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Interleukin-1 down-regulates gene and surface expression of interleukin-1 receptor type I by destabilizing its mRNA whereas interleukin-2 increases its expression

K. YE, K.-C. KOCH, B. D. CLARK & C. A. DINARELLO *Department of Medicine, Tufts University School of Medicine and New England Medical Center, Boston, Massachusetts, U.S.A.*

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SUMMARY

The interleukin-1 receptor type I (IL-1RtI) plays an important role in the biological effects of IL-1, but regulation of its surface and gene expression remains unknown. We found that occupancy of 2–15% of the IL-1 surface receptor results in dramatic down-regulation of IL-1RtI both at the mRNA and cell surface level in murine D10S cells, a subline of T-helper type 2 cells. At these low occupancy levels (3×10^{-12} to 3×10^{-13} M), the reduction in IL-1RtI surface expression appears at 24 hr and continues to 48 and 72 hr. At the mRNA level, low occupancy of the IL-1R results in decreased IL-1RtI mRNA stability; steady state half-life of the IL-1RtI mRNA is reduced from 6 to 1 hr after exposure to 3×10^{-12} M IL-1. This down-regulation of IL-1RtI by IL-1 is blocked by cycloheximide, suggesting *de novo* protein synthesis is necessary for decreased RNA stability. Low concentrations of human IL-1β, murine and rabbit IL-1α or β similarly down-regulated IL-1RtI, whereas low concentrations of human IL-1α failed to reduce the receptor surface expression, despite inducing a full proliferative response. We also observed that the effect of IL-1 on this down-regulation was not through protein kinase C (PKC), since PMA rapidly increased IL-1RtI mRNA levels within 30 min and persisted for 24 hr. IL-2 up-regulated IL-1RtI in D10S cells at both mRNA and protein levels. These results demonstrate that low occupancy of IL-1 receptors induces down-regulation of IL-1RtI surface as well as mRNA expression. The regulation of IL-1RtI gene expression may be one of the mechanisms by which IL-1-mediated events are controlled.

INTRODUCTION

Interleukin-1 (IL-1) possesses diverse biological effects, mediating inflammatory and immunological responses.[1] However, many aspects of the interaction of IL-1 with its receptors, post-receptor signal transduction and subsequent nuclear events remain unknown. At least two distinct IL-1 receptors exist: an 80,000 MW glycoprotein, termed IL-1R type I (IL-1RtI), predominantly found on thymocytes, T cells, fibroblasts, keratinocytes and hepatocytes,[2] and a 68,000 MW glycoprotein, named IL-1R type II (IL-1RtII), and found mainly on pre-B cells, B cells, macrophages[2] and neutrophils.[3] The IL-1RtI have been cloned.[4]

In previous studies, understanding of surface expression of the IL-1R has been carried out almost exclusively using the binding of radiolabelled IL-1. Several reports demonstrate that at receptor saturation, IL-1 reduces the binding of more IL-1 to murine T-cell clones and that the ligand may accumulate in the nucleus.[5] We have shown previously that the IL-1 receptors are expressed in large numbers in murine D10S cells (a subline of D10.G4.1) and that binding of radiolabelled IL-1 to these cells is reduced by prior incubation with IL-1.[6] The regulation of IL-1 surface receptor expression is still a largely unknown process; however, several factors mediate their expression. Platelet-derived growth factor increases the number of IL-1 binding sites four- to fivefold and IL-1RtI mRNA 20–30-fold in murine 3T3 fibroblasts.[7] Transforming growth factor beta decreases IL-1 binding on bone marrow progenitor cells,[8] whereas interferon-gamma (IFN-γ) increases IL-1 receptor numbers in keratinocytes.[9] However, because these studies are based on binding of radiolabelled IL-1, it is unclear which IL-1 receptor is being regulated and the effect of varying receptor occupancy. Therefore, we have studied the surface expression of the IL-1RtI using a specific anti-IL-1RtI which detects the surface receptor regardless of occupancy by IL-1.

The importance of studying the regulation of IL-1 receptors involves several issues. IL-1 plays a key role in the pathogenesis of inflammatory and autoimmune diseases. Pretreatment with IL-1 can prevent, ameliorate or improve a variety of disease

Abbreviations: CHX, cycloheximide; IL-1, interleukin-1; IL-1RtI, interleukin-1 receptor type I; PKC, protein kinase C.

Correspondence: Dr C. A. Cinarello, Dept. of Medicine, New England Medical Center, 750 Washington St., Boston, MA 02111, U.S.A.

processes in experimental animal models. For example, administration of a single small dose of IL-1 24 hr prior to lethal bacterial infection prevents death.[10] Pretreatment with a low dose of IL-1 reduces radiation- and hyperoxia-induced death. However, the mechanism(s) of this non-specific resistance to infection and injury is not known. Studies have ruled out a role for circulating factors. It is possible that part of the protection mechanism is at the receptor level.[11]

We report here that the IL-1RtI in D10S cells can be down-regulated by low concentrations of IL-1 (2% receptor occupancy); in constrast, the receptor is up-regulated by IL-2. Both cytokines regulate IL-1RtI expression at both the cell surface and mRNA level. Our results indicate that IL-1 decreases the receptor mRNA level by a mechanism of shortening its mRNA half-life and this IL-1-mediated event is not through protein kinase C.

MATERIALS AND METHODS

Reagents

Human recombinant IL-1β[12] was kindly provided by Repligen Corp. (Cambridge, MA). Human recombinant IL-1α was a gift from Hoffmann-LaRoche Inc. (Nutley, NJ). Murine IL-1α, IL-1β, rabbit IL-1α, rabbit IL-1β were gifts from Glaxo (Geneva, Switzerland). Human recombinant IL-2 obtained from Cetus Corp. (Emeryville, CA). Rat anti-mouse IL-1RtI monoclonal antibody (35F5) (IgG1) was a generous gift from Dr Richard Chizzonite (Hoffmann-LaRoche Inc.). Fluorescein isothiocyanate (FITC)-conjugated goat anti-rat IgG was obtained from Caltag Laboratories (South San Francisco, CA). Anti-IL-2R (p55) monoclonal antibody (mAb) 7D4, FITC-Mar-18.4 (for staining of 7D4) and a rat anti-murine IFN-γ mAb (IgG1) used as an isotype control were gifts from Dr Brigitte Huber (Tufts University, Boston, MA). Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma Chemical Co. (St Louis, MO). Cycloheximide and antinomycin D were purchased from Boehringer Mannheim (Mannheim, Germany).

cDNA probes

A 2·2 kilobase cDNA for murine IL-1RtI was a kind gift of Dr Ueli Gubler (Hoffmann-LaRoche Inc.). cDNA for chicken β-actin was kindly provided by Dr Brigitte Huber (Tufts University).

Cell culture

D10S is a subline of the murine T-helper type 2 line, D10.G4.1. This subline does not require feeder cells and proliferates to subfemtomolar concentrations of either form of human IL-1.[13] D10S cells were maintained in RPMI-1640 medium (Microbiological Associates, Walkerville, MD) supplemented with 5% heat-inactivated foetal calf serum (FCS, Hyclone, Logan, UT) and 10% T-cell growth factor derived from Concanavalin A (Con A)-stimulated mouse spleen cells. The cells were split (1:10) when the cell density reached 10^6 cells/ml (approx. 7 days). D10S cells were used 4–7 days after the split. For experiments, cells were adjusted to 10^6 viable cells/ml. The CDC.25 cell line was a kind gift of Dr D. C. Parker (University of Massachusetts Medical School, Worcester, MA). This cell line responds to rabbit IgG in context with I-$A^{k\,d}$ and was maintained as described.[14] Briefly, CDC.25 cells were stimulated every 10–14 days with antigen, irradiated splenotyes from C3D2F_1/J mice (Jackson Laboratories, Bar Harbour, ME) as antigen-presenting cells and human recombinant IL-2. For the experiments, CDC.25 cells were separated from splenocytes by Ficoll–Hypaque gradient centrifugation at Days 10–14 after antigenic stimulation. EL4-6.1 cells were obtained from ATCC (Rockville, MD) and maintained in complete medium at 10^6/ml.

RNA extraction and Northern/dot-blot analyses

Total RNA was extracted using guanidine-isothiocyanate-CsCl method.[15] For Northern hybridization, 20 μg of total RNA was subjected to electrophoresis in a 1·2% agarose–formaldehyde gel and transferred by capillary action to nylon membranes (Amersham Corp., Arlington Heights, IL). Dot-blot analysis also employed nylon membranes. RNA was fixed to the membrane with UV light as suggested by the manufacturer. cDNA were labelled using a random primed DNA labelling kit (Boehringer Mannheim). Membranes were typically exposed to Kodak XAR5 at -70° for 4 days (with DuPont Cronex intensifying screen).

IL-1RtI detection by FACS analysis

D10S cells were washed twice in cold phosphate-buffered saline–bovine serum albumin (PBS–BSA) (0·25% w/v). Cell number was adjusted to 5×10^5 cells per sample. Cells were stained with anti-IL-1RtI mAb for 30 min at 4°, washed with PBS–BSA, and then stained with fluorescein isothiocyanate (FITC)-labelled goat anti-rat IgG secondary antibody. Cells were then either fixed in formaldehyde (0·37%) and stored at 4° for several days, or cells were stained with propidium iodide dye before FACS analysis. Fluorescence staining was determined by data acquisition on a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Five thousand or 10,000 cells were collected per sample. Non-relevant antibody controls were performed in parallel with anti-IL-1RtI staining. Results are displayed as histograms indicating relative fluorescence versus viable cell number, or percentage change of mean fluorescence channel number in stimulated compared to unstimulated cells. At 4°, saturating concentration of IL-1 (10^{-9} M) had no effect on the ability of the anti-IL-1RtI antibody to stain cells (data not shown).

RESULTS

Effect of IL-1 on D10S IL-1RtI surface expression

We used a rat anti-murine IL-1RtI monoclonal antibody for cell staining to determine specifically whether the IL-1RtI can be down-regulated by maximal receptor saturation with IL-1. At 37°, human IL-1α (hIL-1α) and human IL-1β (hIL-1β) at a saturating concentration of 17 ng/ml (10^{-9} M) rapidly down-regulated IL-1RtI in D10S cells by 80 and 60% within 1 hr respectively (three separate experiments, data not shown) and this persisted with further down-regulation of >90% by 24 hr. This is in agreement with observations that the IL-1R can be internalized under complete receptor occupancy, since at 10^{-9} M IL-1, all the IL-1 receptors are occupied.[6]

We next used decreasing concentrations of IL-1β to examine whether partial IL-1R occupancy had an effect. As shown in Fig. 1a, IL-1β at 50 pg/ml (3×10^{-12} M) down-regulated IL-1RtI. At this concentration, the down-regulation process was slow compared to high doses of IL-1. After 72 hr the down-regulation reached the same reduction as a 300-fold greater concentration

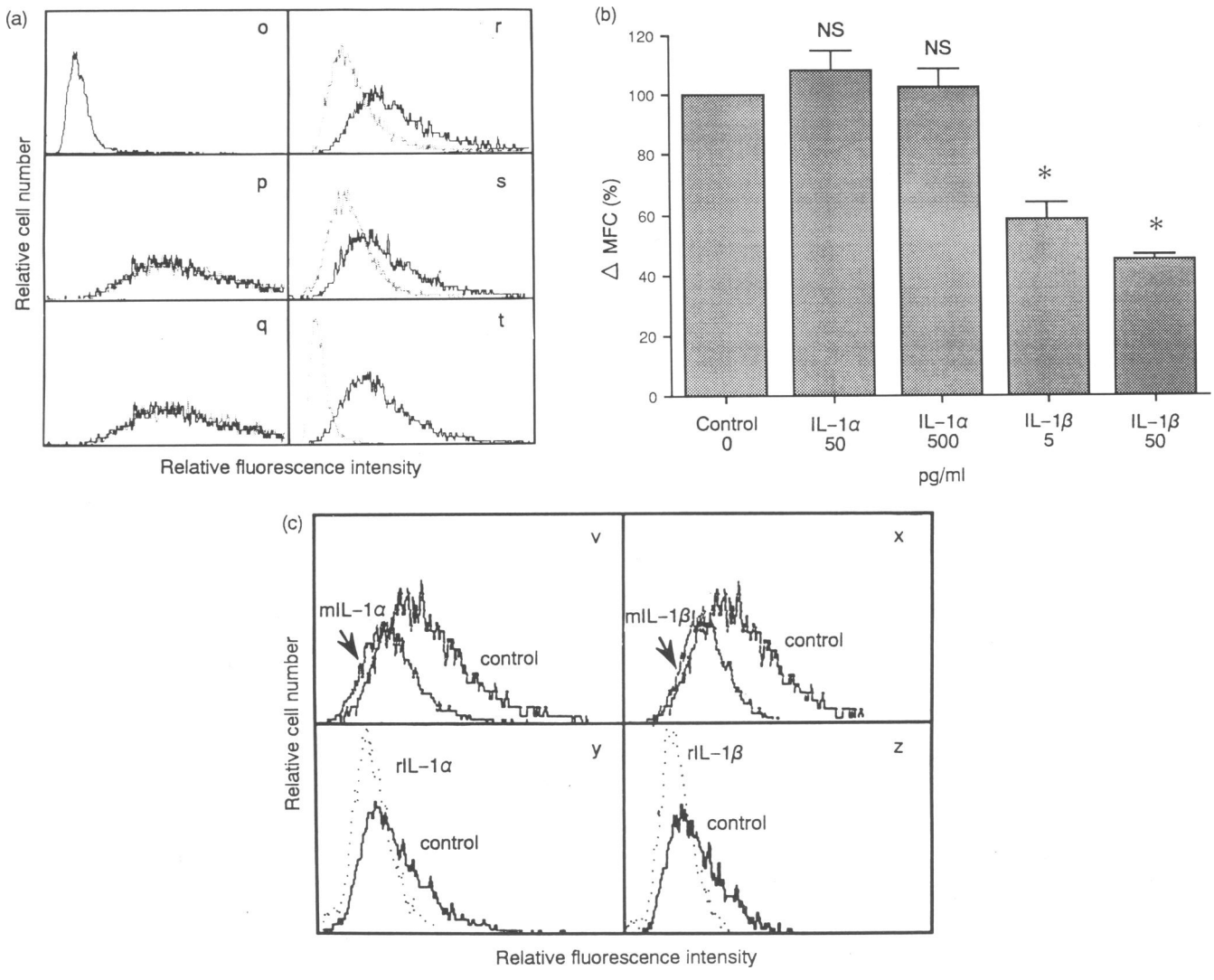

Figure 1. FACS analysis of IL-1RtI surface expression on D10S cells. Untreated D10S cells incubated for the same time periods as controls. At each time-point after incubation with IL-1, mean fluorescence channel (MFC) numbers were compared and each control was set as 100%. Relative cell number versus fluorescent intensity is shown. (a) (o) Dotted line is isotype control and solid line is background fluorescence. Incubation time of 2, 4, 24, 48 and 72 hr with hIL-1β (50 pg/ml) for (p), (q), (r), (s) and (t) are shown in dotted lines whereas control is solid line. (b) Cells were incubated with 50 or 500 pg/ml of human IL-1α or 50 and 5 pg/ml of hIL-1β for 24 hr and change in per cent of MFC of IL-1RtI was measured. Data are shown as mean $\pm$ SEM of three experiments as analysed by Student's *t*-test for paired samples (*$P < 0{\cdot}05$). (c) 50 pg/ml of murine IL-1α (mIL-1α) (v), murine IL-1β (mIL-1β) (x), rabbit IL-1α (rIL-1α) (y) or rabbit IL-1β (rIL-1β) (z) were incubated with D10S cells for 24 hr.

of IL-1 (at 1 or 24 hr). At 50 pg/ml, only 15% of the IL-1 receptors are occupied, based on a conservative assessment of 11,000 receptors per cell.[6] In contrast, hIL-1α at 50 and 500 pg/ml have no effect on IL-1RtI after 24 hr incubation (Fig. 1b) or after 48 and 72 hr of treatment (data not shown). However, as previously reported[13,16,17], a number of D10.G4.1 subclones (D10S, MD10, D10A) proliferate to pg/ml of human IL-1α. We repeated these previous experiments to demonstrate that the current D10S cells proliferate as well to 5 pg/ml of hIL-1α as to hIL-1β (data not shown). More strikingly we observed that even lower concentrations, 5 pg/ml of IL-1β (3×10^{-13} M) can significantly down-regulate the surface IL-1RtI by 30% in D10S cells after 24 hr (Fig. 1b). At this concentration, less than 2% of the receptors are occupied but over 30% of the receptors were down-regulated. On the other hand, using similar concentrations of IL-1β, surface CD4 antigens on D10S cells were not affected over the same 72-hr period (data not shown).

We also examined whether this down-regulation of IL-1RtI has a species-specificity effect. As in Fig. 1c (v) and (x), 50 pg/ml of either murine IL-1α and IL-1β were equally effective in down-regulating the surface IL-1RtI after 24 hr treatment. Similarly, 50 pg/ml of either rabbit IL-1α and IL-1β down-regulated IL-1RtI as in murine IL-1α and IL-1β [Fig. 1c(y) and (z)].

We also tested whether IL-1 down-regulates its surface receptor in two other cell lines, CDC.25, another murine T-helper type 2 cell line and EL4-6.1, a murine thymoma cell line.

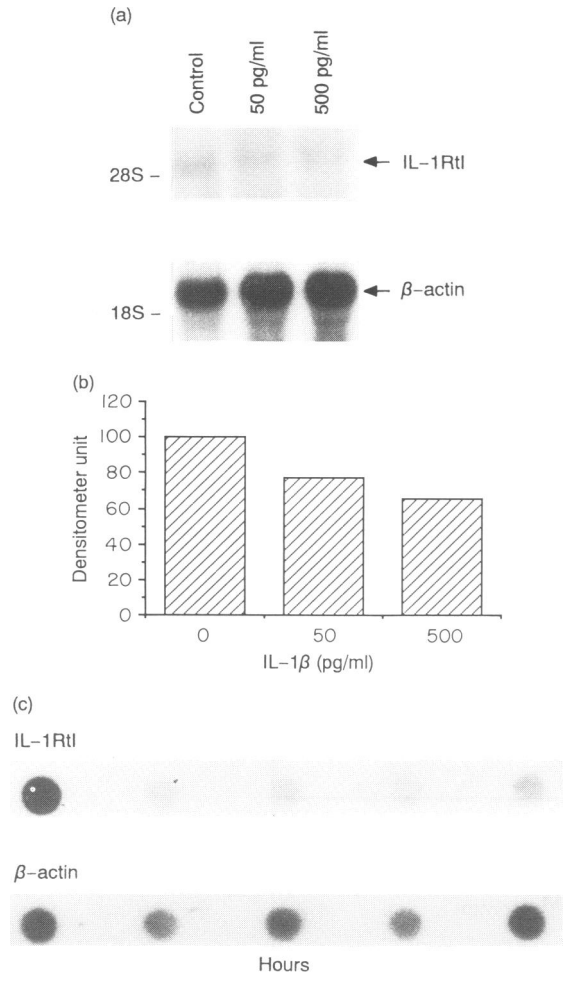

Figure 2. Effect of IL-1β on mRNA levels for IL-1RtI. (a) Northern analysis of IL-1β on IL-1RtI mRNA. Total RNA was isolated from 1×10^7 D10S cells. Twenty micrograms of RNA was loaded per lane. D10S cells were treated for 24 hr without IL-1β (lane 1), with 50 pg/ml (lane 2) and 500 pg/ml (lane 3) IL-1β. β-actin mRNA served as a standardization control (48 hr exposure). (b) Densitometry reading of IL-1RtI mRNA from data shown in (a). Densitometer readings from untreated cells were set at 100% and each sample was compared to β-actin mRNA. (c) Dot-blot analysis of time-course of IL-1β on IL-1RtI mRNA. Cells were either untreated or treated with IL-1β (50 pg/ml) for 2, 4, 24 and 48 hr. Twenty micrograms of total RNA was loaded per well. (IL-1RtI blot was exposed for 4 days, β-actin blot was exposed for 24 hr.)

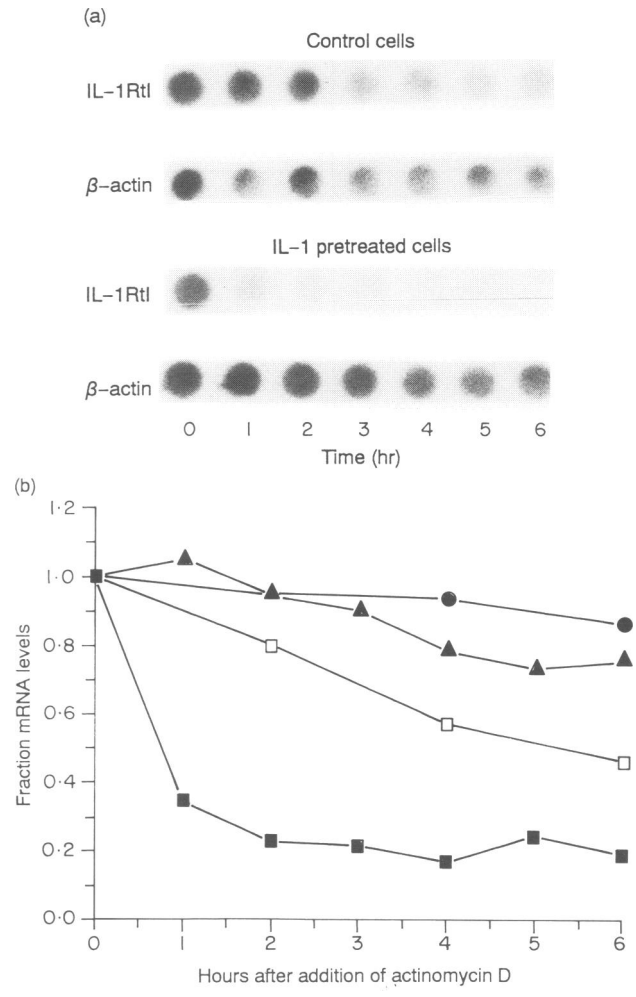

Figure 3. IL-1RtI mRNA half-life analysis. (a) D10S cells were incubated either without or with 50 pg/ml IL-1β for 16 hr and then 10 μg/ml of actinomycin D was added. Total RNA was extracted at each hour and dot-blot analysis was carried out. Ten micrograms of RNA was loaded per well (IL-1RtI blot wax exposed for 3 days, β-actin blot was exposed for 24 hr). (b) Dot-blots from (a) as quantitated by densitometry. Readings of mRNA from D10S incubated in the presence of IL-1 for time intervals shown was set as 100%. Data represent one of two separate experiments. IL-1RtI in IL-1 pretreated cells (■); IL-1RtI in control cells (□); β-actin in IL-1 pretreated cells (▲); β-actin in control cells (●).

Both of these cell lines differ from D10S in that they do not proliferate to IL-1 alone but require an additional signal. Down-regulation of IL-1RtI in these cell lines was not observed with human IL-1β at 50, 500 pg/ml and 1 ng/ml (data not shown). In EL4-6.1 cells, using 17 ng/ml of human IL-1β for 24 hr can only down-regulate IL-1RtI by 20% (data not shown).

Effect of IL-1 on IL-1RtI mRNA expression in D10S cells

The above observations prompted us to test whether IL-1 down-regulates its receptor at the mRNA level. We used hIL-1β in the following experiments in order to be consistent. Figure 2 shows in Northern hybridization analysis that IL-1β at 50 pg/ml (3×10^{-12} M) decreased IL-1RtI mRNA in a dose-dependent fashion. Next we examined the time-course of this reduction in mRNA using dot-blot analysis. The suppression of IL-1RtI mRNA began as early as 2 hr after IL-1 treatment and remained low for the next 48 hr (Fig. 2c). After 72 hr there was still demonstrable reduction. β-actin mRNA level was unaffected by IL-1 throughout the time-period.

IL-1RtI mRNA half-life

We established the half-life of IL-1RtI mRNA in unstimulated cells by arresting mRNA synthesis using actinomycin D. The steady state IL-1RtI mRNA half-life is approximately 6 hr; β-actin is 10–15 hr (Fig. 3). When D10S cells were pretreated with IL-1β for 16 hr and then subjected to half-life analysis, we observed a dramatically shortened half-life to 1 hr. β-actin

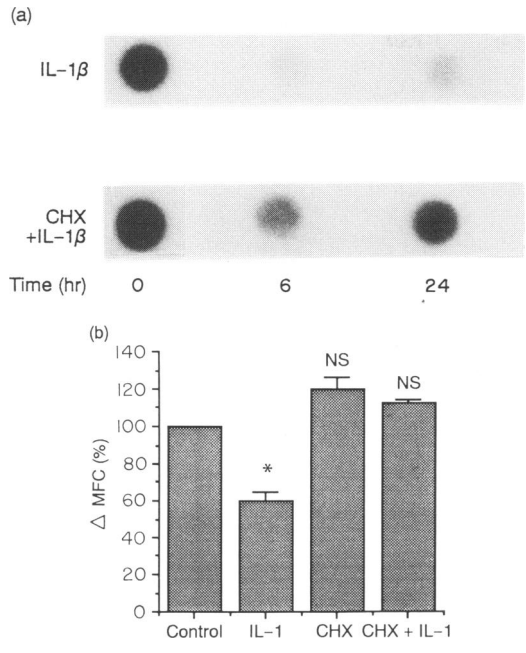

Figure 4. Effect of CHX on IL-1RtI surface and gene expression. (a) Dot-blot analysis of IL-1RtI mRNA after CHX treatment. D10S cells were incubated either without or with 10 μg/ml CHX for 2 hr and then 50 pg/ml of IL-1β was added. The viability of D10S cells after 24 hr CHX treatment is about 65% by trypan blue exclusion. After 6 and 24 hr total mRNA was analysed. Ten micrograms of RNA was loaded per well (2 days exposure). (b) D10S cells were pretreated with CHX for 2 hr and then IL-1β was added and incubated for 24 hr. FACS analysis of IL-1RtI was performed as previously described. Live cells were distinguished from dead cells by propidium iodide staining. Data are shown as mean $\pm$ SEM of three experiments and analysed by Student's *t*-test for paired samples ($*P < 0{\cdot}05$).

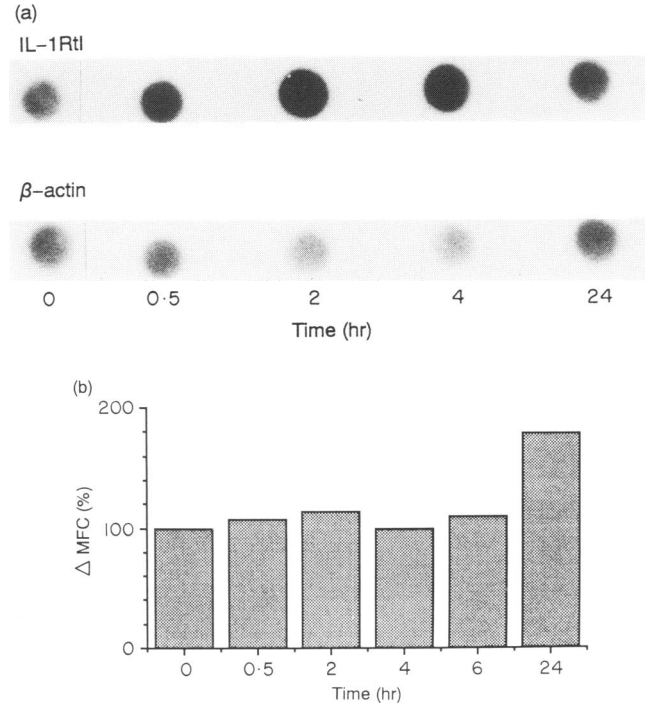

Figure 5. Effect of PMA on IL-1RtI. (a) Dot-blot hybridization of IL-1RtI and β-actin mRNA after 10 ng/ml PMA treatment. Twenty micrograms of RNA was loaded per well (IL-1RtI blot was exposed for 2 days and β-actin blot was exposed for 12 hr). (b) FACS analysis of IL-1R type I after stimulation of 10 ng/ml of PMA. Results represent one of three separate experiments.

mRNA was unaffected (Fig. 3). In additional experiments, we also observed that IL-1β reduced its receptor mRNA half-life as early as 4 hr after treatment with IL-1β (data not shown).

To obtain further information of the post-transcriptional regulation of the IL-1RtI in D10S cells, we used cycloheximide (CHX) to study both IL-1RtI mRNA and cell surface IL-1RtI. FACS analysis showed that CHX alone did not significantly affect surface expression in resting cells after 24 hr treatment (Fig. 4b). However, CHX prevented the down-regulaton of IL-1RtI by IL-1β (Fig. 4b). CHX also blocked the down-regulation of the IL-1RtI mRNA by IL-1β in D10S cells (Fig. 4a) suggesting that down-regulation of surface and gene expression of the IL-1RtI by IL-1 may require new protein synthesis.

Down-regulation of IL-1RtI surface and gene expression by IL-1 is not mediated by protein kinase C

Reports suggest that PKC may be one of the pathways for IL-1 signal transduction in T lymphocytes;[17] other studies propose that IL-1 does not activate PKC.[18] Therefore, we used the PKC activator PMA (since PMA activates virtually all PKC isoforms) to investigate its effect on IL-1RtI expression. FACS and dot-blot analyses of the IL-1RtI expression were performed after D10S cells were incubated with PMA. PMA rapidly stimulated IL-1RtI mRNA levels in 30 min (60% increase above the baseline) which continued to increase over the subsequent 2–4 hr (150% increase) (Fig. 5a). Messenger RNA for IL-1RtI after 24 hr of PMA stimulation showed a 30% increase above baseline at time zero hour. FACS analysis showed that PMA does not affect the IL-1RtI surface level at earlier time-points (0·5, 2, 4, 6 hr) but up-regulates the receptor surface level after 24 hr (Fig. 5b). Based on the above results, we conclude that PMA activates IL-1RtI gene expression and that the down-regulation of IL-1RtI by IL-1 is not through PKC.

Regulation of IL-1RtI surface and gene expression by IL-2 in D10S cells

Using FACS analysis and a mAb to the p55 (TAC) antigen of IL-2R, D10S cells, like the parent line, express IL-2R on their surface (data not shown). However, D10.G4.1 or D10S cells do not produce IL-2 under antigen or mitogen stimulation[13] but proliferate in response to IL-2. Furthermore, D10.G4.1 cells in the presence of IL-2 show increased proliferation to IL-1.[19] As shown in Fig. 6a and b, IL-1RtI surface expression on D10S cells was increased twofold when incubated in the presence of 1 U/ml of IL-2; 10 U/ml of IL-2 increased IL-1RtI surface expression three- to fourfold. After 48 hr of IL-2 presence, mRNA for the IL-1RtI was enhanced in parallel with surface expression (Fig. 6c). In two experiments, we observed that IL-2 increased IL-1RtI surface expression began after 6 hr (Fig. 6d), whereas IL-2 increased IL-1RtI mRNA levels after 4 hr and steadily increased the message over time (Fig. 6e).

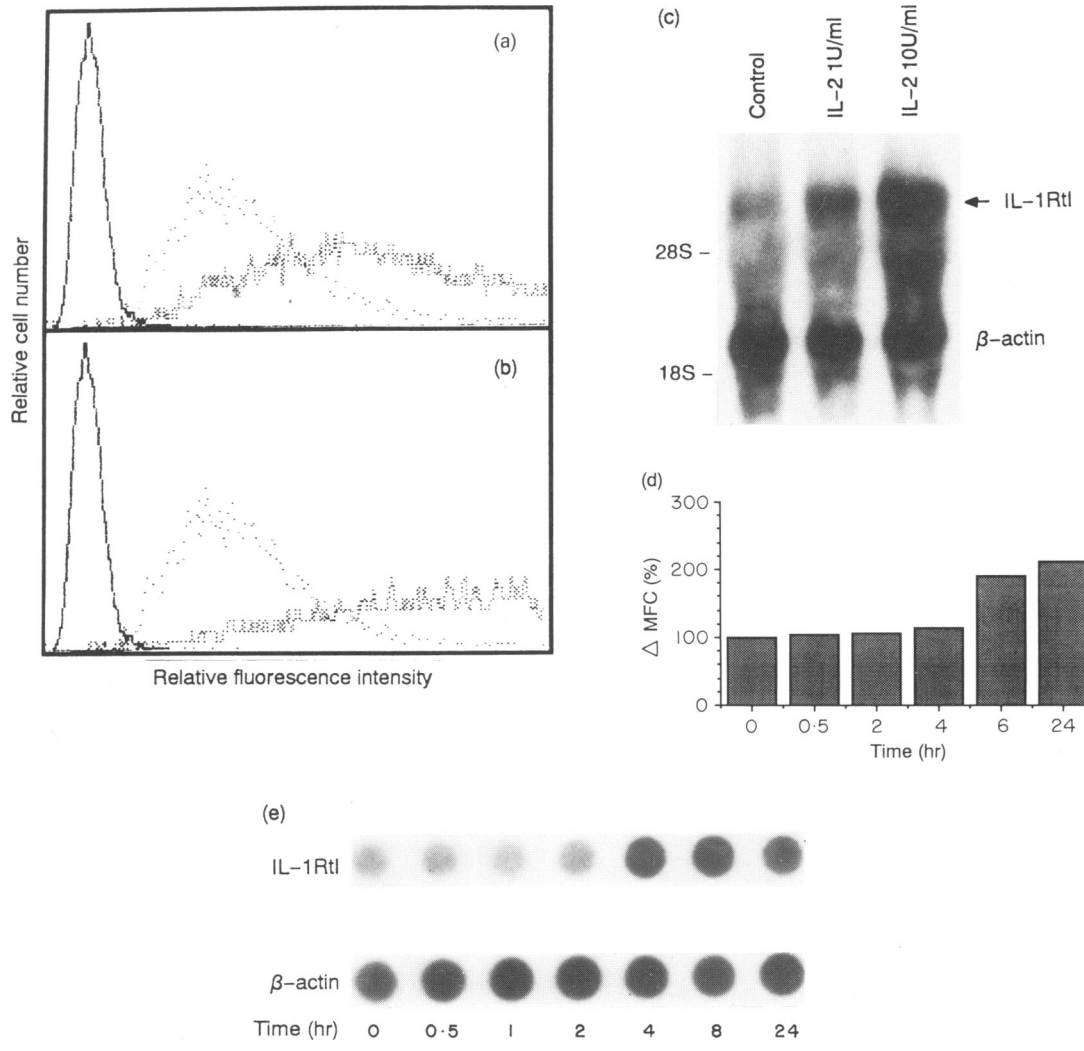

Figure 6. Effect of IL-2 on IL-1RtI gene and surface expression. (a) and (b) FACS analysis of IL-2 on D10S IL-1RtI surface expression. Solid line depicts D10S background fluorescence (no anti-IL-1R type I antibody). D10S cells were treated 48 hr without IL-2 (individual dots) or with IL-2 (dense dotted line). (a) D10S cells incubated with 1 U/ml IL-2; (b) with 10 U/ml IL-2. (c) Northern analysis of IL-2 effect on IL-1RtI mRNA from D10S cells shown in (a) and (b). Total RNA was extracted from D10S cells incubated without IL-2 treatment for 48 hr (lane 1) or with 1 U/ml (lane 2) or 10 U/ml (lane 3) (3 days exposure). (d) Time–course of the effect of IL-2 on IL-1R type I. FACS analysis of IL-1RtI following 10 U/ml of IL-2 stimulation. (e) Time–course of IL-1RtI mRNA under IL-2 (10 U/ml) stimulation. Ten micrograms total RNA was loaded per well (IL-1RtI blot was exposed for 3 days, β-actin blot was exposed for 2 days).

DISCUSSION

We present evidence that the IL-1RtI in a subline of murine Th2 cells can be down-regulated by IL-1 but up-regulated by IL-2. Low concentrations of IL-1 which occupy 15% of the surface receptors on these cells down-regulated the surface expression of the IL-1RtI in 24 hr to the same extent as saturated receptor occupancy. Even a lower concentration (2% receptor occupancy at 3×10^{-13} M) reduced IL-1RtI surface expression. Although these same concentrations of human IL-1α induced the same degree of proliferation in these cells as that of human IL-1β, human IL-1α failed to down-regulate IL-1RtI at low receptor occupancy whereas human IL-1β, murine IL-1α, β or rabbit IL-1α, β down-regulated IL-1RtI equally as well under low receptor occupancy. The down-regulation of the IL-1RtI occurs at the level of receptor mRNA and IL-1 presumably decreases its surface and mRNA level by increasing the rate of IL-1RtI mRNA degradation. This mechanism requires *de novo* protein synthesis as suggested by the addition of cycloheximide and we speculate that IL-1 may trigger its receptor to induce a RNase. PKC is apparently not involved in the down-regulation of IL-1RtI gene expression, nor in the signal triggering decreased surface expression. On the other hand, IL-2 up-regulates both the IL-1RtI on the surface of the cell and the mRNA level.

D10S cells maintain a high level of IL-1RtI mRNA and surface receptors. These cells are highly sensitive to IL-1 as assayed by proliferation in the presence of attomolar concentrations of IL-1 in the absence of any co-stimulant or mitogen.[13] Peincubation with IL-1 reduces subsequent proliferative response to IL-1.[6] On other cells, IL-1RtI can be internalized by direct ligand binding.[5] This process is relatively fast (1 hr) and is thought to be mediated by rapid autophosphorylation of the receptor through a putative endocytotic pathway.[20,21] However,

that event requires saturated receptor occupancy which does not explain the potent biological effects mediated by IL-1 in pM and fM ranges. Based on our findings, we postulate that another internalization pathway might exist for the IL-1RtI, in which unoccupied receptors can be internalized, triggered by low occupancy levels by IL-1. This concept is supported by studies showing there are two kinetically distinct internalization pathways for the epidermal growth factor receptor; one is independent of receptor occupancy.[22] For the IL-1RtI, the process of receptor down-regulation by low concentrations of IL-1 is relatively slow, requiring 24 hr or longer. IL-1 reduces it receptor mRNA and prevents new receptor synthesis, while the existing surface receptors are spontaneously being internalized (through a non-ligand triggered pathway) and undergo receptor degradation. It has been reported that the IL-1 surface half-life in unstimulated cells is about 11 hr.[5] We observed a relatively long lasting down-regulation of IL-1RtI by IL-1 (up to 48 and 72 hr); however, we can not rule out the possibility that the internalized IL-1 could be recycled back to the surface, since the internalized IL-1 may not be degraded for hours and can be transported to the nucleus.[5]

The present data on differences of low concentrations of human IL-1α and human IL-1β to down-regulate IL-1RtI may suggest different capabilities of IL-1α and IL-1β to trigger a signal transduction pathway upon binding to the murine IL-1RtI. There are several reports that IL-1α and IL-1β have different biological activities depending on different cell types investigated.[23,24] In addition, several studies have shown that thre are discrepancies between different species of IL-1 for some biological effects whereas others are unaffected.[25-27] A single amino acid substitution in human IL-1α (Asp^{151} changed to Tyr^{151}) results in the failed induction of PGE_2 production in a human osteosarcoma cell line (MG-63) but, on the other hand, continues to induce PGE_2 in the rabbit dermal fibroblast cell line, RAB-9.[28] A single amino acid change of human IL-1β (Arg^{127} To Gly^{127}) blocks procollagenase induction in fibroblasts normally observed with wild type human IL-1β.[29] In our studies, it may be that rabbit and murine IL-1α are capable of down-regulating the murine IL-1RtI whereas human IL-1α is not, possibly due to slight changes in the amino acid sequences.

We failed to observe the down-regulation of IL-1RtI by IL-1 under low receptor occupancy in CDC.25, another murine Th2 cell line and in EL4-6.1, a murine thymoma cell line. However, in a mouse neuronal cell line, IL-1β in concentrations of 10–100 pg/ml also down-regulated the IL-1RtI mRNA (D. Neumann, personal communication). These observations may reflect the fact that the IL-1R in these cell lines may be structurally different or lack an accessory receptor. On the other hand, the differences in regulation of IL-1RtI in these cell lines may be explained by the differences of signal transduction mechanisms. D10S cell can proliferate in response to IL-1 alone similar to other subclones isolated from the parent D10.G4.1.[17,30] On the other hand, CDC.25 and EL4-6.1 cell lines require an additional signal for optimal IL-1 activities.[31,32] It has been reported that in a similar D10 subline, IL-1 has an additional signal pathway compared to EL4 cells.[17,33]

The down-regulation of the IL-1RtI gene by IL-1 itself might take place at the transcriptional level. Our data suggest that IL-1 increases the degradation of IL-1RtI mRNA, the mechanism of which is not clear (induction of a RNase?). However, the effect of IL-1 is clearly not through protein kinase C since the PKC activator PMA increases the expression of IL-1RtI gene in 30 min. Our data are consistent with other observations that PMA does not affect the IL-1RtI surface level at earlier time-points,[34] but up-regulated the receptor surface level dramatically by 24 hr (Fig. 5b). One can argue that this later event may be due to the fact that PMA activates other genes which secondarily increase the receptor level. However, the short time interval of 30 min strongly suggests that PMA directly activates IL-1RtI gene expression via direct transcriptional expression.

Opposite to the effect of IL-1, we observed that IL-2 up-regulates IL-1RtI both at protein as well as mRNA levels. Whether this is at a transcriptional or post-transcriptional level remains to be investigated. The up-regulation of the IL-1RtI surface expression is consistent with the report that D10.G4.1 cells are more sensitive to a proliferative signal from IL-1 in the presence of IL-2.[19]

There may be a physiological significance for these differences in regulation of IL-1RtI by IL-1 itself or by IL-2. Pretreatment with low doses of IL-1 can protect animals by increasing non-specific resistance to lethality from radiation, injury, inflammation or infection. If IL-1 participates in the 'lethal event', part of this protection mechanism may be through reduced expression of the IL-1RtI. IL-2 increases IL-1RtI mRNA and surface expression and by maintaining more IL-1RtI in immune competent cells, T-cell responses could be amplified. However, this hypothesis remains speculative and awaits *in vivo* animal experiments.

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