Complex Binding of Leukemia Inhibitory Factor to Its Membrane-Expressed and Soluble Receptors (43762)

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Abstract. **The complex interaction of leukemia inhibitory factor (LIF) with its specific receptor present on the cell surface, in isolated membranes and in solution, has been examined in detail. Several aspects of this complexity have been highlighted, including the presence of high- and low-affinity murine LIF receptors, biphasic dissociation of human LIF from apparently homogeneous high- or low-affinity human LIF receptors, and unusual species cross-reactivity. The unusual species cross-reactivity observed between murine and human LIF has also been exploited to map an important receptor binding epitope on human LIF.** [P.S.E.B.M. 1994, Vol 206]

Leukemia inhibitory factor (LIF) is a glycoprotein that was initially purified and cloned based on its ability to induce the differentiation of the myeloid leukemic cell line, M1 (1). Subsequently, LIF has been shown to elicit a diverse range of effects on many cell types. These effects include suppressing the differentiation of embryonal stem cells, thereby allowing these cells to retain their pluripotentiality, increasing the production of acute phase proteins by hepatocytes, stimulating a switch from adrenergic to cholinergic neurotransmitter phenotype in neurons, inhibiting the production of lipoprotein lipase in adipocytes and stimulating the survival and/or proliferation of megakaryocyte progenitors, certain hemopoietic cell lines, myoblasts, and primitive germ cells (1).

All LIF responsive cells examined to date express high-affinity receptors for LIF at their cell surface (2–4). Some cells, however, also express low-affinity LIF receptors (3, 4). Recently, a cDNA clone encoding a low-affinity LIF receptor was described by Gearing and colleagues (5). This group also demonstrated that a high-affinity LIF receptor could be generated by the interaction of the low-affinity LIF receptor with a nonbinding subunit termed gp130 (6). The gp130 subunit also appears to be important for the generation of high-affinity IL-6, IL-11, CNTF, and oncostatin-M receptors (7, 8). The situation is made even more complicated by the observation that the high-affinity CNTF and oncostatin-M receptors also contain the low-affinity LIF receptor (7).

In this paper we describe a series of binding studies that have revealed additional complexities present in the LIF/LIF receptor system. The results are discussed in terms of a model for this interaction.

Materials and Methods

Cells were prepared and binding experiments were carried out essentially as described previously (4, 9). LIF-binding protein was purified as described elsewhere (9). Chimeras between human and murine LIF were created and analyzed as described previously (10).

Results and Discussion

High- and Low-Affinity Murine LIF Receptors. A single class of high-affinity receptor was detectable on the surface of almost all LIF-responsive murine cells (Table I). The apparent equilibrium dissociation constant (K_D) for the interaction between LIF and this class of receptor ranges from 20 to 200 pM (Table I). The kinetics of this interaction appeared to be simple

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Table I. Equilibrium and Kinetic Constants Governing LIF Binding

Receptor source[a]	Treatment	[^{125}I]LIF species[a]	K_D (pM)	k_d (min^{-1})	k_a (min^{-1} M^{-1})
m Hepatocytes	Intact cells	mLIF	105 ± 45	0.0007 ± 0.0004	
	Membranes	mLIF	37 ± 11	0.0012 ± 0.0006	5.4×10^8
			1819 ± 665	0.42 ± 0.25	8.1×10^8
	Detergent-solubilized	mLIF	1711 ± 421	0.33 ± 0.28	—
m 3T3-L1 preadipocytes	Intact cells	mLIF	31 ± 19	0.0007 ± 0.0003	$4.9 \pm 1.1 \times 10^8$
m PC13 cells	Intact cells	mLIF	150–250	0.0002	3×10^8
		hLIF	20–30	0.0004	8×10^8
m Macrophages	Intact cells	mLIF	46 ± 8	0.0006 ± 0.0002	$7.2 \pm 2.7 \times 10^8$
			1420 ± 495	0.43 ± 0.37	$4.2 \pm 1.1 \times 10^8$
	Membranes	mLIF	41 ± 18	0.0008 ± 0.0003	—
			1017 ± 316	0.65 ± 0.41	—
	Detergent-solubilized	mLIF	1554 ± 326	—	—
m LIF binding protein	Aqueous	mLIF	1000–4000	0.5	3×10^8
		hLIF	10–20	0.0008	4×10^8
h Allen 1 cells	Intact cells	mLIF	Not detectable	Not applicable	Not applicable
		hLIF	8	0.3–0.4	$1–2 \times 10^7$
				0.001–0.002	$2–4 \times 10^8$
h COS LIF rec	Intact cells	mLIF	Not detectable	Not applicable	Not applicable
		hLIF	300–1000	0.2–0.3	$1–2 \times 10^7$
				0.008	$1–4 \times 10^8$
h COS sol LIF rec	Intact cells	mLIF	Not detectable	Not applicable	Not applicable
		hLIF	700–1000	0.1–0.2	$1–2 \times 10^7$
				0.002–0.004	$1–2 \times 10^8$

[a] m, murine; h, human.
Binding experiments were carried out as described in Hilton and Nicola, 1992; Layton *et al.*, 1992.

and to be governed by single association and dissociation rate constants (k_a = 2–9 × 10^8 min^{-1} M^{-1}, k_d = 0.002–0.0012 min^{-1}; Table I). Certain populations of activated macrophages did not bind LIF in this simple high-affinity manner. Scatchard transformation of saturation isotherms of LIF binding to these cells resulted in concave plots. These results cannot be explained by an underestimation of nonspecific binding or in terms of negative cooperativity; rather these macrophages appear to express both high-affinity receptors (K_D = 20–60 pM) and low-affinity receptors (K_D = 900 pM–2 nM; Table I). The difference between high- and low-affinity receptors in this system was due to a difference in the kinetic dissociation rate (k_d = 0.0004–0.0011 min^{-1} vs k_d = 0.06–1.06 min^{-1} respectively; Table I), however, the kinetic association rate constants governing binding of murine LIF to high- and low-affinity LIF receptors were similar (k_a = 3–9 × 10^8 min^{-1} M^{-1}; Table I).

Further complexity was apparent from experiments in which intact and detergent-solubilized membranes were prepared from cells that expressed only high-affinity LIF receptors. In contrast to the single class, high-affinity receptors present on the cell surface, both high- and low-affinity receptors were detected on membranes, but exclusively low-affinity receptors were present when membranes were solubilized in nonionic or zwitterionic detergents. The binding characteristics of the high- and low-affinity receptors generated by disruption of cell integrity were indistinguishable from those receptors expressed on the surface of activated macrophages (Table I). A low-affinity LIF-binding protein was also detected in high concentration (1–30 μg/ml) in murine serum. This molecule was purified and shown to have identical binding characteristics to detergent-solubilized cell membrane receptors (i.e., K_D = 1–4 nM, k_a = 3–6 × 10^8 min^{-1} M^{-1}, k_d = 0.2–0.4 min^{-1}).

These results led us to speculate that LIF receptors might be composed of a low-affinity binding subunit and a second "affinity-converting" subunit. The molecular basis of LIF receptor heterogeneity was clarified by a series of experiments by Gearing and colleagues (5, 6, 8), in which the low-affinity LIF receptor was cloned and shown to be a member of the cytokine receptor family, and the high-affinity LIF receptor was demonstrated to be a complex between the low-affinity LIF receptor and the affinity converter gp130. Moreover, N-terminal amino acid sequencing of the LIF-binding protein purified from murine serum demonstrated that it was identical to the predicted N-terminal sequence of the cloned low-affinity LIF receptor (9). The composition of the LIF receptor also provided an explanation for the shared biological effects of LIF, interleukin-6, oncostatin-M, and ciliary neutrophic factor (1), since the receptors for these cy-

tokines contain gp130 and, in the case of oncostatin-M and ciliary neurotrophic factor receptors, also the binding subunit of the LIF receptor (7).

Biphasic Dissociation of Human LIF from High- and Low-Affinity Human LIF Receptors. Analyses of equilibrium binding of human LIF to cellular and soluble human LIF receptors revealed a similar picture to that observed for the interaction between murine LIF and its receptor (3; Layton *et al.*, in preparation). Binding of human LIF to the Ewings sarcoma cell line Allen 1, revealed the presence of a single class of high-affinity receptor (K_D = 5–20 p*M*), whereas binding of human LIF to full-length or truncated soluble LIF receptors, expressed transiently in COS cells, revealed only low-affinity LIF receptors (K_D = 0.3–1.0 n*M*). In contrast to the situation at equilibrium, binding of human LIF to the human LIF receptor differed from the murine system in terms of dissociation kinetics. While dissociation of murine LIF from high-affinity murine receptors was uniformly slow, and from low-affinity receptors was uniformly fast, biphasic dissociation of human LIF was observed from both high- and low-affinity receptors, with only the proportion of slowly to rapidly dissociating complexes differing between receptor types. Consistent with this observation, association kinetics were also biphasic to both high- and low-affinity human LIF receptors.

One interpretation of the heterogeneity of human LIF receptor/human LIF system is that the human LIF receptor is capable of isomerization and interaction with gp130 to generate high-affinity LIF receptors. In this way, the ratio of slowly to rapidly dissociating complexes would reflect the rate constants governing isomerization of the LIF receptor and the rate constants governing interaction of the low-affinity LIF/receptor complex with gp130.

Species Cross-Reactivity. Both murine and human LIF are equally capable of eliciting a biological response from murine cells (e.g., inducing the differentiation of M1 myeloid leukemic cells), but only human LIF can act upon human cells. The biological cross-reactivity of LIF was recapitulated in binding experiments; however, there was an additional, quite unexpected level of complexity. Not only was murine LIF unable to bind to either high- or low-affinity human LIF receptors, but human LIF was capable of binding more strongly to murine LIF receptors than murine LIF itself. On high-affinity murine LIF receptors, such as those expressed by the embryonal carcinoma cell line PC13, the K_D for human LIF binding was 20–30 p*M*, while for murine LIF it was 150–250 p*M*. The difference was even more pronounced when examining binding to the soluble murine LIF binding protein purified from serum. In this case, the K_D for the interaction of murine LIF with the murine LIF binding proteins was 1–4 n*M*, while the K_D for binding human LIF was 10–20 p*M*, similar to that for the interaction with the high-affinity murine LIF receptor. This represents a 50- to 500-fold difference in primary binding affinity.

On the basis of their primary binding affinities, unlabeled human LIF should be a 50- to 500-fold better competitor than murine LIF when binding to the murine LIF-binding protein, irrespective of whether labeled human or murine LIF was used as a tracer. This was not observed (Layton *et al.*, in preparation). Rather, both murine and human LIF were not as potent competitors when the heterologous, instead of the homologous, ligand was used as a tracer. Thus human LIF was a 5000- to 10,000-fold better competitor than murine LIF when human LIF was used as a tracer, but only 5- to 10-fold better when murine LIF was used as a tracer. One explanation of this phenomenon was that binding of human and murine LIF to the murine LIF-binding protein may not be mutually exclusive events.

Mapping a Receptor Binding Epitope of Human LIF. Chimeras of human and murine LIF have been constructed to exploit the unusual species cross-reactivity of LIF. These chimeras have been used to map the epitopes present on human LIF that are responsible for its ability to bind to the human LIF receptor and its capacity to bind to the soluble murine LIF-binding protein with a higher affinity than does mouse LIF (10). Since all chimeras should have been equally capable of inducing differentiation in myeloid leukemic cells and of competing with murine LIF for binding to the murine LIF binding protein, it was possible to screen mutants for their correct overall topology.

An initial panel of 15 murine/human LIF chimeras was generated, purified to homogeneity and found to induce M1 differentiation with a specific activity in the normal range; $0.8–2.9 \times 10^8$ U/mg (10). Likewise, each was capable of inhibiting the binding of labeled murine LIF to the murine LIF-binding protein. Remarkably, all the chimeras which showed enhanced capacity to compete with labeled human LIF for binding to the murine LIF-binding protein were also capable of binding to human LIF receptors. On the basis of these results, it is possible to define three regions within the human LIF molecule that are responsible for its enhanced ability to bind to murine LIF receptors as compared with murine LIF, and for its capacity to bind to the human LIF receptor (10). These regions lie in the predicted C-D loop, the C helix and the B-C loop. Further chimeras have been generated which will define more closely the residues that are critical for these effects.

The results generated using interspecies chimeras of human and murine LIF suggest that the interaction of mouse LIF with the mouse LIF receptor involves a different site from the interaction of human LIF with

the human LIF receptor, but that the interaction of human LIF with the mouse LIF-binding protein involves both sites.

Conclusion

Any model of the LIF/LIF receptor interaction must explain the following observations: (i) the presence of high- and low-affinity receptors formed, respectively, by the low-affinity LIF receptor and a complex between the low-affinity LIF receptor and gp130; (ii) biphasic dissociation of human LIF from soluble low-affinity human LIF receptor and cellular high-affinity human LIF receptors; (iii) the capacity of both murine and human LIF to bind to the murine LIF receptor, but only of human LIF to bind to the human LIF receptor; (iv) the higher affinity of human LIF than murine LIF for the murine LIF receptor; and (v) the apparently lower affinity of human and murine LIF for the low-affinity murine LIF receptor when present together in the binding reaction.

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