ml), onto 96-well plates at 1, 3, 10, or 30 cells/well (24 wells/group). Eighteen hours later, 100 μl of medium containing 400 U/ml recombinant IL-2, either alone or in combination with 100 irradiated APCs, and 2.4 nmol of antigenic peptide (NH$_2$-ASNENMDAM-COOH), were added to each well. After a 2-wk incubation at 37°C in a 5% CO$_2$ incubator, plates were scored for the presence of live proliferating cells. The frequency of cells able to proliferate in each experimental condition determined as mean $\pm$ SD were estimated by interpolating the frequency that contains an average of one precursor cell (F = 0.37) on a semi-logarithmic plot containing the estimated cells/well and the frequency of negative cultures on a log scale. This analysis has been done online using the Bioinformatics facility of The Walter & Eliza Hall Institute of Medical Research (Melbourne, Australia) (accessed at http://bioinf.wehi.edu.au/software/limdil/index.html).
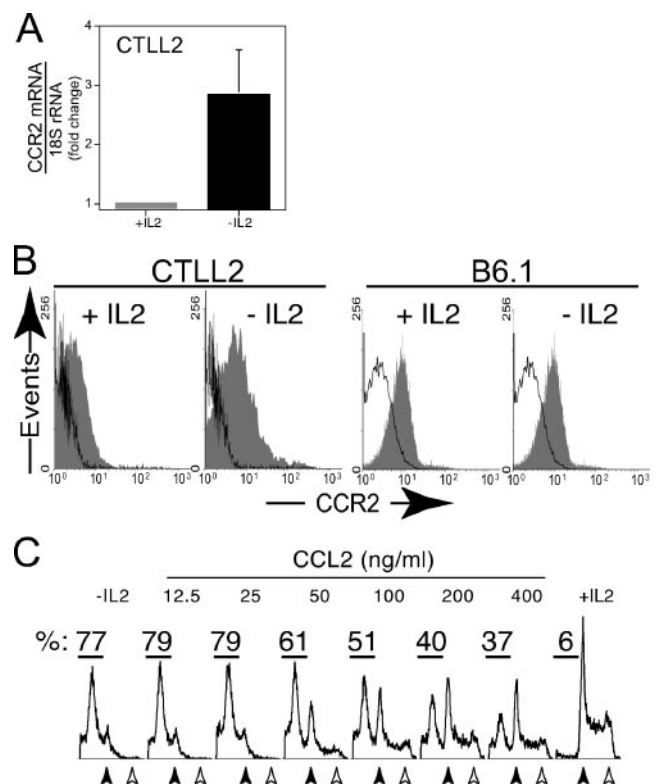

FIGURE 1. CCR2 chemokine receptor induction in response to IL-2 deprivation. *A*, Normalized CCR2 mRNA expression, determined by quantitative RT-PCR, on CTLL2 cells after 4–6 h deprivation of the growth factor (−IL-2), taking as 1 the expression level of the exponentially growing (+IL-2) CTLL2 cells, represented as mean $\pm$ SD ($n = 3$). *B*, FACS analysis of CCR2 expression in the plasma membrane of the PI$^-$Annexin V$^-$ CTLL2 and B6.1 cells (gray-filled histogram) grown in the presence of IL-2 (+IL-2) or upon 18 h IL-2 deprivation (−IL-2). IL-2 withdrawal leads to an increase on CCR2 expression in CTLL2 cells upon IL-2 deprivation, but not with B6.1 cells, which undergo a reversible G$_1$-arrest upon IL-2-deprivation. Control represents cells stained only with the secondary FITC-conjugated anti-goat Ab (black line histogram). *C*, CCL2 prevents apoptosis of IL-2-deprived CTLL2 cells (20 h) on a dose-dependent manner, as determined by the decrease on the percentage (above each histogram) of cells with hypodiploid DNA content (sub G$_0$/G$_1$) by FACS analysis. Cells maintained in the presence of IL-2 were used as control. Filled arrowhead indicates the location of the G$_1$ peak and open arrowhead the G$_2$ peak.

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[4] Abbreviation used in this paper: PI, propidium iodide.

Results

CCR2 up-regulation upon growth factor deprivation

The CCR2 mRNA is up-regulated in CTLL2 cells 4–6 h upon IL-2 deprivation (2.8-fold), as demonstrated by quantitative RT-PCR (Fig. 1A). This up-regulation took place in cells that have not died, as demonstrated by the increase on CCR2 cell surface expression within the cell population devoid of any apoptosis sign (PI $^-$Annexin V$^-$), as determined by FACS analysis (Fig. 1B). Conversely, in the cytotoxic T cell line B6.1, where IL-2 deprivation leads to a reversible proliferation arrest (20), this receptor was not up-regulated (Fig. 1B).

A CCR2 up-regulation upon IL-2 deprivation might enable the cell response to low chemokine levels. This response would be physiologically relevant if the CCR2 ligand, CCL2, interfered with the apoptosis program and modulated the outcome of the cells. Thus, the effect of different concentrations (400–12.5 ng/ml) of CCL2 (JE, MCP1) was assessed. IL-2 deprivation for 20 h led to apoptosis in a large fraction of CTLL2 cells. The fraction of apoptotic cells decreased in a dose-dependent manner by CCL2 (Fig. 1C). Indeed, FACS analysis of permeabilized PI-stained cells allowed to quantify the subG$_0$/G$_1$ cell fraction and to determine that CCL2 concentrations >25 ng/ml had an apoptosis blocking effect, reaching a plateau at 200 ng/ml (Fig. 1C).

### Kinetics of apoptosis inhibition by CCL2

A BrdU pulse labeled cells in S phase and facilitated following them as a synchronized population for a complete cell cycle. BrdU$^+$ CTLL2 cells reached the G$_1$ phase of the next cell cycle 7 h after the pulse, independent of the presence or absence of either IL-2 or chemokines (Fig. 2). At this point, cells maintained in the presence of IL-2 continued cycling (Fig. 2B), whereas in the absence of IL-2 cells died, determined by the gradual increase in the sub G$_0$/G$_1$ cell fraction (Fig. 2B). In IL-2-deprived cells, addition of CCL2 led to a 50% reduction in the fraction of dead cells (Fig. 2, B and C). CCL5 and CXCL9, neither alone nor in combination with CCL2, had any detectable effect in the fraction of dead cells (Fig. 2B), despite the up-regulation of their receptors (CCR5 and CXCR3) in these cells following IL-2 deprivation (data not shown), thus demonstrating specificity of CCL2. The CCL2-mediated inhibition of apoptosis in IL-2-deprived cells was not specific to cells that were in S phase of the cell cycle at the time of deprivation (BrdU$^+$ cells), as the same effect was observed in BrdU$^-$ cells (Fig. 2C). Thus, these results suggest that CCR2 up-regulation upon IL-2 deprivation of CTLL2 cells can change the cell outcome, as demonstrated by the CCL2 inhibition of apoptosis.

CCL2 inhibits apoptosis in primary T cells

We ascertained whether CCR2 up-regulation upon IL-2 deprivation and CCL2 responsiveness were particular traits of CTLL2 cells, rather than a general characteristic of Ag-activated primary CD8$^+$ T lymphocytes. Primary CD8$^+$ T cell activation is characterized by an initial phase of exponential proliferation (8–10 cell divisions) concomitant with the acquisition of effect or functions. A second phase (plateau) in which the cells exert their effect or functions, and a third phase characterized by the apoptotic death of the vast majority of the cells, in a process wherein IL-2 deprivation have been implicated. To analyze CCR2 expression and the effects of CCL2 during the latter phase of the primary antigenic response, naive CD8$^+$ T cells from F5-TCR$^{+/+}$ Rag1$^{-/-}$ mice were activated with Ag in the presence of x-ray irradiated APCs and IL-2. Under these conditions >95% of the cells were activated and exponentially proliferated for the first 7 days, obtaining a 150- to 300-fold expansion (eight to nine cell divisions). Afterward, the cells reached the proliferation plateau and were maintained in the same medium for another week. At this point, T cells were seeded in fresh medium and maintained for 18 h in the presence or absence of IL-2. Anti-CCR2 mAb staining showed an increase on CCR2 expression upon IL-2 deprivation (Fig. 3A). In contrast, anti-CCR5 mAb revealed a very weak, but reproducible, staining on primary activated T cells, which was significantly up-regulated upon IL-2 deprivation (Fig. 3A). IL-2 deprivation led to apoptosis of a large fraction of these cells (60–80%), addition of recombinant CCL2, although did not trigger cell expansion, decreased the apoptotic cell fraction by 50% (Fig. 3B), whereas neither CXCL9 nor CCL5 had any effect (Fig. 3B). These data demonstrate that CCL2 prevents IL-2 deprivation induced apoptosis in Ag-activated primary CD8$^+$ T lymphocytes.

To determine whether CCL2 is able to rescue functional T cells, the cloning efficiency of IL-2-deprived primary T cells either in the presence or absence of CCL2 was investigated. Cells were FACS sorted, directly plated at different cell concentrations and deprived of IL-2 either in the presence or absence of CCL2, on limiting dilution analysis. After 18 h IL-2 deprivation, each well was supplemented with fresh IL-2 (Fig. 4A) and cultures were scored for cell proliferation 7–14 days later. The frequency of positive cultures was 2-fold higher in the presence of CCL2 (1/4.88) than in its absence (1/11.5) (Fig. 4B). The cloning efficiency of these cultures was 1/3.33 (+IL-2). A similar effect of CCL2 was observed when Ag-activated T cells, after 18 h of IL-2 deprivation, underwent a secondary antigenic response (Fig. 4C). In this case, the frequency of positive cultures in the presence of CCL2 was also higher

FIGURE 2. Kinetics of apoptosis inhibition by CCL2 in IL-2-deprived CTLL2 cells. *A*, A BrdU pulse (labeling S-phase cells) was used to follow a synchronous CTLL2 cell population and the fate of the cells was determined by FACS analysis following PI staining. *B*, Kinetic analysis of IL-2 deprivation-induced apoptosis in BrdU$^+$ CTLL2 cells maintained in the presence or absence of the chemokine ligands CCL2 (200 ng/ml), CCL5 (100 ng/ml), or CXCL9 (10 ng/ml). Cells grown with saturating concentrations of IL-2 were used as a control. *C*, Quantification of data in *B* is presented as a percentage of cells without hypodiploid DNA content, analyzed in the BrdU$^+$ and BrdU$^-$ populations.
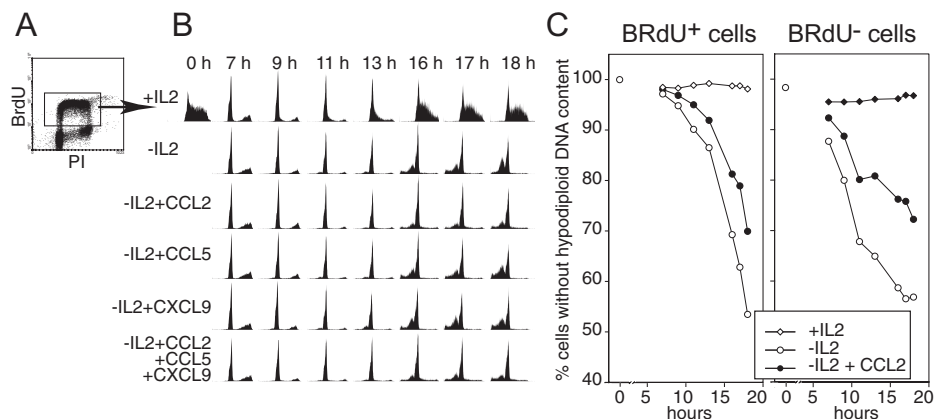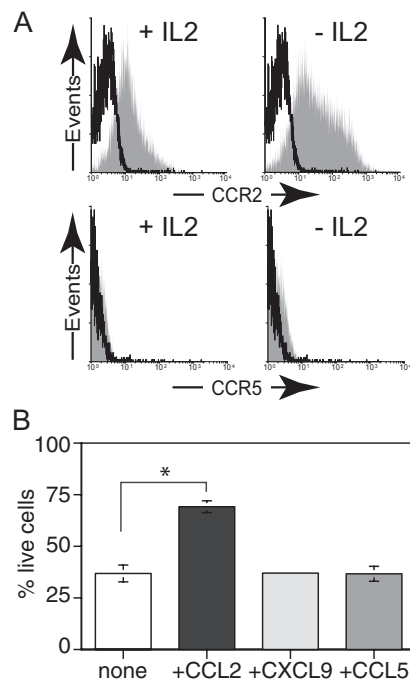
FIGURE 3. CCR2 expression and CCL2 function on Ag-activated primary T cells. Primary CD8$^+$ T cells were activated with Ag and were used for these experiments at the end of the exponential proliferation phase ($\sim$2 wk). Cells were washed and incubated for 18 h in fresh medium supplemented or not with saturating concentrations of exogenous recombinant IL-2. *A*, CCR2 and CCR5 expression was assessed using the appropriate mAbs and FACS analysis. Control staining with the secondary Ab (black line histogram) and staining of the cells incubated in the presence of IL-2 or in the absence of IL-2 (gray-filled histogram) are shown. *B*, Ag-activated primary CD8$^+$ cells deprived of IL-2, in the absence or presence of the chemokine ligands CCL2 (200 ng/ml), CCL5 (100 ng/ml), or CXCL9 (10 ng/ml) for 18 h, were stained with PI and analyzed by FACS. The effects of the chemokines on the cells are presented as the percentage of live cells (G_1+S+G_2+M cells) following the different treatments, using as positive control cells maintained in the presence of saturating concentrations of IL-2 (100% live cells). Data were analyzed using the Student's *t* test. *, *p* < 0.0001.

(1/1.85 vs 1/3.98) (Fig. 4*D*). The cloning efficiency of these cultures was 1/1.17 (+IL-2) (Fig. 4*D*). Thus, in Ag-activated primary CD8$^+$ T cells, CCL2 blocks growth factor deprivation-induced apoptosis, rescuing a higher frequency of cells able to proliferate in response to IL-2 and/or Ag, and therefore, behaving as fully functional T cells.

Discussion

Chemokines, in addition to their chemoattractant role, exhibit critical functions in diverse physiological processes including cell death and survival. Indeed, CCL5 has been shown to mediate apoptosis in T cells and virus-infected macrophages (27, 28); XCL1 costimulates the apoptosis of human CD4$^+$ T cells (29), and CXCL12 has been implicated both in survival and apoptosis of T cells (30). Conversely, CXCL8 inhibits neutrophil apoptosis (31) and induces B cell chronic lymphocytic leukemia cell accumulation (32). It has also been suggested that CCL2 might regulate pancreatic cancer progression (33), protect cardiac myocytes from hypoxia-induced apoptosis (34), and inhibit activation-induced cell death in HIV-infected individuals (35).

The data presented demonstrate an up-regulation of CCR2 mRNA and protein in both CTLL2 cells and Ag-activated primary T cells following growth factor deprivation (Figs. 1–3). This up-
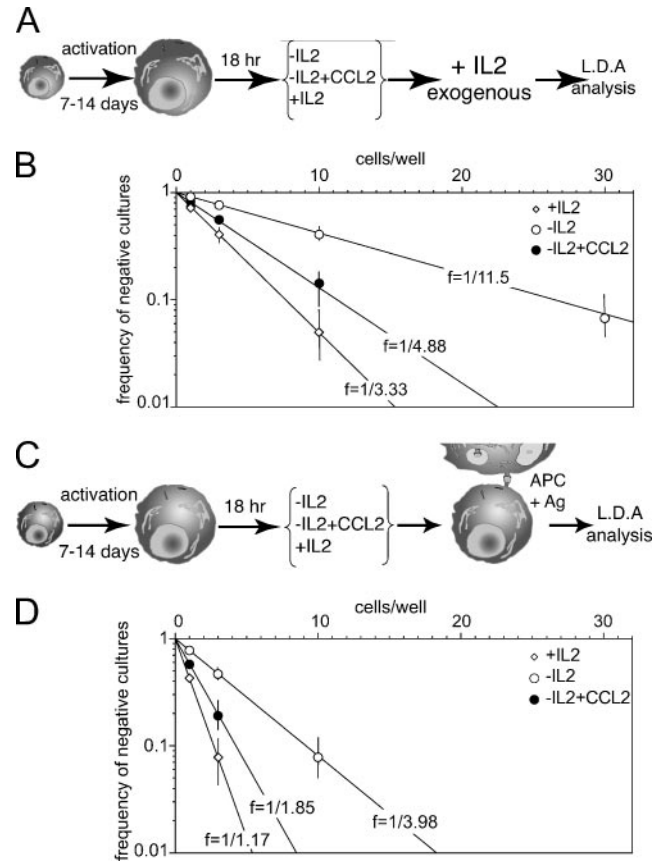

FIGURE 4. CCL2 increases the frequency of cells, which following growth factor-deprivation, are able to proliferate in response to either exogenous IL-2 or secondary antigenic responses. *A*, Representation of the experimental set-up used to determine by limiting dilution analysis (LDA) the cloning efficiency, in response to exogenous IL-2, of Ag-activated primary T cells. *B*, The frequency of negative cultures under each condition, +CCL2 (●) or −CCL2 (○), was plotted vs the input cell number per well, represented as mean ± SD. These data were used to calculate the frequency (f) of responding cultures and the regression line, in response to exogenous IL-2. *C*, Representation of the experimental set-up used to determine by limiting dilution analysis, the cloning efficiency, in response to secondary antigenic stimulation, of Ag-activated primary T cells. *D*, The frequency of responding cultures to secondary antigenic responses were determined as described. Cells maintained in the presence of IL-2 were used as a positive control (◇). Twenty-four wells per group were used.

regulation, however, did not occur in the B6.1 T cell line (Fig. 1*B*), which upon growth factor deprivation, instead of triggering the apoptosis program, undergoes a reversible arrest in the G_1 phase of the cell cycle (19, 20). Thus, suggesting that the increased CCR2 receptor expression might be associated with triggering of the apoptotic program. The CCR2 up-regulation is detectable in both CTLL2 and Ag-activated primary T cells within the cell population devoid of any apoptosis sign (PI$^-$Annexin V$^-$), suggesting that this up-regulation rather than induced by, precedes apoptosis. Data presented in Fig. 1*C* apparently suggests a proliferation stimulatory activity of CCL2. A more careful analysis using a synchronized cell population (cells in the S phase of the cell cycle, labeled with BrdU on a 30 min pulse), in which the CCL2 effects upon IL-2 deprivation were analyzed over time (Fig. 2), suggests that the CCL2-dependent death sparing could be dissociated from effects on cell cycle progression.

IL-2 deprivation leads to CCR2 up-regulation, and its ligand CCL2 changes the outcome of the cells on a dose-dependent manner (Fig. 1*C*), leading to a 50% increase on the fraction of live cells

following growth factor deprivation both in CTLL2 and Ag-activated primary T cells, as determined by the increase in the fraction of cells showing a nonhypodiploid DNA content (Figs. 2 and 3).

It has been demonstrated that peptide caspase inhibitors can efficiently block caspase activation and inhibit cell death in response to a variety of stimuli. In some cases, however, although inhibition of caspases delayed the morphological changes of apoptosis, it did not alter the eventual fate of the cell (36–38). This finding is also true for proteins such as Bcl-2 that inhibited 90% of the apoptosis induced by gamma irradiation, but the clonogenic efficiency was only 30% of the controls (39), or the baculovirus p35 protein, which is able to efficiently block caspases and inhibit thymocyte cell death ex vivo, but does not block negative selection in vivo (40). Because the loss of clonogenicity is separable from the appearance of apoptotic markers (41), we wished to determine whether CCL2 in IL-2-deprived cells was able to rescue cells fully, or whether it merely delayed the death of cells that would eventually succumb. For this purpose, the clonogenic potential of these cells was determined in limiting dilution analyses, demonstrating that CCL2, in addition to decrease the apoptosis by 50%, led to a 2-fold increase on the cloning efficiency of the cells in response to either IL-2 or Ag (Fig. 4).

The apparent differences in the frequency of proliferating cells between IL-2-restimulated and secondary antigenic stimulation (Fig. 4, *B* and *D*) are due to the higher cloning efficiency in response to Ag (Ag-pulsed APCs) as compared with IL-2 (2.63- to 2.89-fold higher), although the -IL-2/-IL-2+CCL2 ratio is similar in both experiments (2.35-fold for IL-2-restimulation vs 2.15-fold for antigenic restimulation). This response indicates that addition of CCL2 upon IL-2 deprivation is able to rescue >50% of the cells.

It is unlikely that CCR2 overexpression is a generalized event induced by apoptotic stimuli because some apoptotic insults, such as CD95-induced apoptosis, are strictly independent of de novo transcription and translation (24). We cannot formally exclude, at this time, that other apoptotic signals dependent of transcription and translation, such as glucocorticoid-induced apoptosis (15, 42), could also induce a CCR2 overexpression. Importantly, CCL2 can effectively interfere with apoptosis induced by IL-2 deprivation. It is possible that CCL2 effects are due to interaction with its receptor CCR2, which is up-regulated in these conditions, although the data presented in this study do not allow us to formally exclude other mechanisms. The signaling pathways involved remain unknown due to the difficulty of biochemical analyses on signaling molecules when a fraction of cells undergo apoptosis.

On the basis these findings, we propose that under conditions in which IL-2 levels are too low to sustain cell proliferation and survival, CCL2-mediated responses may be physiologically relevant during the CD8$^+$ T cell contraction phase of the immune response because the response limits suicide and, in the presence of other inflammatory sites, enhances survival of the cells. Thus, up-regulation of chemokine receptors before triggering of apoptosis might allow effector T lymphocytes to detect low concentrations of their ligands. CCL2 rescues fully functional cells from the apoptotic program and might promote their migration toward a new inflammation site, where growth factors or Ags might be available.

Acknowledgments

Disclosures

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