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RECEPTOR MEDIATED ENDOCYTOSIS AND INTRACELLULAR FATE OF INTERLEUKIN 1

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Abstract—We have studied the receptor mediated endocytosis of interleukin 1 (IL1) by the murine thymoma cell line EL4. These cells express the Type I IL1 receptor which binds its ligand with both high (K_d = 65 pM) and low affinity (K_d = 14.5 nM). We have shown that the two affinity states of the receptor have different rates of turnover both in the absence and presence of ligand. The biological responses of cells to IL1 stimulation are rapid and occur at low levels of receptor occupancy, whereas receptor mediated endocytosis of IL1 is relatively slow. Internalized IL1 appears to accumulate within cells in a non-degraded form and a proportion of this is associated with a detergent insoluble intracellular fraction, which may reflect transport to the nucleus. In this article, we review our previous findings and discuss the possible biological significance of IL1 internalization and nuclear targeting.

IL1α,‡ IL1β and IL1ra are three members of a family of polypeptide cytokines which play a central role in inflammation and immunoregulation. Despite only limited primary sequence homology, two of these cytokines IL1α and IL1β are full agonists. They bind to the same cell surface receptors with equivalent affinity and elicit an almost identical profile of biological responses in target cells [1–5]. The IL1ra gene product, which is more distantly related to IL1α and IL1β [6], binds to the same cell surface receptors but fails to elicit a biological response [7–9], and consequently can be considered as a naturally occurring receptor antagonist. In addition to the three IL1 gene products, there are two distinct IL1 receptors known as the Type I and Type II. Both are members of the immunoglobulin gene superfamily with three extracellular immunoglobulin folds and a single transmembrane domain [10, 11]. The Type I receptor is the predominantly expressed form in fibroblasts and T-cells, and has been shown to be effective in signal transduction. The Type II receptor, which is predominantly expressed on B-cells, monocytes and neutrophils, binds IL1 with lower affinity than the Type I receptor and is not thought to transmit a signal. The biological function of the Type II receptor is unclear, and recent evidence suggests that it may bind IL1 in a non-productive fashion and so act as a negative regulator [12].

We, [13–15] and others [16–22] have shown that following binding of IL1α and IL1β to the Type I receptor, the ligands are internalized and accumulate within the cell. In contrast to this, the IL1ra does not appear to undergo receptor mediated endocytosis [23]. There is some evidence that the Type II receptor also internalizes ligand, albeit at a low rate [24]. Findings that IL1α and IL1β may accumulate in the nucleus of the cells [13, 14, 19, 21, 22] and attempts to correlate IL1 internalization with induction of gene expression [20], have led to suggestions that IL1 endocytosis may be important for its biological actions. In this article, we review our previously published observations and discuss them in light of a possible biological role for IL1 internalization.

MATERIALS AND METHODS

Materials. Na^{125}I, ^{125}I-labelled IL1β and ^{125}I-labelled Bolton and Hunter reagent, Amersham (Amersham, U.K.); Millilitre plates, Millipore (Bedford, MA, U.S.A.); PD10 columns, Pharmacia (Milton Keynes, U.K.); and Myoclone foetal-calf serum (FCS) and RPMI 1640 were from Gibco (Uxbridge, U.K.).

Cell culture. EL4 6.1 and CTLL cell lines were routinely grown in suspension culture in RPMI 1640 medium supplemented with 10% FCS, penicillin (100 U/mL), streptomycin (100 μg/mL), 2 mM-glutamine, and 20 μM 2-mercaptoethanol (2-ME). CTLL cells were additionally supplemented with IL2 (2 U/mL).

Preparation of recombinant-derived IL1. Point mutations were introduced into the human IL1β molecule by site-directed mutagenesis as described previously, and wild-type IL1α, IL1β and site-directed mutants were all purified as described previously [14]. The concentration of all IL1 preparations was measured by both colorimetric methods and by determination of the A_{280} as described previously [14].

Iodination of IL1. IL1α was iodinated as described

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‡ Abbreviations: IL1, interleukin 1; IL1ra, interleukin 1 receptor antagonist; IL2, interleukin 2; IL2Rα, interleukin 2 receptor α chain; ConA, concanavalin A; EMSA, electrophoretic mobility shift assay.

previously [14]. Briefly, 9.25 μg of recombinant-derived human IL1α [in phosphate-buffered saline (20 mM sodium phosphate/150 mM NaCl), pH 7.5] were incubated with 37 MBq of $Na^{125}I$ (diluted 1:1 in 0.5 M phosphate buffer, pH 7), in a glass tube coated with 0.5 μg of iodogen. The reaction was allowed to proceed on ice for 30 min, then the incubation mixture was desalted on a PD10 column [equilibrated with phosphate-buffered saline and 0.2% bovine serum albumin (BSA)]. Fractions (500 μL each) were collected, and the appropriate fractions pooled. In all cases, the incorporation of ^{125}I was in the range of 60–80%, and the final products exhibited a specific radioactivity of 450–2000 Ci/mmol. The preparation of ^{125}I-IL1α was found to be at least 90% bindable. The biological activity of ^{125}I-IL1α was tested in the EL4/CTLL IL2 production assay, and was found to be identical with that of the native unlabelled protein.

M50 was radioiodinated with the di-iodo derivative of the Bolton and Hunter reagent according to the manufacturer's instructions, giving a specific radioactivity of approximately 300 Ci/mmol.

Binding studies. EL4 cells were resuspended in binding medium (RPMI 1640 supplemented with 25 mM Hepes, 5% myoclone FCS and 0.5% NaN_3), and 50-μL aliquots were placed in wells of a 96-well 0.2 μm-pore-size Millititre filtration plate (Millipore). ^{125}I-IL1α and IL1α were added at the concentrations indicated in the figure legends. After incubation to equilibrium for 4 hr at 37°, the unbound ^{125}I-IL1α was separated from the cells by vacuum filtration, followed by four washes with washing medium (RPMI 1640 supplemented with 25 mM Hepes/0.5% NaN_3). The filtration plates were dried for 20 min at 80°, and the filtration membranes were subsequently punched out and counted for radioactivity in an LKB Clinigamma counter.

Endocytosis studies. Receptor-mediated endocytosis and "nuclear" accumulation of ^{125}I-labelled IL1 was quantified as described previously [13]. Radiolabelled ligand was allowed to bind to EL4 cells overnight at 4°, in the presence or absence of a 100-fold molar excess of unlabelled ligand, to determine non-specific binding. After binding, cells were washed extensively with ice-cold medium to remove unbound ligand, and the total radioactivity associated with the cells was determined. The cells were rapidly warmed to 37° and incubated for various lengths of time. At timed intervals, aliquots of cells were removed and washed either with ice-cold acid buffer (0.15 M NaCl/10 mM sodium acetate, pH 3.5) or with medium containing 2% Tritox X-100. Acid washing removed any ligand remaining on the cell surface, and consequently radioactivity remaining in the cell pellet after acid washing was taken to represent internalized ligand. Radioactivity remaining associated with the cell pellet after Triton washing was taken to represent "nuclear" accumulation, as described previously [13]. In order to compare different forms of IL1, internalization and "nuclear" accumulation were calculated as the percentage of the total cell-associated radioactivity at zero time.

To measure receptor-mediated endocytosis in the presence of varying ligand concentrations, EL4 cells were suspended in assay medium, to which 50 or 300 pM ^{125}I-IL1α was added, and incubated to equilibrium for 18 hr at 4°. Non-specific binding was determined by the addition of a 100-fold molar excess of unlabelled ligand. The cells were then washed to remove any unbound ^{125}I-IL1α and rapidly warmed to 37°. At the times indicated, aliquots of cells were removed, and the receptor-mediated endocytosis stopped by vacuum filtration followed by four washes with ice cold washing medium. Radioactivity remaining associated with the washed cells was taken as the total cell associated (T). Surface bound (S) and internalized (I) ^{125}I-IL1α were also measured. Surface bound ^{125}I-IL1α was removed by washing the cells four times with acid buffer (10 mM sodium acetate, 150 mM NaCl, pH 3.5) at 4°; this treatment removed 95% of specific surface bound ^{125}I-IL1α (results not shown). Radioactivity which remained cell associated following acid washing was consequently considered as intracellular (I). Surface-bound ligand was calculated as the difference between total cell-associated and acid resistant counts ($S = T - I$).

IL-2 production assay. EL4 6.1 cells were plated out on a 96-well plate at a cell density of 10^5 cells/well, and incubated with the indicated concentrations of IL1α, IL1β or the mutants M49, M50 and M51. The final volume was 200 μL/well. After 24 hr, 100-μL aliquots of supernatant were removed, taking care not to disturb the EL4 6.1 cells, and assayed for IL2 content. IL2 was measured by the proliferation of an IL2-dependent cell line, CTLL. On the day of passage, CTLL cells were starved of IL2 for 24 hr. The following day, 10^4 cells were added to a 96-well plate, containing 100 μL of test supernatant or 100 μL of IL2 at known concentrations. The cells were incubated at 37° for 24 hr before being pulsed with [^{3}H]thymidine (1 μCi/well). The cells were incubated for a further 24 hr before harvesting on to filter mats using a Scatron semi-automatic harvester. The filter mats were dried for 20 min at 80°, and the [^{3}H]thymidine incorporated into the cells was counted in a LKB 1205 Betaplate liquid-scintillation counter.

Analytical procedures. All analytical techniques have been described previously [13–15]. To measure activation of NFκB, A549 cells incubated with or without human recombinant IL1β, were lysed and nuclear extracts prepared as described previously [25]. A ^{32}P-labelled, double-stranded oligonucleotide containing the ELAM-1 gene NFκB recognition sequence was used to measure DNA binding activity in the extract using an EMSA. Bands were detected by autoradiography or by phosphor-image analysis.

RESULTS AND DISCUSSION

Occupation of the IL1 receptor and induction of signal transduction and biological responses

The relationship between the level of IL1 receptor occupancy and biological response was established for two murine T-cell lines [26]. This was done by comparing the concentration of IL1 required to give a 50% saturation of high affinity receptor binding sites (K_d), and the concentration required to induce a biological response which was 50% of the maximal

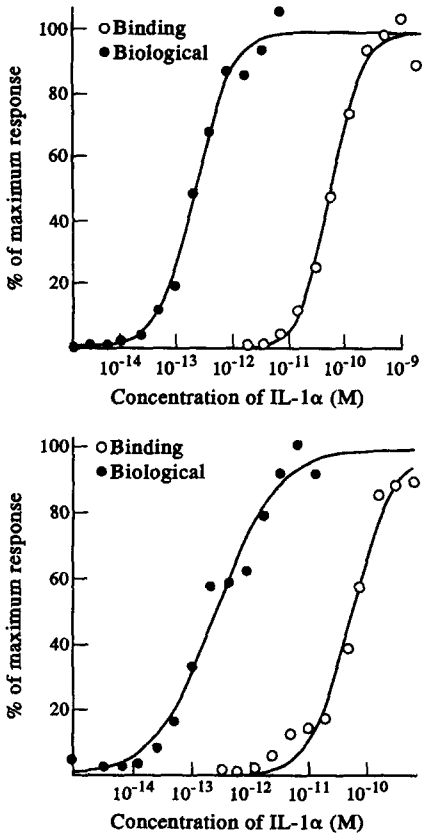

Fig. 1. Comparison of biological responses to IL1 and receptor binding. (Top panel) EL4 6.1 cells were stimulated for 24 hr with varying concentrations of IL1. The cell supernatants were collected and their IL2 content determined in a CTLL proliferation assay (● biological). Saturation binding of ^{125}I-IL1α to the cells was also performed (○ binding). The biological response had an EC_{50} of 208 fM and the saturation binding had a K_d of 53 pM. In the bottom panel similar studies were performed on D10.G4.1 cells. These cells were stimulated for 36 hr with varying concentrations of IL1 in the presence of 2.5 μg/mL Con A. The cells were subsequently incubated with [^{3}H]thymidine to measure proliferation. The biological response had an EC_{50} of 245 fM and the saturation binding had a K_d of 55 pM (from Ref. 26).

(EC_{50}). Examples of such comparisons for EL4 cells and D10.G4.1 cells are shown in Fig. 1. The murine T-cell line EL4 secretes IL2 in response to IL1 stimulation with an EC_{50} of 208 fM. Saturation binding studies revealed that the K_d for the receptor ligand interaction was 53 pM, and that these cells express between 1000 and 2000 receptors per cell. Similarly, the murine T-cell line D10.G4.1 will proliferate in response to IL1 and a sub-optimal concentration of Con A. The EC_{50} for IL1 induced proliferation of these cells is 245 fM and they express between 400 and 1000 high affinity receptors per cell [27]. From these studies we demonstrated that the EC_{50} concentration of IL1 is approximately 250-fold lower than the K_d for receptor–ligand interaction. In both cases, at the EC_{50} concentration, we cannot detect binding of ^{125}I-IL1 to its cell surface receptor. This large difference between concentrations of IL1 required for receptor binding and induction of biological responses has been reported by many workers in the field of IL1, but perhaps most dramatically in the case of IL1 induced diacylglycerol production in Jurkatt cells in the absence of any detectable cell surface receptors [28], and the proliferation of a subclone of D10 cells at attomolar concentrations of IL1 [29].

It is well documented that one of the earliest events in the signal transduction cascade triggered by IL1 binding to its receptor is the activation of the transcription factor NFκB. We have measured the IL1 induced activation of NFκB using an EMSA as previously described [30], to reveal early events following receptor–ligand interaction. Figure 2 shows that following stimulation of A549 cells with 10 pM IL1, the activation of NFκB peaked at 15 min post-stimulation and gradually declined thereafter, but was still detectable at 24 hr. Dose–response curves show that a concentration of IL1 as low as 100 fM is sufficient to induce activation of NFκB (Fig. 3).

We concluded from these studies that following engagement of the IL1 receptor by ligand, the induction of a signal transduction cascade leading to a biological response is both rapid and occurs at very low levels of receptor occupancy.

Analysis of receptor binding studies and receptor mediated endocytosis

Although binding of ^{125}I-IL1α to a high affinity is well characterized, there remains some controversy as to the existence of low affinity binding sites. To address this question, ^{125}I-IL1α and unlabelled IL1α were used to perform competition binding studies on EL4 cells. Azide-treated EL4 cells were incubated with 66 pM ^{125}I-IL1α to equilibrium for 4 hr at 37°, in the presence of increasing concentrations of unlabelled IL1α. Such a competition analysis was required to achieve high enough concentrations of ligand to fully saturate low affinity binding. Figure 4a shows that unlabelled IL1α competed for ^{125}I-IL1α binding in a dose-dependent manner. The data was subjected to Scatchard transformation and was best fit by a two site model (see Fig. 4b) with mean K_ds of 65.5 pM and 14.5 nM and 986 and 10,417 receptors per cell, respectively.

Using receptor affinity labelling techniques we were unable to distinguish biochemically these two affinity states of the receptor [15].

Having established that the IL1 receptor can exist in both high and low affinity states, we went on to examine whether we could detect any functional differences between the two states in terms of ligand internalization. To determine the rate of ligand endocytosis mediated by high affinity receptors, EL4 cells were pretreated with 50 pM ^{125}I-IL1α for 18 hr at 4°. The cells were then washed to remove unbound ligand, rapidly warmed to 37° and incubated for various times. At this low concentration of ligand only high affinity receptor would be occupied by ligand. Endocytosis of ligand by these high affinity IL1 receptors was measured at timed intervals by washing the cells either in ice cold washing medium in order to determine total cell associated

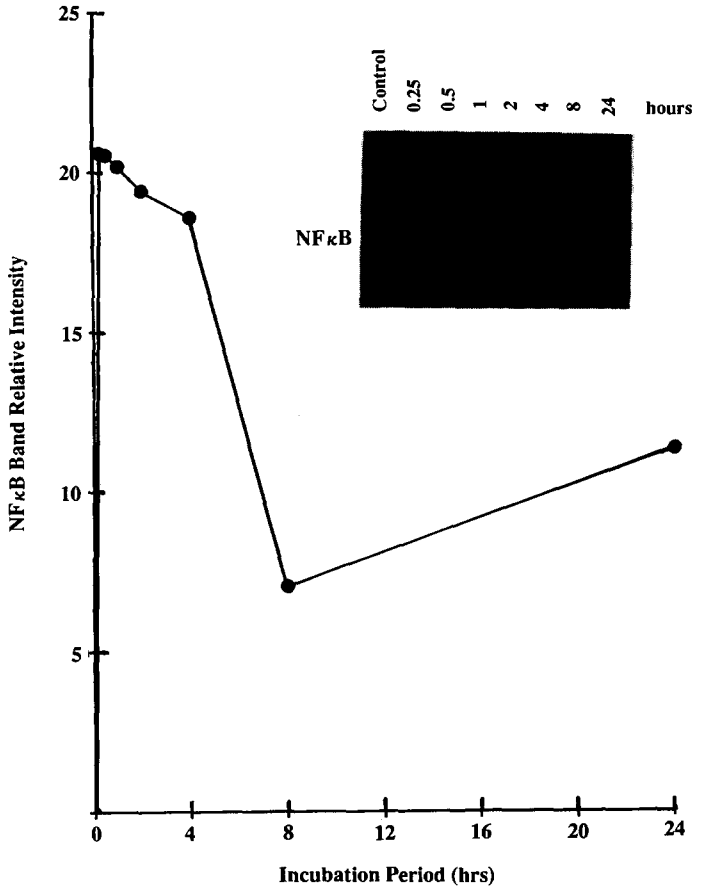

Fig. 2. Time course of IL1 induced NFκB activation. A549 cells were stimulated with 10 pM IL1 for varying periods of time. Following stimulation, the cells were lysed and a nuclear extract prepared. The nuclear extract was incubated with a ^{32}P-labelled double stranded oligonucleotide probe corresponding to the ELAM-1 gene NFκB recognition sequence, and DNA binding activity in the extract was determined by EMSA. The DNA–NFκB complex was quantified by autoradiography of the gel by phosphor-image analysis (from Ref. 25).

radioactivity (T), or in ice cold acid buffer to elute surface bound ^{125}I-IL1α and so determine internalized ligand (I) and surface bound ligand (S).

The results shown in Fig. 5 reveal the removal of receptor bound ^{125}I-IL1α from the cell surface by internalization. We have previously shown that the ligand does not dissociate from the cells over this time period [15]. The results clearly show that receptor-mediated endocytosis of ligand by high affinity receptors was relatively slow. The effect of higher ligand concentrations on receptor-mediated endocytosis was determined by pretreating EL4 cells with 300 pM ^{125}I-IL1α and measuring endocytosis as described above. Under such conditions of higher ligand concentration a proportion of both high and low affinity receptors would be occupied with ligand. The resulting receptor-mediated endocytosis of IL-1 was more rapid, and significantly different from that observed at low ligand concentration.

We went on to examine whether the high and low affinity receptors could internalize and recycle constitutively, that is in the absence of added ligand. In order to do this we pre-incubated EL4 cells for 1.5 hr with 10 μM cycloheximide in order to inhibit *de novo* receptor synthesis. We then incubated the cells for periods of up to 6 hr at 37°, and at timed intervals we removed aliquots of cells, cooled them to 4° and determined cell surface receptor number by binding ^{125}I-IL1α at 50 pM or 10 nM to detect high or low affinity binding sites, respectively. The data presented in Fig. 6 shows that both high and low affinity binding sites are internalized from the plasma membrane constitutively, and that in the case of the low affinity receptor there is evidence that the receptor recycles back to the cell surface.

From these studies we conclude that the IL1 receptor exists in two affinity states which can be functionally distinguished based on their rate of ligand internalization. The low affinity receptor internalizes ligand rapidly, and in the absence of

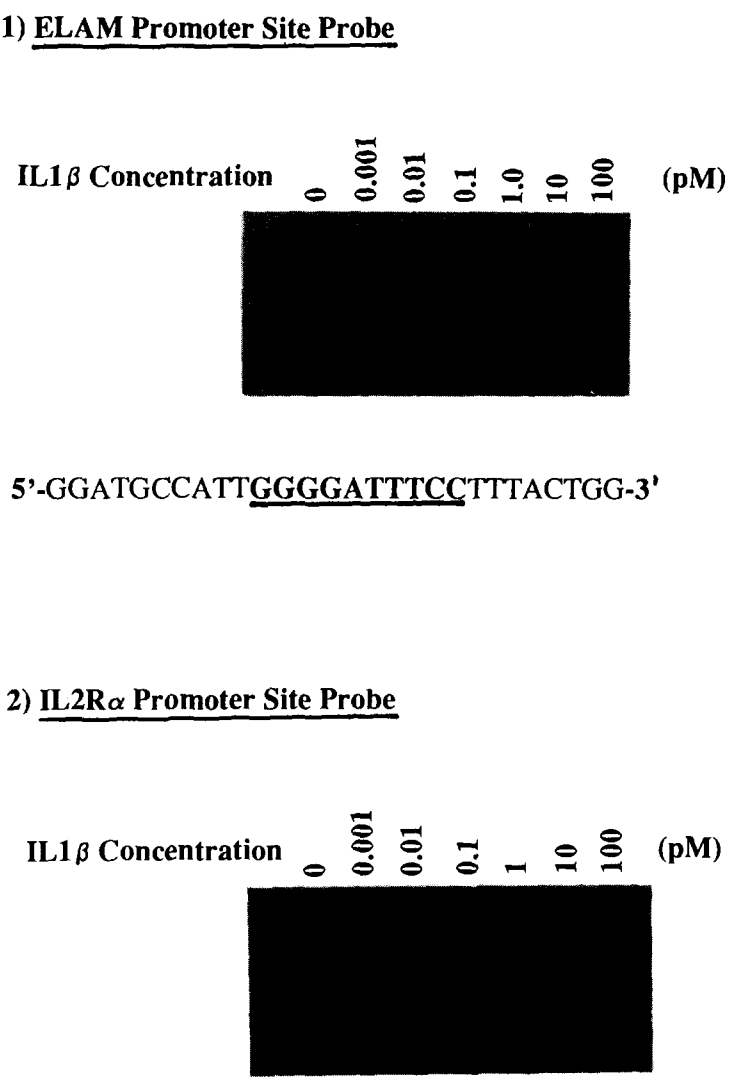

Fig. 3. Dose–response for IL1 induced NFκB activation. A549 cells were stimulated with varying concentrations of IL1. Following stimulation, the cells were lysed and a nuclear extract prepared. The nuclear extract was incubated with a ^{32}P-labelled double stranded oligonucleotide probe corresponding to either the (i) ELAM promoter or (ii) IL2Rα promotor NFκB recognition sequence. The DNA binding activity in the extract was determined by EMSA.

ligand the receptor is constitutively internalized and recycled. The high affinity receptor endocytoses ligand much more slowly and is also constitutively internalized. These studies also showed that over a 5 hr period, almost all of the ^{125}I-IL1α bound to the cell remains cell associated, and is not degraded and released from the cell [13, 15].

Given that the EC_{50} for the biological response of cells to IL1 stimulation is between 100 and 250 fM, it is most unlikely that the low affinity state of the receptor (K_d = 15 nM) is involved in IL1 signal transduction. A more likely explanation is that the function of low affinity state is to rapidly internalize IL1 when present at high concentrations extracellularly. Another possible function is to provide a pool of receptors which can rapidly be converted from a non-functional low affinity state to a functional high affinity state. We have previously presented data in favour of such an "upregulation" hypothesis [15].

The intracellular fate of internalized IL1

In order to examine the intracellular fate of ^{125}I-IL1α following receptor mediated endocytosis we performed the series of experiments outlined below. We first wished to examine whether IL1 remained

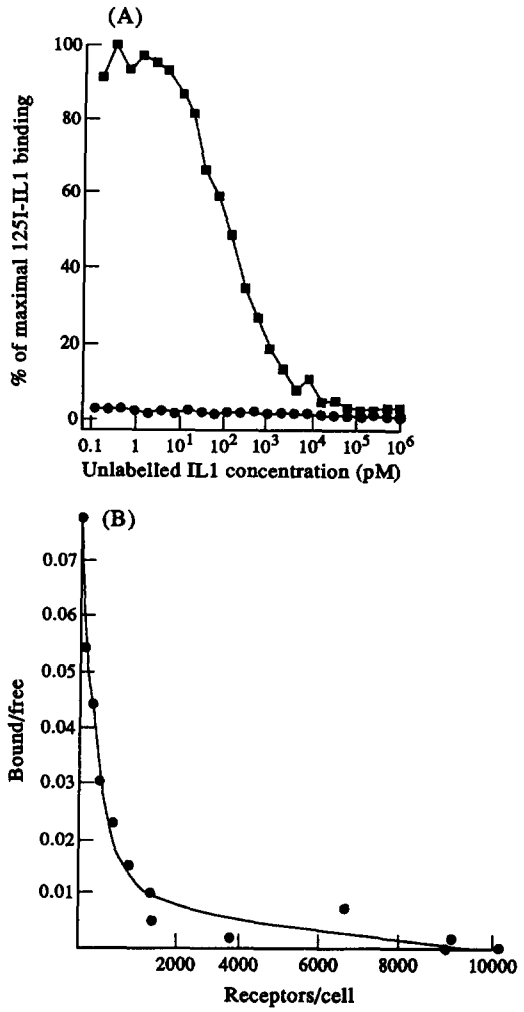

Fig. 4. IL1 receptor binding analysis. (A) EL4 cells were incubated with 66 pM ^{125}I-IL1α in the presence of the indicated concentrations of unlabelled IL1 at 37° for 4 hr to attain steady state binding (■). At that time the cells were vacuum filtered on a 96-well filter plate, washed in washing buffer and the filters punched out of the plate and counted for radioactivity. Each point represents the mean of quadruplicate determinations and is corrected for non-specific binding. In order to examine non-specific binding to the filter mats, the above experiment was repeated in the absence of cells (●). (B) Analysis of the binding data according to Scatchard (from Ref. 15).

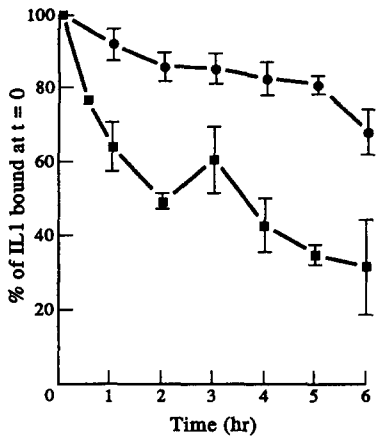

Fig. 5. Internalization of surface-bound IL1 at high and low ligand concentrations. EL4 cells were incubated with 50 pM (●) or 300 pM (■) ^{125}I-IL1α for 18 hr at 4° to achieve equilibrium binding. The cells were washed to remove unbound ligand, rapidly warmed to 37° and incubated for the indicated times. At that time, aliquots of cells (7.5×10^5 cells/well) were removed, washed in ice cold washing buffer to remove unbound ligand and surface bound ^{125}I-IL1α determined by elution of ^{125}I-IL1α with ice cold acid buffer. Each point represents the mean ± SEM of three individual experiments, each conducted in duplicate and corrected for non-specific binding (from Ref. 15).

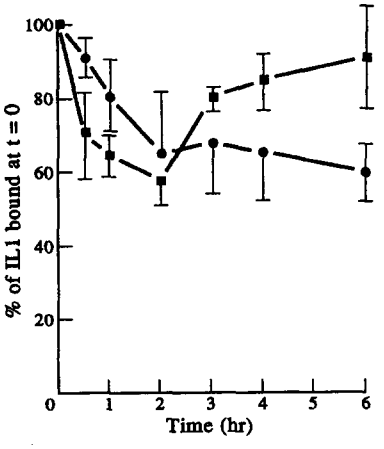

Fig. 6. Constitutive internalization and recycling of the IL1 receptor. EL4 cells were pre-incubated at 37° with cycloheximide for 1.5 hr. The cells were subsequently incubated with cycloheximide for the indicated times. At the indicated time points, aliquots of cells (5×10^6 cells/well) were removed, washed in ice cold washing buffer and assayed at 4° for ^{125}I-IL1α binding with 50 pM (●) or 10 nM (■) ^{125}I-IL1α as described in Materials and Methods. Each point represents the mean ± SEM of three individual experiments, each conducted in quadruplicate and corrected for non-specific binding.

bound to its receptor once internalized, and in order to do this we bound ^{125}I-IL1α to cells overnight at 4°, washed three times with medium to remove unbound ligand, and rapidly warmed to 37°. At timed intervals, aliquots of cells were removed, cooled to 4° and washed in acid buffer to remove surface bound ^{125}I-IL1α—thus the only ^{125}I-IL1α remaining cell associated had been internalized. These cells were then solubilized in 0.05% lubrol, (which we had previously determined did not dissociate receptor–ligand complexes), and centrifuged to remove the detergent insoluble pellet. The first observation we made was that following internalization, a significant proportion of the ^{125}I-IL1α began to accumulate in the detergent insoluble pellet (▲, Fig. 7). The second observation we made was when we examined the detergent-soluble cell

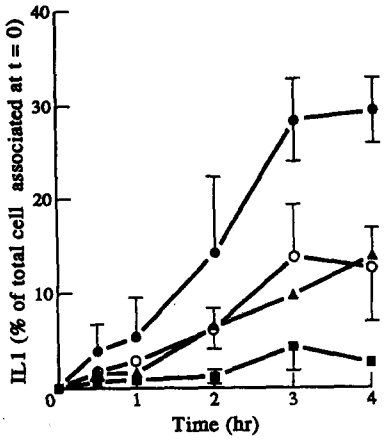

Fig. 7. Intracellular fate of endocytosed IL1. EL4 cells were incubated with 300 pM ^{125}I-IL1α for 18 hr at 4° to achieve equilibrium binding. The cells were washed to remove unbound ligand, and rapidly warmed to 37°. At the times indicated, aliquots of cells (7.5×10^5 cells/well) were removed and washed in ice cold washing medium. Internalized ^{125}I-IL1α (●) was measured by eluting surface bound ^{125}I-IL1α with acid buffer (pH 3.5). The cells were subsequently solubilized in 0.05% Lubrol, and the detergent insoluble pellet was removed by centrifugation. The amount of ^{125}I-IL1α bound to this pellet was (▲) quantified. The ^{125}I-IL1α bound to its internalized receptors (■) was quantified by precipitation of the soluble cell extract with PEG 8000. Internalized free ^{125}I-IL1α (○) was soluble in PEG 8000. Each point shows the percentage of the total cell associated ^{125}I-IL1α at $t = 0$ that is associated with the indicated fraction and represents the mean ± SEM of three experiments, each conducted in duplicate and corrected for non-specific binding.

lysate by precipitation with polyethylene glycol (PEG). We had previously determined that ^{125}I-IL1α when bound in a complex to its receptor was precipitable with PEG, whereas free ^{125}I-IL1α was not. Our data revealed that most of the internalized ^{125}I-IL1α was in fact not precipitable by PEG (○, Fig. 7) so demonstrating that it had dissociated from its receptor. These data suggested that following receptor mediated endocytosis, IL1 dissociated from its receptor and accumulated in a detergent insoluble fraction of the cell.

Based on morphological data, it had previously been observed that internalized IL1 accumulated in the cell nucleus [17, 19], findings which were compatible with our studies showing transport to a detergent insoluble fraction. We went on to examine if this accumulation of ^{125}I-IL1α in a detergent insoluble fraction was an artefact due to non-specific binding of the ligand to the nuclear and cytoskeleton pellet following detergent lysis of the cell. In order to demonstrate that the transport was specific we examined the temperature dependence of ^{125}I-IL1α transport to the detergent insoluble pellet.

EL4 cells were incubated for 18 hr at 4° with ^{125}I-IL1α, followed by extensive washing in ice-cold medium. The cells were then further incubated at 4, 16 or 37° for various periods of time. Internalized ^{125}I-IL1α was determined by acid-washing the cells, and the "nuclear-associated" ^{125}I-IL1α was determined by washing the cells in 2% Triton X-100. Using this technique we demonstrated that at 4° there is no significant endocytosis or transport to the Triton-soluble fraction. This failure to detect ^{125}I-IL1α associated with the Triton-insoluble fraction after binding to the cell surface at 4° suggests that IL1 receptors present on the plasma membrane are effectively solubilized by the Triton wash, and do not remain associated with the "cytoskeleton/nuclear" pellet. At both 16 and 37°, endocytosis of ^{125}I-IL1α can be demonstrated to occur with similar kinetics; however, transport to the Triton-insoluble fraction takes place only at 37°, and not at 16° (Fig. 8). Blockade of transport to the Triton-insoluble fraction at 16° further demonstrates that the "cytoskeleton/nuclear" pellet is not contaminated with membrane-bound IL1 [13].

The inhibition of ^{125}I-IL1α translocation to the "nuclear" fraction by incubation at 16° implied that the internalized ligand was routed through an acidic endosomal/lysosomal compartment. Thus the observed accumulation of radioactivity in the "nuclear" fraction may represent proteolytic fragments of the ^{125}I-IL1α. To examine this possibility, we incubated cells at 37° with ^{125}I-IL1α for 4 hr then analysed the detergent insoluble "nuclear pellet" by 2-dimensional SDS–PAGE [13]. The results clearly show that although some proteolytic fragments did accumulate in this "nuclear fraction", the majority of the radioactivity represented ^{125}I-IL1α that was still intact.

Biological significance of "nuclear transport"

Our studies had shown that following receptor mediated endocytosis, IL1 accumulated intact in a detergent insoluble cell fraction which was probably the nucleus [13]. These findings confirmed previous morphological studies which had come to the same conclusions. Subsequent studies [20], showing that the biological responses of cells to IL1 could be correlated to internalization, led to speculation that nuclear targeting of IL1 could play an important role.

We attempted to address this question using site directed mutants of IL1β [14]. We generated a series of mutants in a highly basic region of IL1β and quantified their receptor binding and biological potency. These mutants had the surprising property of being more biologically potent that wild-type IL1β. One such mutant, M50 (Lys211◊Ala) bound to the IL1 receptor with equivalent affinity to wild-type IL1, but was 80-fold more potent at inducing IL2 secretion from EL4 cells. We were thus able to test whether we could correlate biological potency with receptor mediated endocytosis and "nuclear" accumulation. Our data indicated that M50 was internalized identically to IL1α and IL1β both kinetically and quantitatively. We could therefore conclude that there was no correlation between internalization and biological responses to IL1 stimulation.

To add further weight to these studies, we went on to examine the effect of pertussis toxin on receptor mediated endocytosis and "nuclear

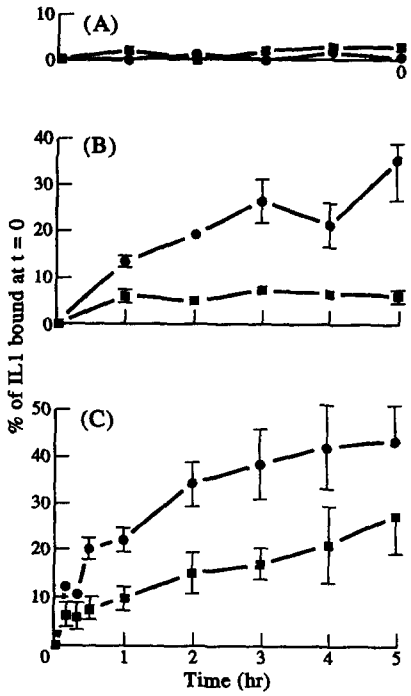

Fig. 8. Internalization and "nuclear" accumulation of IL1. EL4 cells were incubated with 300 pM ^{125}I-IL1α for 18 hr at 4° to achieve equilibrium binding. The cells were washed to remove unbound ligand, rapidly transferred to (A) 4° (B) 16° or (C) 37° and incubated for the indicated times. At that time, aliquots of cells (7.5×10^5 cells/well) were removed, washed in ice cold washing buffer and internalized ^{125}I-IL1α determined by elution of surface bound ^{125}I-IL1α with ice cold acid buffer (●). Nuclear associated ^{125}I-IL1α (■) was determined by further washing with 2% Triton X-100 buffer. Each point represents the mean ± SEM of three experiments, each conducted in duplicate and corrected for non-specific binding (from Ref. 13).

accumulation". We had shown previously [31] that pretreatment of target cells with pertussis toxin was effective at completely abolishing responses to IL1. However, treatment of EL4 cells with pertussis toxin has no effect upon internalization or "nuclear accumulation" (data not shown). From these studies we propose the evidence is highly suggestive that IL1 is internalized and accumulates intact in the nucleus. However, we are unable to demonstrate or correlate any biological role with such transport. Recent studies in which the IL1 receptor has been mutagenized, provide strong evidence that internalization is not required for signal transduction [32]. This leaves us to ponder what the biological significance of "nuclear" transport might be. From our experience, internalization of IL1 is most rapid at high concentrations of ligand, which occupy the low affinity state of the receptor. We would not expect these low affinity receptors to be involved in signal transduction for the reasons discussed. Indeed, we can only detect significant nuclear accumulation when using concentrations of ^{125}I-IL1α (300 pM) far in excess of the maximum required for biological responses. The observed nuclear accumulation could therefore be a response to exposure of cells to supra-optimal concentrations of IL1.

In conclusion, given the number of IL1 receptors per cell (between 400 and 2000) in addition to the fact that biological responses can be elicited at such low levels of receptor occupancy and that the rate of internalization of high affinity receptors is quite slow, it is most likely in our opinion that nuclear targeting of IL1 plays a major role in the responses of cells to IL1 stimulation.

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