THE YALE JOURNAL OF BIOLOGY AND MEDICINE 63 (1990), 87–93

Interleukin 1 Receptors and Biological Responses

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Received June 21, 1989

Interleukin 1 (IL-1) is a polypeptide which possesses a wide variety of biological properties. IL-1 was originally studied as "endogenous pyrogen" and "leukocytic endogenous mediator" and more recently as "lymphocyte activating factor." Within a few minutes after intravenous injection into experimental animals, IL-1 triggers events in the hypothalamus to initiate fever, slow-wave sleep, and the release of a variety of neuropeptides. The nature of the IL-1 receptors (IL-1R) is important to the understanding of IL-1's multiple action in mediating both neural and non-neural events. In this paper, the data are reviewed on the physical nature of the dominant, high-binding 80 kDa IL-1R isolated from murine T cells. In addition, newer studies demonstrate the existence of other IL-1 binding proteins which may participate as functional IL-1 receptors. These are a 68–75 kDa binding protein found on B cells and a 26–30 kDa binding protein found on T cells and mesangial cells. There is a considerable discrepancy between the number and affinities of the 80 kDa IL-1R and biological responses. Little is known about the relationship of the 68–75 or 26–30 kDa IL-R's biological responses. It is possible that, similar to neurotransmitter receptors, multiple chains of different binding proteins participate in the signal transduction of IL-1. The hydrolysis of non-phosphatidyl inositol membrane phospholipids plays an important role in responses to IL-1.

INTRODUCTION

The diverse biological activities of interleukin 1 (IL-1) have attracted interest from a wide range of biological disciplines [1]. After systemic injection into a variety of animals, it seems that IL-1 affects nearly every organ system. Interest falls into two groups: *(1)* those studying the potential beneficial effects of IL-1 on host defense mechanisms, particularly the hemopoietic stem cell, and *(2)* those looking at ways to block the production and/or activity of IL-1, since many of its biological activities are associated with disease processes such as tissue degradation in arthritis and diabetes. Some groups are now preparing to use IL-1 in humans to bolster the stem cell's reactivity to colony stimulating factors, while other groups are in clinical trials with new drugs for pain and arthritis which may be effective by reducing production of IL-1. No doubt the isolation of IL-1 receptors is of vital importance to both areas of investigation.

Abbreviations: CSF: colony stimulating factor DAG: diaclyglycerol IL-1: interleukin 1 IL-1R: interleukin 1 receptor PC: phosphatidylcholine

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CLONING OF THE 80 KDA IL-1 BINDING PROTEIN

An 80 kDa IL-1 specific binding protein has been observed on a variety of cell lines [2–7]; it recognizes both IL-1a and IL-1β. Treatment of this 80 kDa protein with glycanases reduces its size to about 65 kDa, indicating that the variation in molecular weight sizes reported by several investigators may be due to differential glycosylation. In addition, some experiments using tunicamycin and plant lectins suggest that the glycosylation sites of the 80 kDa IL-1 receptor may play a role in receptor affinity [8].

The 80 kDa IL-1 binding protein has been purified to homogeneity from the murine cell line El4 6.1 C10, and the 26 N-terminal amino acids have been determined [9]. Using direct in situ binding a protein has been cloned [10], and the amino acids derived from the cDNA match the purified protein. The actual molecular weight of the 80 kDa IL-1 binding protein is 64,598 daltons. This protein, which does not match any other protein presently reported in the various data bases, is the first IL-1 receptor described. The question remains whether this protein is the sole IL-1 receptor responsible for the multitude of biological activities of IL-1; furthermore, is there a case for the existence of other IL-1 binding proteins which, either alone or in conjuction with the 80 kDa protein, comprise the IL-1 receptor complex?

The 80 kDa IL-1R is a member of the immunoglobulin superfamily. Several groups have been actively involved in studies on the binding of IL-1 to the plasma membranes of various cells and cell lines. Of considerable importance is the finding that the 80 kDa IL-1R is a member of the immunoglobulin superfamily. The murine 80 kDa IL-1R is composed of four sections: a 19 amino acid signal sequence, a 319 amino acid extracellular portion, a 21 hydrophobic transmembrane segment, and a 217 amino acid piece probably located in the cytoplasm. The sequence of the cytoplasmic segment does not reveal any similarities characteristic of protein tyrosine kinases. There is a sequence of Lys-Lys-Ser-Arg-Arg that may represent a protein kinase C phosphorylation site which could regulate receptor activity.

Similar members of the immunoglobulin superfamily, the extracellular segment of the 80 kDa IL-1R may contain several characteristic tertiary structures of two beta sheets connected by disulfide bonds. These structures put the 80 kDa IL-1R into the group of receptors with other growth factors such as platelet-derived growth factor, colony stimulating factors (CSF), and, most recently, IL-6 [11]. IL-1 shares biological activities with platelet-derived growth factor on smooth muscle cells, with CSF-1 on hemopoietic cells, and with IL-6 in the induction of hepatic acute-phase reactants as well as T- and B-cell stimulation.

The extracellular segment of the 80 kDa IL-1R has three domains, with the sequences of domains 1 and 2 related; domain 3 appears less related to 1 and 2. Each domain, however, shares sequence hemology with other members of the immunoglobulin superfamily. As analyzed [10], domain 1 is similar to rat CD8 chain II and domain 3 to the CB of the human T-cell receptor; domains 2 and 3 are similar to the murine platelet-derived growth factor receptor. In the extracellular segment, seven distinct glycosylation sites can be observed. The extent of glycosylation appears to be one explanation of the various molecular weights for the IL-1 binding proteins.

Since the initial molecular cloning of IL-1β [12] and IL-1a [13], it has been clear that these two proteins are, with the exception of a few stretches, not strictly homologous molecules. The three-dimensional structure of human IL-1β has been reported [14], and that study shows completely beta-sheet configuration. Using computerized molecular modeling, IL-1a appears also to be a beta-sheet structure

[Cohen F: personal communication]. Single point mutations and multiple point amino acid addition to the N-terminal or mutations to the mid-region (histidine) results in marked changes in the affinity of IL-1β for receptor binding and biological activity [15,16]. Similar mutations in the IL-1a molecule have no effect, and it is important to recognize the fact that the majority of receptor binding and cross-linking studies have been reported for IL-1a (since this form survives the labeling process better than IL-1β). Thus, although the 80 kDa IL-1R recognizes both forms, specific binding between the ligand and its receptor may differ for the two IL-1 forms. Site-directed mutagenesis of the 80 kDa IL-1R will probably clarify this issue.

In human synovial cells, IL-1β binds with a Kd of 4 pM, whereas IL-1a has a Kd of 66 pM [17]. Despite this 15-fold higher affinity in the binding of IL-1β, the biological response (induction of PGE_2) was only 3.3-fold higher. Similar examples exist in the discrepancy between the EC_{50} (effective concentration for half-maximal responses) for IL-1 and its binding affinities on other cells. For example, IL-1 activates a variety of responses on endothelial cells in the 5–50 pM range without significant specific binding. Similar examples exist for IL-1 activation of fibroblasts, chondrocytes, B cells, and neuroblastoma cells.

TWO CLASSES OF IL-1R

A second class of considerably higher affinity (1–15 pM) IL-1 receptors has been observed by several groups [6] and appears to be distinct from the affinity (>500 pM) of the cloned murine 80 kDa IL-1R. Several possibilities might explain the data on the existence of two classes of IL-1R: *(1)* the binding of the first IL-1 molecule to the 80 kDa IL-1R changes its conformation and increases its affinity for a second IL-1 molecule [Joss U: personal communication]; *(2)* there are monomeric and polymeric forms of the IL-1R [10] with increasing affinities; *(3)* after binding, there is a rapid phosphorylation of non-bound receptors; and *(4)* another binding protein(s) exists which forms complexes during binding, and this characteristic increases the affinity and accounts for the two classes of binding. The MacDonald group was the first to describe two classes of receptors on EL4 cells [3]; however, mitogen-stimulated spleen cells apparently show both high- and low-affinity IL-1 receptors [10]. We have also observed on several occasions two classes on EL4 and D10.G4.1 murine T-helper cells [18].

OTHER IL-1 BINDING PROTEINS

The possibility exists that the IL-1R has other chains, similar to the situation of the IL-2R in that the p55 IL-2R subunit would be comparable to the 80 kDa binding protein (IL-1Rβ) and the 30 kDa would represent a putative second chain (IL-1Ra) which, like the p70 chain, would be a low-affinity binding protein; however, the present 80 kDa IL-1R (single subunit) has an affinity of about 500 pM, which could indicate that a second chain has an even lower affinity; the two-chain complex would then account for the high (5–15 pM) affinity class of receptor. A second IL-1R has been reported from RAJI cells, a human B-cell lymphoma line, which has a Kd of 2.2 nM for IL-1β [16,19]. This putative second IL-1R has a molecular weight of 68 kDa; it appears to be a distinct gene product rather than a breakdown fragment of the 80 kDa IL-1R. Others have isolated monoclonal antibodies to the B-cell surface proteins and one of these monoclonal antibodies immunoprecipitates this 68 kDa IL-1R on B-cells [20].

In cross-linking experiments, other IL-1 specific binding proteins have been observed in addition to the prominent 80 kDa IL-1R. From a structural standpoint, the "two-chain" IL-1R complex has received supporting evidence from Bird et al. [5], Resch [21], and from our own studies [18]. The other IL-1 binding proteins are 26–30 and 43–45 as well as 110 and 220 kDa. Are there other IL-1 binding protein breakdown products or aggregates? These IL-1 binding proteins are specific in that they do not cross-link to radiolabeled IL-1 in the presence of competing unlabeled IL-1.

Clearly the small-molecular-weight proteins which are present at approximately 5 to 10 percent of the total IL-1 binding the 80 kDa receptor may be breakdown products of the 80 kDa IL-1R; however, time-dependent cross-linking experiments seem to rule out this possibility [21]. Furthermore, proteases are present during the cross-linking and subsequent procedures. In addition, cell membrane preparations also reveal these lower-molecular-weight forms which bind IL-1. As occurred in the case of the appearance of two classes of receptors in mitogen-activated spleen cells [10], we have been able to increase the binding (cross-linking) of radiolabeled IL-1 to the 30 kDa protein by the addition of phytohemagglutinin during the binding process at 4°C [18,22]. Mitogens may cross-link the 30 kDa protein to membrane and allow a more efficient binding to take place. We have also produced antibodies to synthetic peptides of the 80 kDa IL-1R, and these antibodies do not recognize either the 68 or the 30 kDa protein [23]. Recently, Resch and co-workers reported the presence of the 30 kDa IL-1 binding protein on mesangial cells in the absence of the 80 kDa protein [Resch K: personal communication]. Thus, we are left with the working hypothesis that these other, smaller IL-1 binding proteins are putative receptors distinct from the 80 kDa IL-1R, which may act alone or in conjunction with the 80 kDa IL-1R.

One possible explanation for the 110 kD binding protein is a complex of the 80 plus the 30 kDa binding proteins, which on SDS-PAGE, would be complexed to IL-1 and appear about 125 kD. The 220 kD cross-linked protein may be a dimer of this; however, this possibility remains speculation at the present time. Nevertheless, these smaller-molecular-weight IL-1 binding proteins are not likely to be artifacts of cross-linking methods since, using Jurkat cells (see below) under the same conditions, we do not observe any significant binding, nor do we observe bands representing the 68 and 30 kDa proteins on SDS-PAGE. This result rules out many cross-linking artifacts.

SIGNAL TRANSDUCTION AND THE IL-1R

Following intravenous injection of IL-1 into a variety of animals, responses such as fever, slow-wave sleep, sodium excretion, hypotension, PGE production, and leukopenia occur within five to ten minutes. The vascular endothelium appears to be the likely target for many of these and other biological effects following intravenous IL-1. *In vitro,* endothelial cells respond to IL-1 at 1–10 pM (or less), but IL-1 specific binding is difficult to demonstrate or is often absent. Similarly, human neuroblastoma cells will release arachidonic acid within seconds after exposure to fM concentrations of IL-1, but there is no significant binding to these cells [Dransfield D, Bernheim H: personal communication]. Thus the response of these and other cells appears to take place without measurable association of IL-1 to a plasma membrane binding receptor. How does IL-1 transduce its signal to these cells? One explanation, of course, is that very few receptors need to be occupied for signal transduction to take place. Another explanation is that the affinity of the small-molecular-weight receptors is too low to allow significant cross-linking to take place.

Recent data, however, demonstrate that IL-1 may be transducing its signal via a novel mechanism of phospholipid hydrolysis [24]. Using Jurkat cells, IL-1 will rapidly increase the liberation of diacylglycerol (DAG), but this increase in DAG takes place in the absence of hydrolysis of phosphatidylinositol 4,5-biphosphate. There is no increase in cytosolic calcium in these cells as others have reported with other cells following exposure to IL-1 [25]. Similarly, there is no increase in inositol triphosphate generation; however, Jurkat cells will release phosphorylcholine from phosphatidylcholine within seconds of exposure to IL-1. The hydrolysis of phosphatidylcholine (PC) at very low concentrations of IL-1 (EC_{50} = 30 fM) reaches maximal levels at 100 fM. The response is specific; that is, TNF has no effect and either IL-1a or IL-1β is equally effective. This turnover in PC has also been measured in D10.G4.1, EL4, and human peripheral blood T-cells. Jurkat cells do not express detectable receptors for IL-1 (either at 4°C or 37°C), whereas D10.G4.1 and EL4 cells possess thousands of receptors, and peripheral blood T cells have only marginal numbers of receptors.

These findings suggest that the presence of the 80 kDa binding protein is only indirectly related to signal transduction. Could a putative, second very-low-affinity chain of the IL-1R complex be linked to PC turnover? A similar case may be observed in YT202 cells, which possess only the low affinity p70 IL-2R and when occupied transduce a signal leading to Na^+/H^+ exchange. In such a model for the IL-1R, the 80 kDa IL-1R would bind IL-1 and internalize the molecule or induce a distinct class of signal transduction mechanisms as proposed by Mizel et al. [26]. On the other hand, the low-affinity IL-1Ra chain would not bind with a suffcient affinity for observed binding, but would nevertheless transduce a signal via PC hydrolysis, DAG liberation, and increased protein kinase activation.

The above model is only a working hypothesis, and the existence of other chains of IL-1R requires evidence other than cross-linking data. Nevertheless, it does help to explain the rapid and dramatic biological response of IL-1 *in vivo* without significant binding to endothelium *in vitro*. We have not been able to show that radiolabeled IL-1 binds to or penetrates the organum vasculosum laminae terminalis, the likely area of IL-1/CNS interaction [Blatteis CM, Dinarello CA: unpublished observation] and [27]. Within a few minutes after intravenous injection, however, a variety of biological effects are easily observed in animals. Interestingly, these are, for the most part, associated with the rapid synthesis of prostaglandins (particularly PGE_2). The release of ^{14}C arachidonic acid from labeled cells occurs within seconds after exposure of IL-1, and one source of this arachidonic acid could be DAG, which might possibly be liberated by IL-1-induced hydrolysis of PC. Household cyclo-oxygenase would rapidly bring about the formation of PGE_2.

One interesting aspect of this model is that it separates IL-1-induced rapid increases of PGE_2 from those that require new gene expression for cyclo-oxygenase [28]. The increase in PGE_2 production by a variety of cells *in vitro* following IL-1 exposure occurs too late to explain the rapid onset of fever, neutropenia, or hypotension following IL-1 *in vitro*. Even the appearance of IL-1-induced phospholipase A_2 [29], a calcium-activated enzyme, occurs too late to explain the release of arachidonic acid from cells incubated with IL-1. This finding is supported by numerous studies showing that IL-1 fails to activate intracellular calcium.

One attractive hypothesis to explain the linkage of a putative IL-1R to signal transduction via PC turnover would be to propose that this chain is a glycosylphospholipid anchored cell-surface protein [30]. These interesting cell surface proteins do not

have an intracellular segment and are linked to membrane phosphatidylinositol via their carboxyl terminus to ethanolamine and a carbohydrate. Recently a truncated form of the platelet-derived growth factor receptor has been shown to be anchored to the membrane via glycosylphosphatidylinositol linkage [31]. The THY-1 alloantigen on murine T-cells is also a glycosylphosphatidylinositol-anchored protein and plays an important role in IL-1 effects on T cell activation [32]. It is possible that there is an IL-1R chain which is a PC-anchored protein.

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