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Interleukin 1 signaling occurs exclusively via the type I receptor

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ABSTRACT **Two receptors for the proinflammatory cytokine interleukin 1 (IL-1) have been cloned and characterized biochemically. While it has been well established that the type I (80-kDa) IL-1 receptor can mediate responses to IL-1, the function of the type II (60-kDa) IL-1 receptor has been unknown. In this manuscript we describe experiments designed to ask whether the type II receptor is capable of delivering a biological signal. We have examined two types of experimental situation: responses to IL-1 in cells which express predominantly the type II receptor, and responses to IL-1 which have been suggested previously in the literature to be mediated by type II receptors. In both situations we find that the responses instead are mediated via type I receptors. A blocking antibody against the type II receptor never inhibits, and in fact sometimes enhances, the responses. We conclude that a very small number of type I receptors is sufficient to mediate all of the actions of IL-1 which we have examined here and that the function of the type II receptor may not be to transduce signals.**

The interleukin 1 (IL-1) family of cytokines has a central role in regulating the host response to infection and injury. Production of the biologically active hormones IL-1α and IL-1β results in fever, the acute-phase response, mobilization of the immune system, and other leukocyte-mediated defense mechanisms, as well as connective-tissue degradation and remodeling (1).

There are two known receptors for IL-1, which differ in both size and tissue distribution (2–4). The ligand-binding portions of the two receptors are similar, but whereas the cytoplasmic region of the type I (≈80-kDa) receptor contains ≈215 amino acids, that of the type II (≈60-kDa) receptor is only 29 amino acids long. It is well established that the type I receptor is capable of mediating biological responses (5, 6). In studies reported elsewhere, it has also been clearly shown that the type I and type II receptors are not subunits of a multimeric receptor complex but instead bind IL-1 independently of one another (7). This raises the possibility that each receptor might couple to different signal transduction pathways. In the studies reported here, however, we have been unable to find any evidence that the type II receptor signals at all.

MATERIALS AND METHODS

Cytokines. Recombinant human IL-1α and IL-1β were expressed, purified, and labeled with ^{125}I as described (8, 9). IL-1α was used for stimulation of murine cells (70Z/3 and D10 cell lines), since it binds comparably to both type I and type II murine IL-1 receptors (IL-1Rs) (3). Because in the human system the type II IL-1R binds IL-1β substantially better than it binds IL-1α, IL-1β was used in attempts to demonstrate signaling via human type II receptors (neutrophils, monocytes, and THP-1, HepG2, and Raji cell lines).

Antibodies. The rat anti-mouse type I IL-1R IgG2a antibody M15 (6), hamster anti-mouse type I IL-1R antibody JAMA147 (10), mouse anti-human type I IL-1R IgG1 antibody M1 (3), and rat anti-human type I IL-1R IgG2b antibody M4 (11) have been described. Mouse anti-human type I IL-1R IgG1 antibody M10 was obtained in the same screen as antibody M1 (3). Rat anti-human type II IL-1R IgG2b antibody M22 was generated as described (7). All antibodies were able to block completely the binding of either form of IL-1 to the appropriate receptor and were specific for that receptor at the concentrations used.

RESULTS

Cell Lines Expressing Predominantly Type II IL-1Rs. To ask whether the type II IL-1R was capable of mediating biological responses, we initially chose to examine responses in cells in which the only detectable IL-1Rs were type II receptors. We first looked at the murine pre-B-cell lymphoma 70Z/3, which has rearranged heavy- and light-chain immunoglobulin genes and transcribes the μ heavy chain constitutively but does not express surface IgM (sIgM) because the κ light chain is not expressed (12). Treatment with IL-1 leads to activation of DNA binding by transcription factor NF-κB, synthesis of κ-chain mRNA, and expression of surface IgM (13). By crosslinking studies, 70Z/3 cells appear to express only type II IL-1Rs (14). They have easily detectable type II IL-1R mRNA, and it is very difficult to detect type I IL-1R mRNA (14, 15), although in some 70Z/3 cell populations it is possible to find trace amounts of type I IL-1R mRNA by careful Northern blot (J.E.S. and K. Bomsztyk, unpublished data) or RNase protection (K. Weisser and R. Overell, personal communication) experiments. Since the 70Z/3 cell line responds to IL-1 and expresses almost entirely type II IL-1Rs, it was a good candidate for a cell line which would utilize type II receptors for IL-1 signaling.

The preponderant expression of type II IL-1R on the 70Z/3 cells used in these experiments was confirmed by antibody blocking. Monoclonal antibody M147, which blocks binding of IL-1 to murine type I receptors but not to murine type II receptors, was unable to block detectably any ^{125}I-IL-1α binding to 70Z/3 cells (Fig. 1*A*). Nevertheless, when cells were treated with IL-1 and examined after overnight incubation for induction of sIgM expression, the antibody completely prevented the response (Fig. 1*B*). The antibody had no effect on induction of sIgM expression by LPS (Fig. 1*C*), showing that its effect was specific for IL-1. Thus, the induction by IL-1 of κ light-chain mRNA transcription in 70Z/3 cells, and subsequent expression of sIgM, appear to be mediated not by the type II IL-1R, which is readily detectable

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Abbreviations: IL, interleukin; IL-1R, interleukin 1 receptor; LPS, lipopolysaccharide; sIgM, surface IgM.

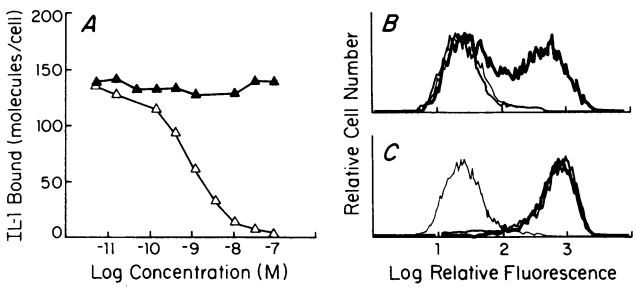

FIG. 1. (*A*) Inhibition of binding of ^{125}I-labeled IL-1α (0.3 nM) to 70Z/3 cells by either unlabeled IL-1α (△) or by anti-type I IL-1R antibody JAMA147 (▲). Binding experiments were done as described (8). (*B* and *C*) sIgM expression on 70Z/3 cells after treatment with either IL-1α or lipopolysaccharide (LPS). The 70Z/3 cells were incubated overnight at 6.7 × 10^5 per ml in culture medium with no additions, with 10 pM recombinant human IL-1α, or with LPS (catalogue no. L2630, Sigma) at 10 μg/ml. When present, the monoclonal antibody JAMA147 was added at 0.1 μM 1 hr before the IL-1 or LPS. The cells were then prepared for analysis by staining with fluorescein-conjugated goat-anti mouse IgM (Tago). In *B*: light line, no additions; heavy line, IL-1; medium line, IL-1 plus antibody. In *C*: light line, antibody alone; heavy line, LPS; medium line, LPS plus antibody.

on these cells, but rather by trace amounts of the type I receptor.

We next looked at the human monocytic cell line THP-1. Resting THP-1 cells express undetectable levels of IL-1Rs. Type II IL-1R expression can be induced by treatment with phorbol ester followed by dexamethasone plus prostaglandin E_2. Type I receptor protein has never been observed on this cell line, although low levels of mRNA can be induced by drug treatment (11, 16). Because the induced cells are very difficult to work with, we chose to use uninduced cells, given that, regardless of the level of receptor expression, there is no evidence for any cell surface type I receptors being present.

THP-1 cells respond to IL-1β by synthesizing mRNA for IL-8. The message is present at 45 min after IL-1 addition and is gone by 2 hr; the induction occurs with IL-1β over the range 0.1–10 ng/ml (data not shown). When we attempted to block the response by using antibodies capable of blocking binding to each receptor, the induction of IL-8 mRNA was blocked by antibodies directed against the type I IL-1R but was not inhibited at all by an antibody against the type II IL-1R (Fig. 2) (in fact, the response was slightly enhanced). This was true not only at near-saturating levels of IL-1β (Fig. 2) but also at low levels of ligand (0.3 ng/ml; data not shown).

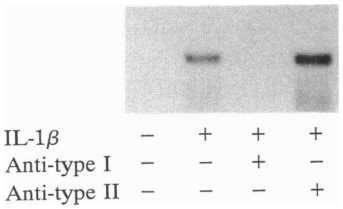

FIG. 2. Northern blots showing steady-state levels of IL-8 mRNA in THP-1 cells, either untreated or after induction by IL-1β. Where present, blocking monoclonal antibodies to the type I receptor (M1, M4, and M10, each at 3.3 μg/ml) or to the type II receptor (M22, 10 μg/ml) were added 15 min before IL-1β (3 ng/ml). Ten micrograms of total RNA, extracted from cells 45 min after stimulation with IL-1β, was loaded in each lane of a 1.2% agarose/0.25 M formaldehyde gel. The RNA was blotted onto HyBond-N (Amersham), and the filter was stained with methylene blue to verify the evenness of RNA loading and transfer. Hybridization was performed in 50% (vol/vol) formamide/0.75 M NaCl at 63°C for 16 hr, and the filters were washed at 63°C in 0.015 M NaCl and exposed to x-ray film. The probe was a ^{32}P-labeled antisense RNA transcribed from the entire coding region of the human IL-8 cDNA.

The two antibodies were without effect in the absence of IL-1β, and the viability of the cells was not altered by the presence of either antibody (data not shown). Thus, as with the 70Z/3 cells, trace amounts of type I receptors seem to mediate the response to IL-1, even though type II receptors are the only receptors which can be detected on THP-1 cells.

Human neutrophils express a readily detectable level of type II IL-1R (16, 17). Surface type I IL-1Rs on neutrophils have never been reported, nor have we been able to observe them in this study, either by flow cytometry of neutrophils stained with anti-type I receptor antibodies or by antibody blocking of IL-1 binding (data not shown). Neutrophils treated with IL-1 produce a number of cytokines, including IL-6, IL-8, and tumor necrosis factor α (ref. 18; M.R.A., unpublished data). When neutrophils were incubated with IL-1β in the presence of antibodies capable of specifically blocking IL-1 binding to either receptor type, production of all of these cytokines was inhibited by anti-type I receptor antibodies (Table 1). Antibodies to the type II receptor either had no effect or had a slight enhancing effect on cytokine production.

Human monocytes detectably express only type II IL-1R on the cell surface (11). They contain predominantly mRNA for the type II receptor, although some type I receptor mRNA can also be found (ref. 11; F.C., F.R., and A.M., unpublished data). Incubation of monocytes with IL-1β leads to secretion of IL-6 and IL-8. There is a report in the literature suggesting that cytokine induction by IL-1 in human monocytes is mediated via the type II receptor (21). In our hands, however, production of these cytokines could be blocked by anti-type I receptor antibodies, but not by antibodies which block the type II receptor (Table 2).

Cells Suggested in the Literature to Respond via Type II IL-1Rs. The human hepatoma cell line HepG2 expresses detectable amounts of both type I and type II receptors (3, 7, 24). It has been suggested, by use of mutant versions of IL-1β to distinguish binding to the two receptors, that the induction of IL-8 in this cell line is mediated via the type II receptor (25). We have looked at the induction of IL-8 mRNA by IL-1β in HepG2 cells and found it to be inhibitable by anti-type I receptor antibodies, but not by anti-type II receptor antibodies (Fig. 3*A*). In addition, we have looked at two other responses to IL-1β in HepG2 cells: the acquisition of DNA-binding ability by the transcription factor NF-κB (Fig. 3*B*) and the induction of α_1-acid glycoprotein mRNA (data not shown) and protein (Fig. 3*C*). In these cases as well, the

Table 1. Cytokine production by human neutrophils

Treatment	IL-6, pg/ml	IL-8, pg/ml	TNF-α, pg/ml
Medium	<5	39	<5
M1	<5	27	<5
M22	<5	51	<5
IL-1 (0.5 ng/ml)	36	207	37
IL-1 + M1	<5	50	<5
IL-1 + M22	85	282	43
IL-1 (2 ng/ml)	65	339	51
IL-1 + M1	<5	99	<5
IL-1 + M22	101	749	41
LPS	350	2741	346
LPS + M1	468	3510	365
LPS + M22	393	2370	494

Neutrophils were purified from normal donors as described (19) and incubated overnight with IL-1β at the indicated levels or with LPS in the presence or absence of blocking antibodies to the type I (M1) or type II (M22) receptor, as indicated. Where present, antibodies were added 30 min prior to IL-1 or LPS. After culture, cytokines in the medium were measured by enzyme-linked immunosorbant assay (19, 20). The data are representative of three separate experiments. TNF, tumor necrosis factor.

Table 2. Cytokine production by human monocytes

Treatment	IL-6, units/ml	IL-8, ng/ml
Medium	<50	25.2 ± 5.4
M4	<50	21.2 ± 2.3
M22	<50	20.8 ± 3.7
IL-1 (0.05 ng/ml)	553 ± 651	64.8 ± 1.6
IL-1 + M4	<50	17.0 ± 4.9
IL-1 + M22	2,501 ± 657	114.9 ± 4.9
IL-1 (0.5 ng/ml)	3,383 ± 2,593	160.8 ± 10.1
IL-1 + M4	<50	62.3 ± 10.1
IL-1 + M22	12,295 ± 2,445	314.2 ± 45.0

Human monocytes were isolated from blood mononuclear cells by using discontinuous Percoll gradients as described (22) and were cultured (10^6 cells per ml) for 24 hr in RPMI-1640 medium containing 1% fetal bovine serum, with the indicated additions. Antibodies were added to 10 μg/ml. IL-6 in the supernatant was measured as hybridoma growth factor activity (23), and IL-8 was measured by radioimmunoassay (F.C., F.R., and A.M., unpublished data). The data are representative of three separate experiments.

responses were blocked by antibodies to the type I IL-1R, but not by an antibody to the type II IL-1R. It was previously reported that the activation of a mitogen-activated protein kinase by IL-1 in HepG2 cells depends on signaling via the type I receptor (27).

The human B-cell lymphoma line Raji has been used by numerous investigators as a source of type II IL-1R (3, 28, 29). This line has been reported to respond to IL-1 by an increase in the transcription of mRNAs encoding the c-*myc*, c-Ha-*ras*, and IL-2R p55 (α chain) gene products (30). In experiments looking at steady-state RNA levels, we have been unable to reproduce these results in our line of Raji cells: c-*myc* and c-Ha-*ras* mRNAs were expressed constitutively, with their levels unaltered by IL-1β stimulation, and IL-2R p55 was not induced by IL-1β (data not shown). Relevant to these results is the finding by McMahan *et al.* (3) that Raji was the only type II IL-1R-positive human cell line examined that failed to express any detectable type I IL-1R mRNA.

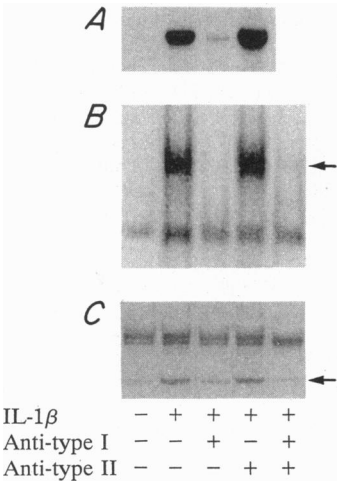

FIG. 3. Responses to IL-1β in HepG2 cells: induction of IL-8 mRNA visualized by Northern blot (*A*), NF-κB DNA-binding activity detected by gel shift (*B*), and α_1-acid glycoprotein detected by Western blot (*C*). (*A*) HepG2 cells were treated with or without IL-1β (1 ng/ml) for 45 min at 37°C. Addition of antibodies where indicated and analysis of IL-8 RNA were performed exactly as described in the legend to Fig. 2, except that antibody M10 was omitted and 10 μg of RNA was loaded in each lane. (*B*) Cells were incubated with or without IL-1β (0.2 ng/ml) for 30 min at 37°C, and nuclear extracts were prepared as described (26). When added, antibodies (M1 and M4, each at 15 μg/ml; M22, 30 μg/ml) were present for 1 hr prior to addition of IL-1. The ^{32}P-labeled oligonucleotide used for the gel shift analysis had the sequence 5′-TGACAGAGGGGACTTTC-CGAGAGGA-3′. (*C*) Cells were incubated overnight with or without IL-1β (0.2 ng/ml), and proteins in the culture medium were separated by SDS/8–16% PAGE, transferred electrophoretically to nitrocellulose membrane (Bio-Rad), and probed with rabbit antiserum to human α_1-acid glycoprotein followed by biotinylated goat anti-rabbit IgG and streptavidin-conjugated alkaline phosphatase (all from Sigma). Where indicated, antibodies (M1 and M4, each at 15 μg/ml; M22, 30 μg/ml) were added 30 min before IL-1.

Muñoz *et al.* (6) looked at responses to IL-1 in the murine T-cell lymphoma D10, which expresses both type I and type II IL-1Rs (24). They found that proliferation, induction of mRNAs for IL-5, c-*myc* and c-*myb*, and production of cAMP were inhibitable by the anti-type I IL-1R antibody M15, which blocks binding of IL-1 to murine type I receptors but not to murine type II receptors (D. J. McKean, J.L.S., J.E.S., and S.K.D., unpublished data). However, induction of c-*fos* mRNA, as well as phosphorylation of an 80-kDa substrate of protein kinase C, was not inhibited by the antibody, suggesting that these latter responses might be mediated via the type II IL-1R. We have reexamined the induction of c-*fos* mRNA by IL-1α in D10 cells, taking care not to introduce serum when adding the IL-1, and have found it to be inhibitable in our hands by the anti-type I receptor antibody M15 (Fig. 4). A control non-blocking anti-type I receptor antibody had no effect (data not shown). The M15 antibody also prevented induction of c-*myc* mRNA and inhibited cell proliferation (data not shown), as reported (6). The latter effect could be overcome at higher IL-1 concentrations, demonstrating that the antibody was not nonspecifically toxic.

DISCUSSION

To ask whether the type II IL-1R was capable of mediating biological responses, we focused on two types of experiments. The first, exemplified by studies using human neutrophils and monocytes as well as the 70Z/3 and THP-1 cell lines, looked at cells in which the only detectable IL-1Rs were type II receptors. This seemed the most likely circumstance in which type II receptors would be used for signaling. The second set of experiments (involving the cell lines HepG2, D10, and Raji) were directed toward situations in which other investigators had suggested that type II receptors might be mediating biological responses to IL-1. In both sets of experiments, however, antibody blocking studies uni-

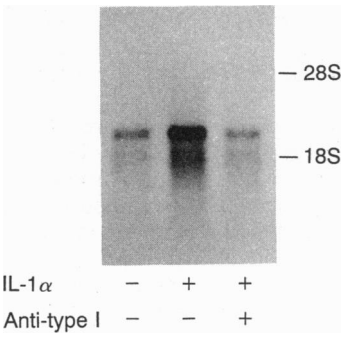

FIG. 4. Induction of c-*fos* mRNA in D10 cells. D10 cells, normally grown in the presence of IL-2 at 10 ng/ml and passaged weekly on a feeder layer of irradiated C57BL/6 mouse spleen cells, were grown for an additional 5 days in the presence of IL-2 alone, followed by culture for 24 hr in serum-free medium. One million cells for each sample were then cultured with no addition or with M15 (60 μg/ml) antibody for 30 min prior to the addition (where indicated) of IL-1α (0.6 ng/ml). Cells were harvested 30 min later, RNA was prepared, and 2 μg was analyzed as described in the legend to Fig. 2. The probe was a ^{32}P-labeled antisense RNA probe generated from a 1.1-kb coding region segment of the murine c-*fos* cDNA. Positions of 28S and 18S rRNAs are indicated at right as size markers.

formly indicated that the type I receptor was responsible for all of the observed biological effects of IL-1.

For the human cells, a specific blocking antibody was available to confirm that the type II IL-1R played no role in the responses examined. Unfortunately, we do not have a corresponding antibody against the murine type II receptor. However, experiments reported elsewhere (7) have demonstrated (*a*) that the complex pattern of high- and low-affinity binding sites seen for IL-1 is not caused by the association of the two receptors as subunits of a multimeric receptor complex and (*b*) that the type I and type II receptors are incapable of simultaneously binding IL-1 with a $K_a > 10^3$ M^{-1}. The latter finding also rules out a scheme whereby large numbers of nonsignaling type II receptors act to enhance responses by capturing IL-1 and "feeding" it without dissociation to a small number of signaling type I receptors. Therefore, the ability of the anti-type I receptor antibody to block a response can be taken as presumptive evidence for the lack of involvement of the type II receptor, despite the absence of a blocking anti-murine type II receptor antibody. That the type II receptor does not play a subsidiary, enhancing role was confirmed by demonstrating its irrelevance to IL-8 induction in THP-1 cells not just at high IL-1β concentrations, where the type I receptor might be able to act independently, but also at subsaturating IL-1β concentrations, where an enhancing role for the type II receptor would be more evident (Fig. 2; data not shown).

In fact, rather than blocking responses to IL-1, the anti-type II receptor antibody usually resulted in a slight enhancement of the response (see Figs. 2 and 3 and Tables 1 and 2), which was more pronounced at low IL-1 concentrations. We suggest that in these instances, the antibody prevented IL-1 from binding to inactive type II receptors and thus effectively raised the concentration of IL-1 available to signal via the type I receptor.

Results which complement and confirm the conclusions of this paper have recently been reached in other studies. The IL-1R antagonist IL-1ra (31, 32) inhibits the binding of IL-1 to murine type I receptors but does not, except at much higher concentrations, inhibit IL-1 binding to murine type II receptors. Stylianou *et al.* (33) and Iwasaki *et al.* (T. Iwasaki, J.E.S., S.K.D., and K. Bomsztyk, unpublished data) have used IL-1ra to examine IL-1 responses in 70Z/3 cells and have obtained results similar to those shown in Fig. 1. Similarly, McKean *et al.* (D. J. McKean, J.L.S., S.K.D., and J.E.S., unpublished data) have used the M15 antibody to demonstrate that the type I IL-1R mediates the induction of NF-κB and c-*jun* mRNA and proliferation in murine T_{h2} helper T-cell clones that detectably express only the type II IL-1R.

In 70Z/3 cells, type II receptors are clearly and routinely demonstrable, whereas it has usually been difficult to show the presence of any type I receptor protein or mRNA. There is good evidence that some RNA samples isolated from 70Z/3 cells have a level of type I receptor mRNA which is effectively zero (ref. 15; C. J. McMahan and J.E.S., unpublished data). It is likely that the expression of type I receptor, always low, fluctuates over time and even within a population. Indeed, it is possible that the reason only a fraction of 70Z/3 cells respond to IL-1 by inducing surface IgM (Fig. 1*B*; S.K.D. and K. Bomsztyk, unpublished data) is that only this fraction is expressing type I IL-1 receptors at the time of the experiment. The presence or lack thereof of a very low level of type I receptors, either because expression is intermittent or because it varies between sublines, may also explain the discrepancies between the results Horuk and McCubrey (30) obtained with Raji cells and those reported here.

In both THP-1 and 70Z/3 cells, IL-1 responses were mediated by very low numbers of receptors (most likely fewer than 20 receptors per cell, our limit of detection). The sensitivity of IL-1 signaling has previously been well established (34–36). It is noteworthy that in all IL-1R-bearing cell lines examined by McMahan *et al.* (3) except Raji, no matter how much type II IL-1R was present, there was always a low level of type I IL-1R mRNA. Despite its low abundance, this level of type I receptor mRNA is most likely sufficient to allow responses to IL-1.

We have examined a representative sampling of IL-1 responses and cell lines and have found no instance in which a response was mediated via the type II IL-1R. Our data are negative and thus are inherently incapable of proving that there is no response which is mediated by the type II receptor in any cell type. Nevertheless, the data do suggest that the type II receptor is not a signaling receptor. Others have detected soluble IL-1-binding proteins which are most likely to be fragments of the type II IL-1R (37, 38). Recent experiments in our laboratory (J.G.G., M.R.A., S.K.D., and J.E.S., unpublished data) suggest that the true role of the membrane form of the type II IL-1R may be to serve as a precursor for a shed, soluble receptor, which could act similarly to the recombinant soluble type I IL-1R (39) in antagonizing or otherwise regulating IL-1 action. In this context it is intriguing that vaccinia and cowpox viruses encode soluble IL-1-binding proteins very similar to the type II receptor (40, 41), which are effective at attenuating the host defense mechanisms.

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