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Coexpression of type I and type II IL-1 receptors in the murine T helper 2 cell line D10N

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IL-1 receptor heterogeneity in murine lymphocytes was investigated by cross-linking to [^{125}I]IL-1α and competition with IL-1 receptor antagonist, and the molecular identity of the IL-1 receptors was identified with PCR using primers specific for type I and type II IL-1 receptors. The thymoma cell line EI4 6.1 exhibits exclusively the 80 kDa receptors which proved to be the type I receptor according to PCR analysis. In the pre-B cell line 70Z/3, predominantly a 60 kDa type II receptor but also a trace of type I receptor can be identified by PCR. The Th2 cell line D10N expresses both types of IL-1 receptors in equivalent amounts according to cross-linking experiments and PCR. The proliferative response of D10N cells to IL-1 is inhibited by IL-1 ra which according to cross-linking affects the binding to the type I receptor only. It is concluded that coexpression of both types of IL-1 receptors might be a characteristic of murine Th2 cells and that their growth-dependence on IL-1 is mediated by the type I receptor.

Interleukin-1 receptor; Interleukin-1; Interleukin-1 receptor antagonist; Mouse T helper cell

1. INTRODUCTION

IL-1 exerts its biological effects by means of at least two different receptors (for review see [1]). Two IL-1 receptors from human and murine cell lines have been cloned and sequenced, an 80 kDa protein with an intracellular domain of 217 amino acid residues [2], the type I IL-1 receptor, and a second one of a 60 kDa protein with an extraordinarily short intracellular domain of 29 amino acid residues, the type II IL-1 receptor [3]. For both types of receptors the mode of signaling remains to be established.

Characteristically, the IL-1 receptors are distributed in a tissue-specific, often mutually exclusive manner [4,5]. In a murine T helper 2 (Th2) cell line D10A, a subline of D10.G4.1, however, IL-1 was shown to activate separate signal transduction pathways [6]. Correspondingly the coexistence of 2 different IL-1 receptors on such cells has been implicated [6] and corroborated by differential blockade of IL-1 responses by M15, a monoclonal antibody directed against the 80 kDa type I IL-1 receptor [7]. In fact, an IL-1 receptor with a lower molecular weight apart from the 80 kDa IL-1 receptor has been detected in D10S cells [8] and D10.G4.1 cells [9] and according to peptide mapping analysis it was suggested to be a related, probably processed form of the type I IL-1 receptor [10]. The identity of this second receptor in T helper cells thus appears not yet unequivocally established. Also the possible coexistence of different IL-1 receptors in T cells merits consideration since more recently the dogma that the type I receptor characterizes T cells, whereas the type II receptor is only found in B cells, has been challenged [11].

Here we have analyzed the IL-1 receptor heterogeneity of D10N cells, another subline of D10.G4.1 [12] by cross-linking experiments, complemented with inhibition studies using the IL-1 receptor antagonist (IL-1ra). Further, we provide proof of identity of two IL-1 receptors in D10N cells by PCR technique.

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2. MATERIALS AND METHODS

2.1. *Reagents*

The murine T-helper (Th2) D10N line is a subline of the D10.G4.1 and was obtained from S.J. Hopkins, University of Manchester Rheumatic Disease Center, Salford, UK.

Human recombinant IL-1α, IL-1β and IL-1ra were kind gifts from Dr. W.-D. Schleuning, Schering AG, Berlin, Dr. S. Gillis, Immunex, Seattle and Dr. D. Boraschi, Sclavo, Siena.

2.2. *Cell cultures*

The murine D10N cell line was cultured in RPMI, containing 10% (v/v) fetal calf serum (FCS), 50 μM β-mercaptoethanol, 100 U/ml penicillin, 10 μg streptomycin/ml, 2 mM glutamine, 1 ng IL-1β/ml, 10 U IL-2/ml and, if indicated, 3 μg ConA/ml at 37°C in a humidified 95% air, 5% CO_2 atmosphere. Before all experiments the D10N cells were 'starved' for 24 h, i.e. incubated as above but without IL-1.

EI 4 6.1 and 70Z/3 cell lines were cultured in RPMI, containing 5% FCS, and β-mercaptoethanol, penicillin, streptomycin and glutamine as described above.

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2.3. *Covalent cross-linking of [^{125}I]IL-1α to cells and gel electrophoresis*

RhIL-1α was labelled by the chloramine T method as described earlier [13]. Cells (2–5 × 10^7) were incubated for 4 h at 4°C with 20 ng [^{125}I]IL-1α in 0.2–0.5 ml serum-free medium alone or in combination with unlabeled IL-1β or IL-1ra as indicated in the figures. They were then washed twice with ice-cold PBS. Dithiobis-succinimidyl suberate (DSS) in DMSO (prepared just prior to use) was added at a final concentration of 5 mM, and incubated for 1 further hour at 4°C. The cells were washed twice with ice-cold PBS and then lysed in PBS containing 1% Triton and 2 mM phenylmethyl-sulfonylfluoride (PMSF) for 15 min. After centrifugation (800 × *g*, 15 min) the supernatent was diluted with an equal volume SDS sample buffer, heated 3 min, and run on a 8% SDS polyacrylamide gel (Laemmli). Gels were dried and autoradiographed.

2.4. *Proliferation assay*

Cells (2–2.5 × 10^4) 24 h starved for IL-1 (see above) were incubated in culture medium in a final volume of 200 μl containing the various concentrations of IL-1α and IL-1ra indicated in Fig. 4. After 72 h the cell proliferation was measured using the MTT procedure [14].

2.5. *PCR*

Total RNA was isolated and cDNA from 1 μg RNA was prepared by reverse transcriptase and amplified using the PCR Gene Amp RNA PCR kit from Perkin-Elmer.

Primers were obtained by the solid-phase oligonucleotide synthesis method of Adams et al. [15] using an Applied Biosystems 380B synthesizer. The location of the primers within the receptor is shown in Fig. 3a. The DNA sequence of the type I receptor was taken from [2], for the type II receptor from the EMBL data base, accession number X59769 [3].

Primers type I: 5′-CTGGAGATTGACGTATGTACAGAATATCCAAAT-3′
and
5′-ATCCCCGGCAATGTGGAGCCGCTGTGGGAAGGTGGCCTGTGT3′

type II: 5′-TTCACCACTCCAACAGTGGTGCACACAGGA-3′
and
5′-GTTCATCCTCTGTACTCCGTCTCTACGAAGTCA-3′

3. RESULTS AND DISCUSSION

3.1. *Two types of IL-1 receptors are present on D10N cells*

Cross-linking of [^{125}I]IL-1α to EI 4 6.1 cells and D10N cells resulted in the previously observed autoradiographic pictures [9]: a band at about 97–120 kDa in EI 4 6.1 (Fig. 1a) corresponding to the type I IL-1 receptor and, in D10N cells a band at about 97–120 kDa plus a band of lower molecular weight (Fig. 1b). This second band was obviously stronger when cells were cultured in the presence of ConA, which was also observed previously by Solari [9] in D10 G4.1 cells, and explained as induction of this type of receptor by ConA. Comparison with the PCR experiments (Fig. 3), however, indicates that the enhancement of the lower molecular weight band might equally well be attributed to an effect of ConA on the cross-linking itself than to a real receptor induction.

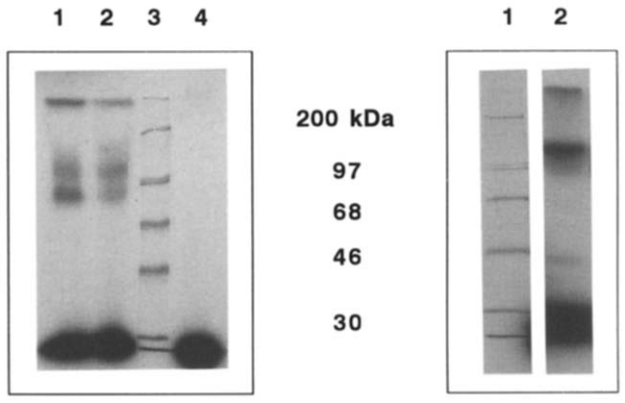

Fig. 1. Left part: Cross-linking of [^{125}I]IL-1α to D10N cells. Lane 1, cells were cultured in the presence of ConA; lane 2, cells were cultured without ConA; lane 3, ^{3}H-labelled protein standards of known molecular weight; lane 4, [^{125}I]IL-1α alone. Right part: Cross-linking of [^{125}I]IL-α to EI4 6.1 cells. Lane 1, standards; lane 2, EI 4 cells.

3.2. *IL-1 ra inhibits cross-linking of IL-1α on D10N cells to the type I IL-1 receptor only*

Since it was shown that the IL-1ra has a greater affinity to the type I IL-1 receptor [16], we performed cross-linking inhibition studies in the presence of IL-1ra to further characterize the nature of the two IL-1 receptors on D10N cells. For this purpose the receptor antagonist was added to the binding reaction together with [^{125}I]IL-1α. In parallel, [^{125}I]IL-1α binding was competed by IL-1β. As can clearly be seen from Fig. 2, IL-1ra in the range of 1–15 times the IL-1α concentration efficiently and selectively antagonized the binding and cross-linking of IL-1α to the 80 kDa IL-1 receptor, whereas binding and cross-linking to the 60 kDa receptor remained practically unaffected. In contrast, IL-1β prevented binding of IL-1α to both receptor types present on

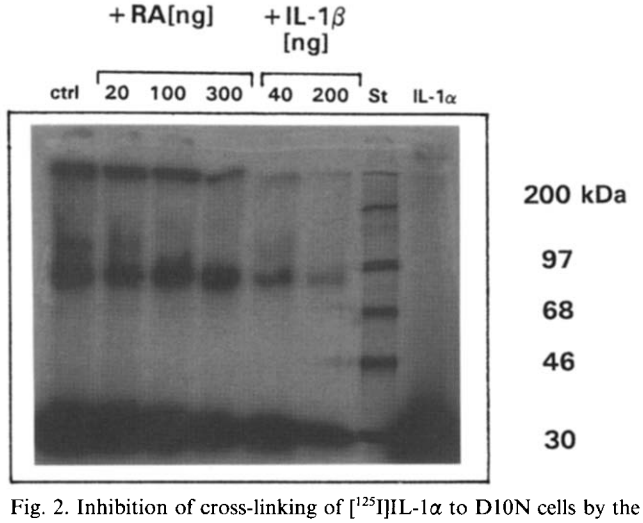

Fig. 2. Inhibition of cross-linking of [^{125}I]IL-1α to D10N cells by the IL-1 receptor antagonist (RA) and IL-1β. In the control (ctrl) 20 ng [^{125}I]IL-1α was used. IL-1ra and IL-1β were present during binding and cross-linking with the concentrations indicated.

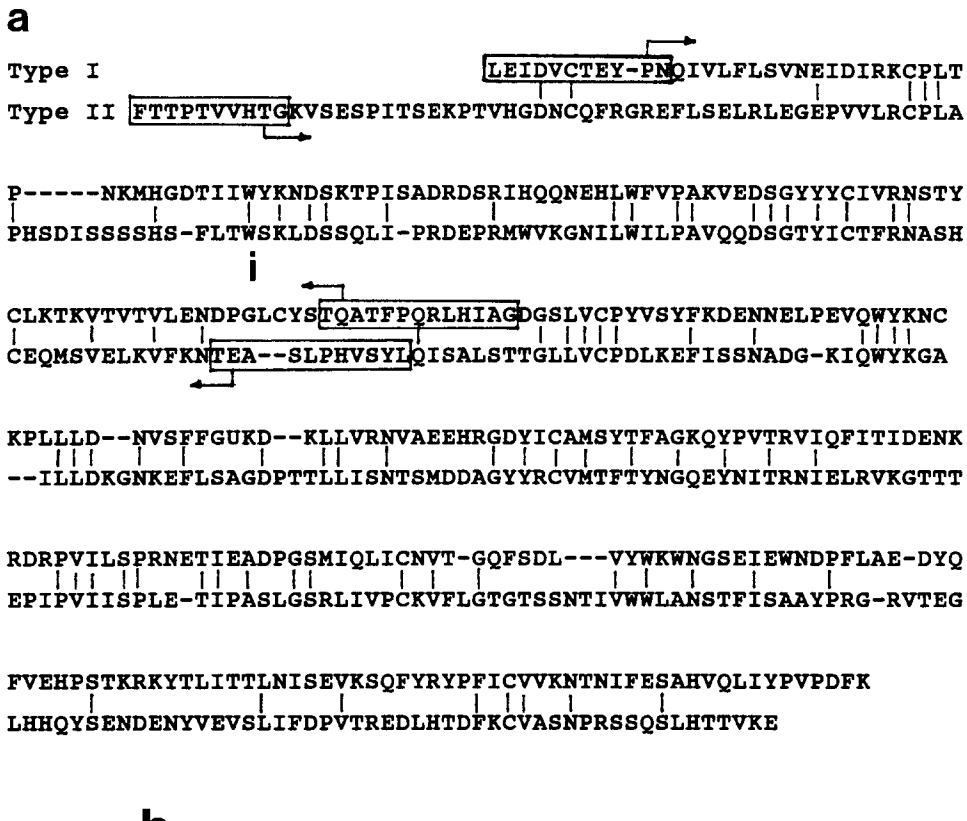

Fig. 3. (a) Amino acid sequences of the extracellular domain of IL-1 receptors type I (2) and II (3). Identical amino acids are indicated by a vertical line, sequences the cDNA was taken from for PCR primers are highlighted. (b) Gel electrophoresis of PCR products. 1 μg RNA was reversely transcribed to cDNA and amplified with the different primer pairs with Taq polymerase in a final volume of 100 μl. 20 μl were transferred to 1.5% agarose gel and electrophorized. Ethidiumbromide was present in the electrophoresis buffer. Lane 1, D10N cells cultured with ConA, primers for the type I IL-1 receptor; lane 2, D10N cells cultured with ConA, primers for the type II IL-1 receptor; lane 3, D10N cells cultured without ConA, primers for the type I IL-1 receptor; lane 4, D10N cells cultured without ConA, primers for the type II IL-1 receptor; lane 5, EI4 6.1 cells, primers for the type I IL-1 receptor; lane 6, EI4 6.1 cells, primers for the type II IL-1 receptor; lane 7, control RNA applied from Perkin-Elmer with the PCR kit; lane 8, standard DNA, pBR 322 × *Hae*III; numbers of base pairs: 587, 540, 504, 458, 434; 267, 234, 213, 192, 184; 124/123, 104, 89, 80, '64; lane 9, 70Z/3 cells, primers for the type I IL-1 receptor; lane 10, 70Z/3 cells, primers for the type II IL-1 receptor; lane 11, standard DNA as in lane 8.

D10N cells in a comparable manner. The results of these cross-linking experiments are in agreement with the characteristic of IL-1ra to preferentially bind to the type I receptor of IL-1 [16].

3.3. *The molecular nature of the IL-1 receptors on D10N cells*

In order to clarify the molecular identity of the IL-1 receptors of D10N cells, we isolated total RNA of D10N, EI4 and 70Z/3 cells, reversely transcribed the RNA to cDNA, and subjected the cDNA to a polymerase chain reaction. The primers used in the PCR were specific for the type I and type II IL-1 receptor, respectively, and were chosen in a way to easily detect the PCR products by size on agarose gels. The sizes of the PCR products and the specificity of the primers used can be seen in Fig. 3a. The T cell EI4 6.1 only showed the PCR product of 363 base pairs corresponding to the type I receptor (Fig. 3b, lane 5) and no trace of type II receptor cDNA (Fig. 3b, lane 6). This is in line with the previous assumption, that T cells express exclusively type I IL-1 receptors. In contrast, the pre B cell 70Z/3, showed a strong band at 414 base pairs with the primer pair for the type II IL-1 receptor cDNA (Fig. 3b, lane 10), but also two faint bands in the PCR reaction with the type I IL-1 receptor primer pair (Fig. 3b, lane 9), from which one corresponds to the expected type I PCR product. This supports the assumption that a minor amount of the type I IL-1 receptors may be expressed on 70Z/3 cells [17] and B cells in general [11]. The data can further explain that IL-1ra binds to 70Z/3 cells [18] although it has been shown that IL-1ra had a very low affinity to the type II IL-1 receptor [16]. In the Th2 D10N cells finally, PCR yielded products with primers for both, the type I and type II IL-1 receptor, with comparable intensity and independent of the presence of ConA in the culture medium (see Fig. 3b, lane 1–4). Thus, both types of receptors are expressed in this T helper cell line in roughly equivalent amounts, at least at the RNA level. In combination with the cross-linking experiments, there is little doubt that both types of IL-1 receptors are present in D10N cells also at the protein level. The data further show that the two prominent IL-1 binding proteins detectable in cross-linking experiments represent the type I and the type II IL-1 receptors, previously thought to characterize T and B cells, respectively.

3.4. *IL-1ra inhibits IL-1-dependent proliferation of D10N cells*

As a presumed physiological function of the type I IL-1 receptor the proliferative response of D10N cells to IL-1α was measured and further specified by demonstration of inhibition by IL-1ra. As is seen from Fig. 4 D10N cells, if previously starved for IL-1, dose-dependently proliferate upon IL-1 addition showing a half-maximum response at about 3 pg IL-1/ml. In the presence of 33 ng IL-1ra/ml the dose-response curve is shifted to roughly 10-fold higher concentrations. This observation reveals that the proliferation of D10N cells by IL-1 is mediated by a IL-1ra-sensitive receptor, i.e. the type I receptor.

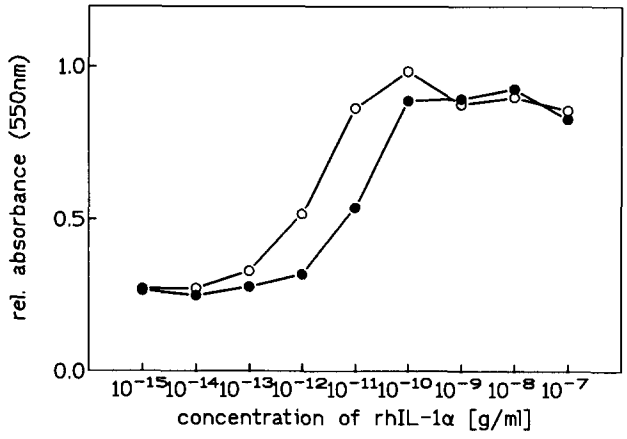

Fig. 4. Inhibition of the IL-1α-dependent proliferation of D10N cells by the IL-1 receptor antagonist. Cells were cultured without (○) and with (●) 33 ng IL-1ra/ml and the indicated concentration of IL-1α. Further details see section 2.

Although the Th2 cell line D10N used in this investigation is not entirely identical to those in which Savage et al. (D10S) [8] and Solari et al. (D10.G.4.1) [9,10] observed a low molecular weight IL-1 receptor, it is tempting to speculate from our molecular characterization of the 60 kDa receptor as type II IL-1 receptor that coexpression is a general characteristic of Th2 cells. Interestingly, this phenomenon is not shared by Th1 cells which according to Solari et al. [10] only express the type II IL-1 receptor, do not express any IL-1 receptor according to Lichtman [19], and do not proliferate upon IL-1 exposure [20]. The expression pattern of IL-1 receptors might therefore be a further criterion to differentiate Th1 from Th2 cells.

The differential expression of IL-1 receptors in T helper cell subsets will certainly also contribute to a better understanding of their function. Although the mode of signaling upon IL-1 binding to any of its receptors and the physiological consequences thereof may be only at the beginning of understanding [21], it appears conceivable that the proliferative IL-1 response of D10N cells is mediated by the type I receptor. This view is also supported by the finding that the proliferative response of D10N cells is inhibited by IL-1ra which hardly, if at all effects binding of IL-1 to the type II receptor (Fig. 2 and [15]). The physiological role of the type II receptor in Th2 cells, however, remains to be established.

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