Fluorescence resonance energy transfer (FRET) was used to investigate whether interleukin-1 (IL-1) causes the aggregation of IL-1 type I receptors (IL-1 RI) at the cell surface. For these experiments, a noncompetitive anti-IL-1 RI monoclonal antibody, M5, was labeled separately with a donor probe, fluorescein isothiocyanate, or with an acceptor carboxcyanine probe, Cy3. Donor-labeled M5 and acceptor-labeled M5 were simultaneously bound to transfected mouse IL-1 RI on either C-127 mouse mammary carcinoma cells or on Chinese hamster ovary (CHO)-K1 cells, and the ratio of acceptor emission at 590 nm (excitation at 488 and 514 nm) was monitored with flow cytometry as an indicator of FRET. Addition of a saturating concentration of human IL-1α at 22 °C causes a time-dependent increase in FRET for both cell lines that indicates IL-1-dependent self-association of IL-1 RI. Binding of the IL-1 receptor antagonist at 22 °C causes little or no FRET for both cell lines, indicating a correlation between receptor aggregation and the ability of the ligand to stimulate a functional response. When donor-labeled and acceptor-labeled Fab fragments of M5 are used to monitor FRET, IL-1α causes efficient energy transfer in the CHO-K1 cells at 22 °C, but not at 4 °C. In contrast, IL-1α causes much less FRET at 22 °C in C-127 cells when the M5 Fab fragments are used instead of the intact bivalent M5. In a striking parallel, IL-1α-dependent activation of prostaglandin E2 production depends on the bivalent M5 antibody in the C-127 cells, but is independent of this monoclonal antibody in the CHO-K1 cells. These results provide a strong correlation between the ability of IL-1 to cause the aggregation of IL-1 RI and the stimulation of a functional response.

Il-1α and β are polypeptide cytokines produced by a variety of cell types in response to injury and infection, and they play central roles in immune and inflammatory responses (1–3). They share similar biological activities and receptor binding properties, and their three-dimensional structures are closely related (4, 5). A receptor antagonist polypeptide, IL-1ra, competes with IL-1α and β for binding to receptors but does not stimulate a functional response (6). There are two known receptors for IL-1. IL-1 RI binds IL-1α or IL-1β to mediate the activation of T cells and hematopoietic cells, and to regulate the synthesis and secretion of acute-phase proteins, prostaglandins, and collagenase (7–10). This receptor is found on almost all cell types and is often involved in immune and inflammatory responses (11, 12). IL-1 RI is found on a more limited number of cell types, including B cells and fibroblasts, and may act as a “decoy” to regulate IL-1 RI-mediated responses (12, 13).

IL-1 RI is a single polypeptide chain with an extracellular segment composed of three immunoglobulin-like domains that make up the IL-1 binding site (14). A single hydrophobic transmembrane sequence connects the extracellular region to an intracellular segment of 219 residues that has sequence homology to the Drosophila gene product Toll (15) but provides no clear prediction of the mechanism by which transmembrane signaling is mediated. Recently, Greenfeder et al. (16) have cloned a 66-kDa polypeptide that interacts with IL-1 RI and exhibits significant sequence homology to this receptor polypeptide. Several recent studies have indicated that IL-1 activates a cascade of serine/threonine phosphorylation (17–19), but the earliest events in signal transduction stimulated by this receptor are still unknown.

A number of different cytokines and growth factor polypeptides have been shown to cause aggregation of their receptors to form dimers and sometimes higher order aggregates. One particular case with structural similarities to IL-1/IL-1 RI is the fibroblast growth factor (FGF) receptor system, in which the binding of FGF causes transphosphorylation of FGF receptors (20) and phosphorylation of downstream substrates on tyrosine residues (21), resulting in a cascade of signal transduction which leads to various physiological responses (22). FGF has a three-dimensional structure that is related to IL-1α and β (23), and it binds to the external domain of the FGF receptor that contains three immunoglobulin domains, in analogy to IL-1 RI (24). The receptor for stem cell factor, c-kit, contains five immunoglobulin-like domains in its extracellular region, and recent studies have shown that binding of stem cell factor to the first three domains cause self-aggregation of the receptors that involves interactions dependent on the fourth immunoglobulin-like domain (25).

There are only limited data to suggest that IL-1 may cause the association of IL-1 RI with each other or with other polypeptides (14, 16). One observation that is consistent with a functional role for ligand-induced receptor aggregation is the ability of the overexpressed mutant IL-1 RI lacking a cytoplasmic segment to inhibit IL-1-dependent activation of PGE2 production by endogenous IL-1 RI in transfected CHO-K1 cells (26). In an analogous situation, co-transfection of excess inac-
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tive c-kit receptors with active receptors was shown to inhibit stem cell factor-dependent signaling in a process involving the co-dimerization of active and inactive receptors (27).

The present study used FRET monitored by flow cytometry to investigate whether IL-1 causes the aggregation of IL-1 RI at the cell surface. For these experiments, a noncompetitive, anti-IL-1 RI mAb or its Fab fragments were labeled separately with the donor and the acceptor probes and bound simultaneously to transfected mouse IL-1 RI on either C-127 mouse mammary carcinoma cells or on CHO-K1 cells. By monitoring the ratio of acceptor emission to donor emission, we can readily detect sensitized acceptor emission and donor quenching that occurs upon addition of IL-1. Our results indicate that IL-1 binding leads to time-dependent aggregation of IL-1 RI, and that this aggregation is likely to play an important role in IL-1-dependent signal transduction.

EXPERIMENTAL PROCEDURES

Materials—Fluorescence isothiocyanate (FITC) was purchased from Molecular Probes, Inc. (Eugene, OR), and the amino-reactive carbocyanine dye Cy3 was purchased from Biological Detection Systems (Pittsburgh, PA). Dithiothreitol and papain were purchased from Sigma. Recombinant IL-1α, IL-1β, and rat mAb anti-IL-1 RI M5 were produced and purified as described previously (28-30).

Cells—CHO-K1 cells stably transfected with wild-type IL-1 receptor (CHO-mu1c), and CHO-K1 cells transfected with cysteolinic acid-truncated IL-1 receptor (CHO-extn) (26) were maintained in Ham’s F-12 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 mg/ml streptomycin, and 250 μg/ml geneticin. They were harvested and resuspended into HBS as described for CHO-K1 cells.

Preparation of Fluorescent M5 mAb Derivatives—The FITC derivatization of M5 mAb was prepared as follows: 25 μg FITC in Me₂SO added to M5 mAb (1 mg/ml) in PBS/EDTA, pH 7.7 (10 mM sodium phosphate, 1 mM magnesium chloride, 5.6 mM calcium chloride, 1.8 mM sodium chloride, 5.0 mM potassium chloride, 100 mM NaCl, 100 μM CaCl₂, 100 μM MgCl₂, and 1 mM EDTA) to give a molar ratio of 100:1 FITC:M5 mAb. This reaction solution was incubated at room temperature in the dark for 36 h. The sample was then microcentrifuged at 9,000 × g to remove unreacted FITC. The supernatant was then microcentrifuged and exhaustively dialyzed in PBS/EDTA, pH 7.4, to remove unconjugated FITC. Molar ratios of coupling were estimated to be between 5.6 and 6.2 to 1 FITC:M5 in four preparations as based on extinction coefficients of 74,000 M⁻¹ cm⁻¹ (495 nm) for FITC, 25,000 M⁻¹ cm⁻¹ (280 nm) for FITC, and 210,000 M⁻¹ cm⁻¹ (280 nm) for M5 mAb.

The Cy3 derivative of M5 was prepared as follows: M5 mAb (1 mg/ml) was dialyzed overnight in borate-buffered saline (BBS: 0.16 M sodium chloride, 0.2 mM sodium borate, pH 9.1). One vial of the Cy3 dye was dissolved in 650 μl of BBS, and 160 μl of this were added to 0.2 ml of the mAb solution and incubated at room temperature in the dark for 45 min. The sample was then microcentrifuged at 9,000 × g to remove unreacted Cy3. The supernatant was then microcentrifuged and exhaustively dialyzed in PBS/EDTA, pH 7.4, to remove unconjugated dye. Molar ratios of coupling were estimated to be 5.9 or 8.8 to 1 Cy3:M5 in two preparations as based on extinction coefficients of 130,000 M⁻¹ cm⁻¹ (552 nm) for Cy3, 650,000 M⁻¹ cm⁻¹ (280 nm) for Cy3, and 210,000 M⁻¹ cm⁻¹ (280 nm) for M5 mAb.

Preparation of Fluorescent M5 Fab Fragments—FITC-M5 or Cy3-M5 were dialyzed overnight in PBS/EDTA at pH 8.0, then dithiothreitol was added to a final concentration of 1 mM. Papain, 1 mg/ml in PBS/EDTA, pH 8.0, was activated by incubation at 37 °C for 10 min in the presence of 1 mg/ml dithiothreitol, then added to the mAb solution at 2.5% (w/w) and incubated in the dark at 37 °C for 3.5 h. N-Ethylmaleimide was then added to a final concentration of 3 mM to alkylate the dithiothreitol and inactivate the papain. After an additional 60 min at 37 °C, the solution was dialyzed overnight in PBS, pH 8.0. Gel permeation chromatography was performed with a Superose 12 column (Phar-
using $^{125}$I-PGE$_2$, anti-PGE$_2$, and a standard curve established with synthetic PGE$_2$ (Dupont NEN).

**RESULTS**

The rat mAb M5 has been previously shown to bind to murine IL-1R1 at a site in the extracellular region that is distinct from the IL-1 binding site (29). This mAb appears to cause the dimerization of these receptors at the cell surface (32), but fails to cause any significant cellular response, and does not interfere with the ability of IL-1α to stimulate these responses in EL-4 cells (29). In initial experiments using flow cytometry, we established that the FITC and Cy3 derivatives of M5 mAb bind to transfected murine IL-1R1 on CHO-K1 cells and on C-127 cells with the properties expected from previous results with $^{125}$I-derivative of M5 mAb (29) (data not shown). In addition, fluorescence microscopy and steady state fluorescence spectroscopy were used to look for endocytosis-mediated quenching due to acidification in endosomes, and we established that >95% of IL-1R1 labeled by these derivatives remains at the surface for at least 1 h at 37 °C in the presence or absence of IL-1 ligands (data not shown). Because of these properties, we were able to bind donor- and acceptor-labeled M5 mAb simultaneously to CHO-K1 cells to monitor time-dependent changes in the ratio of acceptor emission to donor emission ($\frac{\text{Cy3(T)} \text{FITC(T)}}{\text{Cy3(F)} \text{FITC(F)}}$) as a sensitive indicator of FRET at the cell surface. In this experiment, IL-1-dependent aggregation of its receptors might be expected to bring donor and acceptor probes into closer proximity that could be monitored with FRET. This process is revealed by an increase in sensitized acceptor emission and a concomitant decrease in donor emission (33).

As shown in Fig. 1A (○), addition of a saturating amount of IL-1α to CHO-mu1ccells prelabeled with equimolar amounts of FITC-M5 and Cy3-M5 results in a time-dependent increase in the ratio of $\frac{\text{Cy3(T)} \text{FITC(T)}}{\text{Cy3(F)} \text{FITC(F)}}$. In a control sample, labeled cells monitored during the same time period in the absence of IL-1α show a small increase in this ratio over time that generally exhibits a constant slope. This upward drift in acceptor/donor fluorescence ratio in the absence of added ligands is seen to vary in extents in different experiments (see below), and the time-dependent changes due to ligand addition are therefore represented as shown in Fig. 1B (●) as normalized fluorescence ratios by dividing for each time point the measured ratio for the samples with added ligands (○) by the corresponding ratio for the control sample (□). As seen in Fig. 1B, the time-dependent increase in the normalized ratio becomes maximal by about 60 min following the addition of IL-1α, with a halftime of about 20 min. As summarized in Table I, line 1, similar results were obtained in four different experiments with two different preparations of labeled M5 mAb.

![Image](http://www.jbc.org/)

*Fig. 1. IL-1α-dependent FRET between donor FITC-M5 and acceptor Cy3-M5 bound to IL-1 RI on the surface of CHO-mu1ccells. A, a mixture of 5 μM FITC-M5 and 5 μM Cy3-M5 was incubated with CHO-mu1ccells (3 × 10⁶ cells/ml) containing wild-type transfected receptors for 50 min at 22 °C. IL-1α (○) or IL-1ra (□) was added at a final concentration of 30 nM immediately after the time point at t = 0 min (arrow), and changes in the ratio of Cy3-M5 fluorescence to FITC-M5 fluorescence were monitored over time. Changes in this ratio were also monitored for the control sample to which no ligand was added (●). B, normalized fluorescence ratio for cells with added IL-1α (○) or IL-1ra (●) calculated from data in A, as described under "Experimental Procedures."*

IL-1 RI which correlates with the stimulatory capability of this ligand compared to IL-1ra.

Because of the potential for receptor dimerization by the M5 mAb, labeled Fab fragments of M5 were prepared and used to investigate whether IL-1α-dependent aggregation detected by FRET was influenced by M5-mediated dimerization. Fig. 2 (□) shows that the addition of IL-1α causes a time-dependent increase in the normalized fluorescence ratio for CHO-mu1ccells labeled with FITC-M5 Fab and Cy3-M5 Fab. Similar to the results with M5-labeled cells in Fig. 1, the time course of the increase in FRET is relatively slow, reaching a maximal value by 80 min, with half-time of ~40 min. The value for the maximum increase in the normalized ratio of acceptor emission/donor emission in this experiment (~1.5) is greater than the value observed with the M5 derivatives in Fig. 1, suggesting that the extent of aggregation observed with the M5-Fab fragments is at least as great as that observed with the bivalent M5 labels. The maximal values observed for the normalized fluorescence ratios due to IL-1α-dependent FRET were generally found to be very reproducible for a particular set of donor- and acceptor-labeled M5 derivatives, but some variation in this maximal value is observed with different preparations of these derivatives, and with different ratios of donor-labeled and acceptor-labeled M5 derivatives (data not shown). In some experiments we examined the IL-1α dose-dependence of FRET using donor and acceptor-labeled Fab fragments of M5. A concentration of 0.5 nM IL-1α is sufficient to occupy ~50% of the IL-1 RI (data not shown), and this concentration results in a maximal value of FRET that is ~80–90% of that observed with saturat-
Changes in the normalized ratio of Cy3-M5 Fab fluorescence to 
acceptor emission is dependent on several parameters that are
difficult to measure directly in this situation (33). In attempts
to quantify the energy transfer efficiency, several experiments 
were carried out in which cells were labeled with a mixture of 
FITC-M5 Fab and Cy3-M5 Fab in a molar ratio of 0.6:1, to 
maximize detection of energy transfer by donor quenching. 
With this ratio, the donor signal is reduced, but there is an 
increased probability that the donor-labeled Fab will be adja-
cent to an acceptor-labeled IL-1 RI during IL-1 dependent 
aggregation. In these experiments, addition of IL-1α caused 
a maximum quenching of 10 and 11% of the donor emission in 
two separate experiments after 70 min of incubation (data not 
shown). Using the simplest model of FRET between single 
donor-acceptor pairs (34), in one limit we assume that all of 
the donor FITC probes bound to aggregated IL-1 RI are within 10 Å of a Cy3 acceptor probe in these measurements. In this case 
the estimated \( R_0 \) value of 55 Å for the FITC/Cy3 donor-acceptor 
pair predicts that \( E \approx 1.0 \) for these donors, and thus at least 
10% of these are co-aggregated with acceptor-labeled IL-1 RI. 
In the other limit, all of the labeled IL-1 RI donor are assumed 
to be adjacent to acceptor-labeled IL-1 RI due to complete 
aggregation, so that the value of \( R_0 \) estimated and the FRET 
efficiency measured lead to an average of \( \approx 70 \) Å between do-
nors and acceptors bound to aggregated IL-1 RI.

In an effort to understand the molecular basis for IL-1-depen-
dent FRET, we investigated the temperature dependence of 
this process. Fig. 3 (□) shows an experiment in which CHO-K1 
cells were initially labeled with FITC-M5 Fab and Cy3-M5 Fab 
fragments at 4 °C, then the acceptor/donor fluorescence ratio 
was monitored before and after the addition of IL-1α at 4 °C. 
Under these conditions, no significant change in the normal-
ized fluorescence ratio was detected following addition of IL-1α 
for a period of 80 min. In several other experiments, a small 
amount of FRET could be detected following extended incuba-
tion at 4 °C, but this was always substantially less than the 
FRET observed with a parallel sample at 22 °C (data not 
shown). Following the incubation with IL-1 at 4 °C, the tem-
perature of the cells was raised to 22 °C and a substantial 
increase in the normalized fluorescence ratio was observed 
(Fig. 3, □). The maximal value of this ratio occurred after 50 
min of incubation at 22 °C, and was similar in magnitude to 
that obtained with a separate sample of the labeled cells that 
were treated with IL-1α at 22 °C from the outset of the exper-
iment (Fig. 3, ○). These latter cells were cooled to 4 °C after 80 
min of incubation at 22 °C, and we continued to monitor the 
fluorescence ratio for an additional 80 min. As seen in Fig. 3 (○), 
only a small decline in the normalized fluorescence ratio was 
observed during this extended time period at 4 °C. These re-
results are representative of three separate experiments and 
indicate that the IL-1-dependent aggregation process detected 
by FRET is highly temperature dependent, but, once it has 
occurred, it remains stable during incubation of the cells at the 
nonpermissive temperature. In several other experiments, we

**Table 1**

Summary of FRET data and functional responses

| Cell line          | Label | Temperature | Ligand | Fig. no. | FRET | PGE2 production |
|--------------------|-------|-------------|--------|----------|------|----------------|
| CHO-mulc (4)       | M5    | 22          | IL-1α  | 1        | ++   | Yes            |
| CHO-mulc (3)       | M5    | 22          | IL-1ra | 1        | ++   | No             |
| CHO-mulc (5)       | M5-Fab| 22          | IL-1α  | 2        | ++++ | Yes            |
| CHO-mulc (3)       | M5-Fab| 22          | IL-1ra | 2        | ++   | No             |
| CHO-mulc (3)       | M5-Fab| 22          | IL-1α  | 3        | ++   | NA             |
| CHO-extrn (4)      | M5-Fab| 22          | IL-1α  | 4        | ++++ | ND             |
| C-127 (5)          | M5    | 22          | IL-1α  | 6        | +    | ND             |
| C-127 (2)          | M5    | 22          | IL-1ra | 6A       | +    | ND             |
| C-127 (2)          | M5-Fab| 22          | IL-1α  | 6B       | +    | ND             |

a Representative experiment.
b Relative magnitude of FRET at the maximum response in the time course.
c PGE2 secretion stimulated by ligand indicated at 37 °C.
d Number in parentheses is number of experiments.
e Data shown in Fig. 5.
f Data not shown.
g Not applicable.
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found that IL-1α-dependent FRET occurs at 37°C to a similar extent as at 22°C, and the time course for this process is similar (data not shown).

To determine whether interaction of the cytoplasmic segment of IL-1 RI with each other or with other cellular components is necessary for IL-1-dependent aggregation detected by FRET, we carried out energy transfer experiments with a mutant that is lacking the C-terminal 194 residues out of 219 in the cytoplasmic segment (26). CHO-extn cells containing these mutant receptors were initially labeled with donor- and acceptor-labeled M5 mAb, but we found that in this situation, there was a large time-dependent increase in the ratio acceptor emission to donor emission even in the absence of IL-1α, suggesting that the bivalent M5 mAb might be causing efficient aggregation of these mutant receptors (data not shown). When labeled M5 Fab fragments were used instead of the intact M5 mAb, the ratio of acceptor emission to donor emission was found to be nearly constant in the absence of IL-1 (data not shown). Fig. 4 shows the results from an experiment in which IL-1α caused a time-dependent increase in the normalized fluorescence ratio that is somewhat less than that for the wild-type receptor measured in the same experiment. In four separate experiments, we observed a wider variation in the amount of energy transfer with the truncated receptor than with the wild-type receptor, but in all four experiments some energy transfer was observed with this mutant. These results suggest that the cytoplasmic segment of IL-1 RI is not essential for IL-1-dependent aggregation, but that it may regulate the aggregation process.

To relate the FRET results to a functional response mediated by IL-1 RI, we measured the production of PGE2 by the CHO-mu1c cells in response to IL-1α. As shown in Fig. 5A, IL-1α stimulates the production of PGE2 by about 10-fold over that in the unstimulated cells, and neither M5 Fab fragments nor the bivalent M5 mAb had any significant effect on PGE2 secretion in the presence or absence of IL-1α. These results are consistent with previous measurements obtained with EL-4 cells in the presence and absence of M5 mAb (29). We also examined the PGE2 secretion response in a separate IL-1 RI transfected cell line, C-127 mouse mammary cells, which express substantially more receptors on their cell surface than CHO-mu1c cells (data not shown). As shown in Fig. 5B, these cells exhibit virtually no response to IL-1α by itself or in the presence of the Fab fragment of M5, but they show a significant response to IL-1α in the presence of the bivalent M5 mAb. As with the CHO-K1 cells, M5 in the absence of IL-1α did not stimulate PGE2 production.

These results suggest that M5 mAb can facilitate a functional response to IL-1α in the C-127 cells. In order to investigate whether this facilitation is related to an effect on IL-1-dependent receptor aggregation, we monitored FRET in the C127 cells as described above for the CHO-K1 cells. As shown in Fig. 6A, C-127 cells labeled with M5 mAb exhibit a time-dependent increase in the normalized fluorescence ratio in response to IL-1α that is generally smaller in magnitude than that observed with the CHO-K1 cells, but which occurs on a somewhat faster time scale. As for the CHO-mu1c cells, IL-1α causes a much smaller amount of FRET under these conditions, even though it binds and occupies most of the receptors (data not shown). In a separate experiment, we compared the ability of IL-1α to cause a time-dependent increase in FRET in C-127 cells labeled with either bivalent M5 derivatives or with M5 Fab fragments. As shown in Fig. 6B, IL-1α causes little or no increase in the normalized fluorescence ratio for cells labeled with the monovalent Fab fragments, even though the expected amount of FRET is observed with the cells labeled with bivalent M5. This dear difference was observed in two separate experiments and is in striking contrast to the results with CHO-mu1c cells described above, in which cells labeled with the Fab fragments showed at least as much IL-1α-dependent FRET as the same cells labeled with the bivalent M5 derivatives. These M5 valency-dependent differences in IL-1-dependent FRET between the two cell lines parallel the differential requirements of these cell lines for IL-1α-stimulated PGE2 production in the presence and absence of M5 mAb. The results, taken together, provide a strong correlation between the ability to detect IL-1α-dependent aggregation of receptors by FRET and the ability of IL-1α to stimulate a functional response.
DISCUSSION

The molecular mechanism by which IL-1 binding to IL-1 RI causes transmembrane signaling that leads to the activation of pro-inflammatory cellular responses has been difficult to ascertain (14). Recent studies indicate that IL-1, like tumor necrosis factor-α, activates a stress-sensitive cascade of mitogen-activated protein-kinase-related enzymes (35, 36), but the earliest events that follow IL-1 binding are largely unknown. Other recent results indicate that IL-1 binding stimulates serine/threonine phosphorylation of a IL-1 RI-associated 65-kDa substrate (37), and this phosphorylation may play an important role in the initiation of the kinase cascade. Our present results indicate that binding of IL-1α causes co-aggregation of IL-1 RI at the cell surface, and that this aggregation process is highly correlated with the activation of at least one functional response, the production of PGE₂.

Using flow cytometric FRET we have established a sensitive and straightforward method to detect IL-1α-dependent aggregation of IL-1 RI labeled with monoclonal anti-IL-1 RI or their Fab fragments. This method can be applied to other cytokine or growth factor receptor system, provided that a noncompetitive mAb specific for the receptor of interest is available. As demonstrated by our results, this method can readily detect ligand-dependent receptor aggregation when fewer than 10⁴ receptors/cell are present, as for the transfected CHO cells in some of our experiments (data not shown). These experiments were carried out with a simple analytical flow cytometer that simultaneously excites both donor (488 nm) and acceptor (514 nm, at lower intensity), and the detection of changes in the ratio of (sensitized) acceptor emission to (quenched) donor emission provides a sensitive means of detecting small changes in FRET that are readily observed upon addition of ligand.

The time course of FRET that we detect in response to IL-1α binding is slow relative to the time course of IL-1α binding. Under the conditions of our FRET experiments, FITC-IL-1 saturates the IL-1 RI receptors and attains a steady state within about one minute of addition (data not shown). In contrast, the maximum amount of receptor aggregation detected by FRET typically requires ∼60 min for the CHO-mu1c cells at 22 °C, with a half-time of 20–30 min in most experiments. This indicates that there is a rate-limiting step subsequent to binding that is necessary for receptor aggregation to occur. In the presence of bivalent M5, the IL-1-dependent aggregation process is significantly faster in the C-127 cells, suggesting that the rate-limiting step is sensitive to some difference between these two cell lines. This rate-determining difference is unlikely to be lateral mobility, as IL-1 RI actually diffuses faster in CHO-K1 cells than in C-127 cells, as measured by fluorescence photo-
bleaching recovery using labeled M5. It is possible that differences in the stoichiometry of IL-1 RI and the newly discovered 66-kDa receptor-associated polypeptide (16) might affect the kinetics of IL-1 RI aggregation.

Our measurements do not distinguish between the formation of receptor dimers and larger aggregates due to IL-1 RI binding, but under conditions of maximal FRET, IL-1-dependent formation of patches of aggregated IL-1 RI are not detectable by confocal fluorescence microscopy, suggesting that aggregation is limited to substantially less than 1000 receptors per aggregate (data not shown). The strong temperature dependence for aggregation that we observe suggests that the rate-limiting step in the aggregation process has an activation energy barrier with a large temperature coefficient. It is reminiscent of the temperature dependencies reported for epidermal growth factor-dependent epidermal growth factor receptor aggregation (38) and decreased rotational diffusion (39), both observed in plasma membrane preparations. Although the molecular basis for this temperature dependence remains to be determined, it provides a useful experimental strategy for investigating the relationship between IL-1 RI-dependent aggregation and other changes in IL-1 RI that can be monitored by physical and biochemical processes.

Our FRET measurements provide direct evidence for IL-1 RI-dependent association of IL-1 RI with other cells and allow us to examine whether this process is related to the initiation of signaling by this ligand. As summarized in Table I, the ability to detect a functional response as represented by PGE$_2$ secretion in a particular combination of cells, ligand, and mAb is highly correlated with the ability to detect a substantial amount of FRET. Minimal aggregation detected by FRET in response to IL-1a or in response to IL-1α on C-127 cells in the absence of bivalent M5 is apparently insufficient to cause productive signaling, while larger amounts of FRET are required for this temperature dependence remains to be determined, it provides a useful experimental strategy for investigating the relationship between IL-1 RI-dependent aggregation and other changes in IL-1 RI that can be monitored by physical and biochemical processes.

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