Interleukin-1 Receptor Antagonist Competitively Inhibits the Binding of Interleukin-1 to the Type II Interleukin-1 Receptor*

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The interleukin-1 receptor antagonist (IL-1ra) inhibits the binding of interleukin-1 (IL-1) to T-cell lines possessing the type I IL-1 receptor; evidence has been published (Carter, D. B., Deibel, M. R. J., Dunn, C. J., Tomicch, C. S., Laborde, A. L., Slightom, J. L., Berger, A. E., Bienkowski, M. J., Sun, F. F., McEwan, R. N., Harris, P. K. W., Yem, A. W., Waszak, G. A., Chosay, J. G., Siew, L. C., Hardee, M. M., Zurcher-Neely, H. A., Reardon, I. M., Heinrickson, R. L., Truesdell, S. E., Shelly, J. A., Eessalu, T. E., Taylor, B. M., and Tracey, D. E. (1990) Nature 344, 633–638; Hannum, C. H., Wilcox, C. J., Arend, W. P., Joslin, F. G., Dripps, D. J., Heimdal, P. L., Armes, L. G., Sommer, A., Eisenberg, S. P., and Thompson, R. C. (1990) Nature 343, 336–340) that IL-1ra does not bind to the type II IL-1 receptor (IL-1RII). In this study we examined the ability of human recombinant IL-1ra to block the binding of IL-1 to the IL-1RII on human polymorphonuclear leukocytes (PMN) and Raji human B-lymphoma cells. The binding of $^{125}$I-IL-1 to PMN was competitively inhibited by IL-1ra. IL-1ra was more potent in inhibiting the binding of $^{131}$I-IL-1 than $^{125}$I-IL-1. Incubation with $^{125}$I-IL-1ra in the presence of increasing concentrations of IL-1 or IL-1ra showed that IL-1ra was an $\sim 40$-fold more potent inhibitor of binding of $^{125}$I-IL-1ra than unlabeled IL-1ra. The IL-1ra was $\sim 500$-fold less potent in inhibiting the binding of $^{125}$I-IL-1 than IL-1a. IL-1ra was also able to competitively inhibit binding of $^{125}$I-IL-1 to Raji cells. PMN or Raji cells were also incubated with $^{125}$I-IL-1 in the absence or presence of IL-1 or IL-1ra. After cross-linking of IL-1 to cells followed by specific immunoprecipitation, sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed a band at 85 kDa corresponding to the 68-kDa IL-1RtII. However, in the presence of an excess of either unlabeled IL-1 or IL-1ra, the 85-kDa IL-1:IL-1RtII complex was not present. These findings demonstrate that the IL-1ra recognizes and blocks IL-1 binding to the IL-1RtII.

Interleukin-1 (IL-1) is the term used to describe two 17.5-kDa polypeptides, IL-1α and IL-1β, which share a wide spectrum of inflammatory and immunological properties (3). IL-1α and IL-1β possess 25% amino acid similarity and bind to the same receptors. However, IL-1α binds preferentially to the 80-kDa type I IL-1 receptor (IL-1RtI) found on T-cells (4–7), fibroblasts (4), and keratinocytes (8); IL-1β binds best to the 68-kDa type II IL-1 receptor (IL-1RtII) on B-cells (6, 7, 9) and polymorphonuclear leukocytes (PMN) (10, 11). This differential binding of IL-1α and IL-1β is observed even though there is 28% amino acid identity between the extra- cellular portions of the IL-1RtI and the IL-1RtII (12). The cytoplasmic portion of the IL-1RtII is truncated in comparison with the IL-1RtI.

Binding of both IL-1α and IL-1β to the IL-1RtI on T-cells can be inhibited by the IL-1 receptor antagonist (IL-1ra), an 18-kDa protein which has 19% amino acid similarity with IL-1α and 26% amino acid similarity with IL-1β (13). Evidence has also been published that IL-1ra does not bind to the IL-1RtII (1, 2). Using competitive binding and cross-linking studies, we demonstrate that IL-1ra inhibits the binding of both IL-1α and IL-1β to the IL-1RtII on polymorphonuclear leukocytes and Raji human B-lymphoma cells.

EXPERIMENTAL PROCEDURES

Materials

Recombinant human IL-1α was a kind gift of Dr. Peter Lomedico (Hoffmann-LaRoche). Recombinant human IL-1β was donated by Dr. Aldo Tagliabue (ScIavo Research Centre, Siena, Italy). Recombinant human IL-1ra was provided by Dr. Robert C. Thompson (Synergen Inc., Boulder, CO). Radiolabeling of IL-1α and IL-1β with $^{125}$I (Du Pont-New England Nuclear) was performed using the technique of Bolton and Hunter. IL-1α was radiolabeled with $^{125}$I (Du Pont-New England Nuclear) using chloroamine T (Sigma).

RPMI 1640 (Sigma) containing 10 mM t-glutamine, NaHCO$_3$ (Mallinckrodt Chemical Works), 100 units/ml penicillin, and 100 μg/ml streptomycin (Irvine Scientific, Santa Ana, CA), pH 7.4, was ultrafiltered using polysulfone hollow fiber filters (F40, Fresenius AG, Bad Homberg, Federal Republic of Germany). To make the binding assay buffer, bovine serum albumin (Fraction V, Sigma) was added (1% final concentration) to the RPMI 1640, 10 mM HEPES (Sigma), 100 units/ml penicillin, and 100 μg/ml streptomycin (Irvine Scientific, Santa Ana, CA), pH 7.4, was ultrafiltered using polysulfone hollow fiber filters (F40, Fresenius AG, Bad Homberg, Federal Republic of Germany). To make the binding assay buffer, bovine serum albumin (Fraction V, Sigma) was added (1% final concentration) to the RPMI 1640 before readjusting the concentration of HEPES to 20 mM and the pH to 7.2. CHAPS lysis buffer (CLB) was made by adding 1% w/v CHAPS (Sigma) and 2 mM phenylmethylsulfonyl fluoride (Sigma) to phosphate-buffered saline (PBS).

Methods

Human PMN—PMN were prepared from the blood of healthy human volunteers. Blood was drawn into syringes containing heparin (20 units/ml final concentration, LyphoMed Inc., Rosemont, IL). Blood was separated by centrifugation through Ficoll (Sigma) and Hypaque (90%, Winthrop Laboratories, New York). Following aspiration of plasma and mononuclear cells, the PMN were recombined with plasma (1 ml/5 ml red cell mass) in a 250-ml graduated cylinder. An equal volume of 3% dextran (M, 266,000, Sigma) in sterile 0.9% sodium chloride (Abbott) was added and the graduated cylinder

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were resolved by SDS-PAGE with a 10% polyacrylamide gel. Each
performing by exposure of XAR-5 film (Kodak, Rochester, NY) for
After washing twice in CLB, the conjugated IgGSorb was resuspended
was washed twice with CLB before being resuspended in 160  pl  of
Tris-HC1, pH 6.8, 10% w/v glycerol, 1% v/v SDS, and 0.0005% w/v
binding by either IL-1p or IL-lra. By using the Cheng-Prusoff
technology Associates, Birmingham, AL) at 10 rpm for 6  h at 4 °C.
with 50  pg of affinity-purified goat anti-rabbit IgG (Southern Bio-
immunoprecipitate was solubilized in 20 pl of loading buffer (62 mM
Mixed with '/10 volume of rabbit anti-human IL-10 and incubated at
human IL-l@-conjugated IgGSorb or anti-human  IL-la-conjugated
IL-10 complexes. Briefly, 100 pl of
labeled IL-la, IL-10, or IL-lra.  Total volume of cell suspension was
inhibits the binding of IL-1  to  PMN, increasing numbers of
To further investigate the mechanism by which IL-lra
inhibits the concentration of IL-1p on PMN of 320 pm (lo),
KDi
for IL-1ra is 7000  pm.
for IL-lra is 7000  pm. (as well as Figs. 2-4) depicts specific binding. Nonspecific binding
was determined in the presence of 4 p~ IL-lra. Nonspecific
binding was 53% of total binding. This figure depicts one of three
experiments. In these experiments, the ratio of the ICo for 1L-1ra to the ICo for IL-1p ranged from 13 to 15.

FIG. 1. Effect of different concentrations of IL-19 and IL-1ra on binding of 125I-IL-1p to PMN. PMN (2 x 10^6) were incubated overnight at 4 °C with ~450 pm 125I-IL-1p in the presence of increasing concentrations of IL-19 (0) or IL-1ra (C). This figure (as well as Figs. 2-4) depicts specific binding. Nonspecific binding (which was determined using 380 nm IL-1p) was 8% of total binding. This figure shows one of three experiments. In these experiments, the ratio of the ICo for IL-1ra to the ICo for IL-1p ranged from 13 to 15.

FIG. 2. Effect of different concentrations of IL-19 and IL-1ra on binding of 125I-IL-1ra to PMN. PMN (1 x 10^6) were incubated overnight at 4 °C with ~450 pm 125I-IL-1ra in the presence of increasing concentrations of IL-19 (0) or IL-1ra (C). Nonspecific binding was determined in the presence of 4 pm IL-1ra. Nonspecific binding was 63% of total binding. This figure depicts one of three experiments. In these experiments, the ratio of the ICo for IL-1ra to the ICo for IL-1ra ranged from 17 to 40.

PMN (10^6-10^7/150  ml) were incubated with ~350 pm 125I-IL-1ra in the absence or presence of 370 nm IL-1ra. IL-1ra inhibited 125I-IL-1ra from binding to these PMN (data not shown). Subsequently, PMN were incubated with 125I-IL-1ra in the presence of increasing concentrations of IL-1p or IL-1ra. As shown in Fig. 2, IL-1p is an ~40-fold more potent inhibitor of 125I-IL-1ra binding to PMN than is IL-1ra. This finding is consistent with the data presented in Fig. 1.

As shown in Fig. 3, IL-1ra competitively inhibits 125I-IL-1a to PMN. Using 125I-IL-1a, the concentration of IL-1a which effectively inhibits 50% of the specific radioligand binding (ICo) is ~500-fold less than the IC0 for IL-1ra.

To demonstrate that the aforementioned findings are applicable to other cells having the IL-1RtII, Raji human B-

RESULTS

To determine whether IL-1ra is able to interact with the
IL-1RtII, PMN were incubated with ~450 pm 125I-IL-1p in the
presence of increasing concentrations of IL-1p or IL-1ra. Fig. 1 depicts the dose-dependent inhibition of 125I-IL-1p
binding by either IL-1p or IL-1ra. By using the Cheng-Prusoff
relationship (16) and a K0 for IL-1p on PMN of 320 pm (10),
the inhibition constant (K0) for IL-1ra is 7000  pm.

To further investigate the mechanism by which IL-1ra
inhibits the binding of IL-1 to PMN, increasing numbers of

were rotated three times. One hour later the supernatant was removed
and centrifuged at 250 x g for 10 min at 4 °C. Pellets underwent
hypotonic lysis at 4 °C with 0.2% sodium chloride followed 45 s later
by an equal volume of 1.6% sodium chloride. Following centrifugation
at 250 x g for 10 min at 4 °C, hypotonic lysis was repeated. As
detected by (American Scientific Products, Asmada, Puerto Rico) staining, the cell population was 97-99% PMN with
>99% of the cells viable by trypan blue exclusion.

Cell Culture—Raji cells were obtained from American Type Culture
Collection. EL4-6.1 cells were a kind gift of Dr. Robert Newton (E.
I. Du Pont de Nemours & Co., Glenolden, PA). Both cell lines were
cultured in RPMI 1640 containing 5% fetal calf serum.

Receptor Binding Assays—Cells were washed twice in cold RPMI
and resuspended at different concentrations in binding buffer. Ali-
quots of cells were rotated in duplicate at 10 rpm for 4 °C overnight
with radiolabeled ligands and different concentrations of either un-
labelled IL-1a, IL-1p, or IL-1ra. Total volume of cell suspension was
150  μl. Cell-bound radioactivity was separated from free radioactivity
by centrifugation through oil (Silicones, General Electric, Waterford,
NY) at 14,000 x g for 90 s at 4 °C. Tips of the tubes containing the
cell pellet were cut and counted. Radioactivity was measured using a
r-scintillation counter.

Cross-linking 125I-IL-13 to IL-1R—Following two washes in cold
RPMI, cells were resuspended in binding buffer. Either 2 X 10^6 PMN,
3 X 10^7 Raji cells, or 4 X 10^6 EL4 cells were rotated at 10 rpm at 4 °C
overnight with 1 nM 125I-IL-13 and either 1 μM IL-18 or 10 μM IL-
1ra. Similar numbers of PMN, Raji cells, or EL4 cells were incubated
under identical conditions with 1 nM 125I-IL-1a or either 1 μM IL-1a
or 100 μM IL-1. Total volume of cell suspension was 1000  μl. Cells
were then washed with cold PBS and cross-linked using 2.7 mM
bis(sulfosuccinimidyl)suberate (BSs, Pierce Chemical Co.) for 2 h at
4 °C. Following a wash with cold PBS the cells were resuspended in
CLB. After 30 min on ice, cell debris was removed by centrifugation
at 13,000 x g for 15 min at 4 °C.

Immuno precipitation—Using the methods of Rangnekar et al. (14)
and Clark et al. (15), rabbit anti-human IL-1a antibody was used to
immobilize the solubilized IL-13-IL-1p complexes. Briefly, 100 μl of
packed IgGSorb (The Enzyme Center, Malden, MA) was incubated
with 50 μg of affinity-purified goat anti-rabbit IgG (Southern Bio-
technology Associates, Birmingham, AL) at 10 rpm for 6 h at 4 °C.
After washing twice in CLB, the conjugated IgGSorb was resuspended
in 1000 μl of cold PBS. Aliquots of conjugated IgGSorb were then
mixed with %/10 volume of rabbit anti-human IL-1a and incubated at
10 rpm overnight at 4 °C. The anti-human IL-1p-conjugated IgGSorb
was washed twice with CLB before being resuspended in 160  μl of
CLB. Prior to immunoprecipitation, cell lysates were incubated for 30
min on ice with %/10 volume of IgGSorb conjugated with goat anti-
rabbit IgG to remove nonspecific binding proteins. PMN, Raji cells,
and EL4 cell lysates were combined with %/10 volume of either anti-
human IL-1α-conjugated IgGSorb or anti-human IL-1α-conjugated
IgGSorb and then incubated at 10 rpm overnight at 4 °C. Immuno-
precipitates were pelleted by centrifugation at 15,000 x g for 15 min
at 4 °C followed by washing in CLB. Supernatants were frozen at
-70 °C.

SDS-PAGE—The immunoprecipitated IL-1α-IL-1p complexes were resolved by SDS-PAGE with a 10% polyacrylamide gel. Each
immunoprecipitate was solubilized in 20 μl of loading buffer (62 nm
Tris-HCl, pH 6.8, 10% w/v glycerol, 1% v/v SDS, and 0.0006% w/v
bromphenol blue) and heated at 100 °C for 2 min. Prestained protein
molecular weight standards (GIBCO/BRL) were applied to the gel.
The gel was dried with heat and vacuum. Autoradiography was performed by exposure of XAR-5 film (Kodak, Rochester, NY) for
10 days at ~70 °C using an intensifying screen (Cronex Lightening
Plus, Du Pont).
IL-1RtII resulted in a band at 97 kDa. In the presence of increasing concentrations of IL-la (●) or IL-1ra (○). Nonspecific binding determined in the presence of an excess of either unlabeled IL-la or IL-1ra, neither the IL-1β nor the IL-1ra was a more potent inhibitor than IL-1ra; in the human cells IL-1α and IL-1ra were equipotent as competitive inhibitors. However, in neither report was IL-1ra at concentrations up to 50 nM able to block the binding of 125I-labeled IL-1α (●) to or 125I-labeled IL-1α (1) to 70Z/3 cells, a murine B-cell line which expresses the IL-1RII.

Data presented in this paper show that IL-1ra competitively inhibits the binding of 125I-IL-1β to cells with the IL-1RII. IL-1β is 1 order of magnitude more potent than IL-1ra in inhibiting the binding of 125I-IL-1β to PMN and Raji human B-lymphoma cells. In further support of the theory that IL-1β and IL-1ra bind to the same receptor, IL-1β is 1 order of magnitude more potent than IL-1ra in blocking the binding of 125I-1IL-1ra to PMN. Cross linking studies confirm that IL-1ra is able to prevent 125I-IL-1β from binding to a 68 kDa receptor.

Species specificity probably is the reason previous studies did not demonstrate inhibition of IL-1 binding by human IL-1ra to the mouse IL-1RII. We believe that the affinity of the mouse IL-1RII for mouse IL-1ra would be greater than its affinity for human IL-1ra. Ikejima has shown that murine EL4-6.1 cells have a higher affinity for mouse 125I-IL-1β than human 125I-1IL-1β. The capacity of IL-1ra to block the binding of IL-1 to B-cells and PMN has implications for the biology of IL-1. IL-1 has been shown to assist in B-cell activation (18) and proliferation (19). Therefore, IL-1ra is potential therapy for B-cell leukemias (20) and lymphomas, multiple myeloma (21), immune thrombocytopenic purpura, systemic lupus erythematosus (22), insulin-dependent diabetes mellitus, Hashimoto’s thyroiditis (23), and some glomerulonephritides. Although there is controversy regarding its effects upon PMN, IL-1 has been reported to prime for superoxide production (24, 25) and myeloperoxide release (24, 26), as well as promote spreading (25). In addition, IL-1α can act synergistically with other cytokines in inducing stem cell proliferation (27, 28). Consequently, granulocytic leukemias (29-33), rheumatoid arthritis (34), and gout might respond to IL-1ra.

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REFERENCES
1. Carter, B. D., Deibel, M. R. J., Dunn, C. J., Tomich, C. S., Laborde, A. L., Slightom, J. L., Berger, A. E., Bienkowski, M. J., Sun, F. F., McEwan, R. N., Harris, P. K. W., Yem, A. W., Waszak, G. A., Chosay, J. G., Sieu, L. C., Hardee, M. M., Zucker-Neely, H. A., Reardon, I. M., Heinrichson, R. L., Trousdell, S. E., Sibert, J. A., Ressell, E. T., Taylor, B. M., and Tracey, D. E. (1990) Nature 344, 633-638
2. Hannum, C. H., Wilcox, C. J., Arend, W. P., Joslin, F. G., Dripps, D. J., Heimdal, P. L., Armes, L. G., Somner, A., Eisenberg, S. P., and Thompson, R. C. (1990) Nature 343, 366-368
3. Dinarello, C. A. (1991) Blood 77, 1627-1652
4. Dower, S. K., Call, S. M., Gillis, S., and Urdal, D. L. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 1060-1064
5. Kiiian, P. L., Kaftka, K. L., Stern, A. S., Woeche, D., Benjamin, W. R., Dechiara, T. M., Guibler, U., Farrar, J. J., Mizel, S. B.

T. Ikejima, manuscript in preparation.
IL-1ra Inhibits Binding of IL-1 to IL-1RtII

6. Horuk, R., Huang, J. J., Covington, M., and Newton, R. C. (1987) J. Biol. Chem. 262, 16275–16278
7. Scapigliati, G., Ghiara, P., Bartalini, M., Tagliabue, A., and Boraschi, D. (1989) FEBS Lett. 243, 394–398
8. Kupper, T. S., Lee, P., Birchall, N., Clark, S., and Dower, S. (1988) J. Clin. Invest. 82, 1787–1792
9. Horuk, R., and McCubrey, J. A. (1989) Biochem. J. 260, 657–663
10. Borish, L., Rosenbaum, R., McDonald, B., and Rosenwasser, L. J. (1990) Inflammation 14, 151–162
11. Rhyne, J. A., Mizel, S. B., Taylor, R. G., Chedid, M., and McCall, C. E. (1989) Immunol. Immunopathol. 48, 354–361
12. Sims, J., Cosman, D., Curtis, B., Fanslow, W., Widmer, M., and Dower, S. K. (1990) Lymphokine Res. 9, 555
13. Eisenberg, S. P., Evans, R. J., Arend, W. P., Verderber, E., Brewer, M. T., Hannum, C. H., and Thompson, R. C. (1990) Nature 243, 341–346
14. Rangnekar, V. M., Thomas, L. S., and Plate, J. M. (1988) J. Biol. Chem. 263, 16408–16413
15. Clark, B. D., Ikehima, T., Mancilla, J., Sirko, S., Orencole, S. F., Ishii, N., Okuda, K., and Dinarello, C. A. (1989) Cytokine 1, 90
16. Cheng, Y., and Prusoff, W. H. (1973) Biochem. Pharmacol. 22, 3099–3108
17. Seckinger, P., Lowenthal, J. W., Williamson, K., Dayer, J.-M., and MacDonald, H. R. (1987) J. Immunol. 139, 1546–1549
18. Koyama, N., Harada, N., Takahashi, T., Mita, S., Okamura, H., Tominao, A., and Takatsuki, K. (1988) Immunology 63, 271–283
19. Falkoff, R. J. M., Muraguchi, A., Hong, J.-X., Butler, J. L., Dinarello, C. A., and Fauci, A. S. (1983) J. Immunol. 131, 801–805
20. Uckun, F. M., Myers, D. E., Fauci, A. S., Chandan-Langlie, M., and Ambrus, J. L. (1989) Blood 74, 761–776
21. Kawano, M., Tanaka, H., Ishikawa, H., Nobuyoshi, M., Iwato, K., Asaoku, H., Tanabe, O., and Kuramoto, A. (1989) Blood 73, 2145–2148
22. Tanaka, Y., Saito, K., Suzuki, H., Eto, S., and Yamashita, U. (1989) J. Immunol. 143, 1584–1590
23. Bendtzen, K., Buschard, K., Diamant, M., Horn, T., and Svenson, M. (1988) Lymphokine Res. 8, 335–340
24. Ferrante, A., Nandoskar, M., Watzke, M., Oeh, D. H. B., and Kowanko, I. C. (1988) Int. Arch. Allergy Appl. Immunol. 86, 82–91
25. Sullivan, G. W., Carper, H. T., Sullivan, J. A., Murata, T., and Mandell, G. M. (1989) J. Leukocyte Biol. 45, 389–395
26. Dularay, B., Elson, C. J., Clements-Jewery, S., Damsa, C., and Lando, D. (1990) J. Leukocyte Biol. 47, 158–163
27. Kupper, T. S., Lee, F., Birchall, N., Clark, S., and Dower, S. (1988) J. Clin. Invest. 82, 1787–1792
28. Rambaldi, A., Torcia, M., Bettoni, S., Vannier, E., Barbui, T., Dinarello, C. A., and Cozzolino, F. (1990) Blood 76, 114a
29. Sakai, K., Hattori, T., Matsuoka, M., Asou, N., Yamamoto, S., Sagawa, K., and Takatsuki, K. (1987) J. Clin. Invest. 86, 1597–1602
30. Eastgate, J. A., Wood, N. C., di Giovine, F. S., Symons, J. A., Grinling, F. M., and Duff, G. W. (1988) Lancet II, 706–709