

Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein

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A human myelomonocytic cell line, U937, produced an interleukin-1 (IL-1) receptor antagonist protein (IRAP) which was purified and partially sequenced. A complementary DNA coding for IRAP was cloned and sequenced. The mature translation product of the cDNA has been expressed in *Escherichia coli* and was an active competitive inhibitor of the binding of IL-1 to the T-cell/fibroblast form of the IL-1 receptor. Recombinant IRAP specifically inhibited IL-1 bioactivity on T cells and endothelial cells *in vitro* and was a potent inhibitor of IL-1 induced corticosterone production *in vivo*.

INTERLEUKIN-1 (IL-1) has a key role in the body's response to infection, tissue injury, autoimmunity and inflammation^{1,2}. Two biochemically distinct forms of this cytokine, IL-1 α and IL-1 β , bind equally to IL-1 receptors to mediate these activities^{1,2}. The local and systemic activities of IL-1 are vital to a broad spectrum of immunological, endocrinological, haematological, neurological, and metabolic responses that occur in such pathological situations. In some cases, however, high levels of IL-1, often in concert with other cytokines such as tumour necrosis factor (TNF), lead to tissue destruction and chronic inflammation¹⁻³. Evidence exists for several natural feedback loops that may down-regulate IL-1 production or activity. One such loop involves the induction of adrenocorticotrophic and glucocorticoid hormones by way of IL-1 stimulation of the pituitary-adrenal axis⁴ and the subsequent inhibition by glucocorticoids of IL-1 production⁵ and activity, including interleukin-2 (IL-2) production by T cells⁶. Specific IL-1 functional inhibitors have also been described that may represent an alternative mechanism for the regulation of IL-1 activity. A specific inhibitor of IL-1-induced thymocyte proliferation and chondrocyte prostaglandin/collagenase release, which has a relative molecular mass (M_r) of ~22,000 (22K), has been found in culture supernatants of adherent human mononuclear cells stimulated with immobilized immune complexes⁷. Cloning and expression of a gene that apparently codes for this protein, termed IL-1ra, has been reported recently^{8,9}. A human urinary inhibitor of IL-1 activity with M_r 18-25K, which blocks IL-1 binding to its receptor on T-cells, has also been described¹⁰. More recently, an IL-1 receptor binding inhibitor of M_r ~23K (IL-1 INH) was shown to be released from cultured human monocytes after stimulation with granulocyte macrophage colony-stimulating factor (GM-CSF) (ref. 11).

We have purified an IL-1 receptor antagonist protein (IRAP) of apparent M_r ~25K from U937 human myelomonocytic cells treated with the differentiating agent phorbol myristate acetate (PMA) and stimulated with GM-CSF. The U937 cell line was

derived from a human histiocytic lymphoma and has phenotypic markers of a monoblast precursor of mononuclear cells¹². We obtained enough peptide sequence from the purified protein to clone the gene coding for the IRAP. Subsequent expression in *E. coli* of the cDNA coding for the mature translation product has provided large quantities of the recombinant IRAP for studies of IL-1 receptor binding activity and of *in vitro* and *in vivo* biological activities of the protein.

IRAP induction, purification and sequencing

Cell-free supernatants from cultured U937 cells were assayed for IL-1 antagonist activity using an IL-1 receptor binding assay (RBA) (ref. 13) with human YT-NCl cells and a sensitive bioassay, the 1A5 T-cell IL-1 antagonist assay⁶ (1A5 assay), which measures the reduction in IL-2 production by LBRM-33-1A5 murine thymoma cells stimulated with IL-1 (ref. 14). Unstimulated U937 cell supernatants did not contain measurable IL-1 inhibitory activity. Exposure of U937 cells to PMA for 48 h induced cell differentiation, as evidenced by increased adherence to culture vessel surfaces. These PMA-differentiated U937 cells produced low levels of IL-1 inhibitory activity which were increased at least 10-fold after stimulation of the cells for an additional 48 h with GM-CSF.

A single activity eluting from a size exclusion column with M_r ~25K was detected in supernatants from stimulated U937

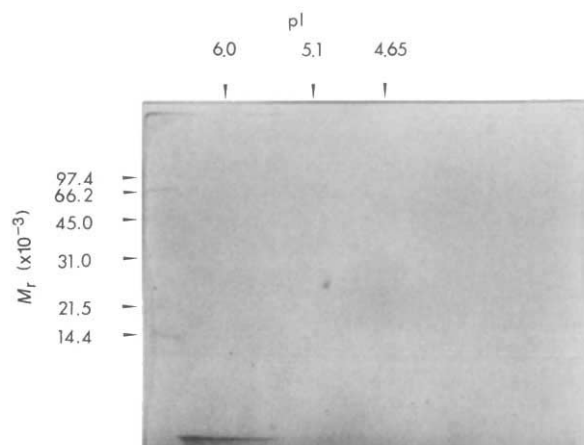


FIG. 1 Two-dimensional gel electrophoresis of purified U937 IRAP (fraction 6, Table 1). Two-dimensional electrophoresis was conducted using 1-mm diameter capillary-tube gels (7% polyacrylamide) in the first dimension (4-8 °C; 1,200 V, constant voltage) for 10-12 h. Ampholines having a pH range of 4-6 were used (Bio-Lytes, 2% w/v, Bio-Rad Laboratories). Focused sample gels were electrophoresed onto a second dimension of 18% polyacrylamide (9 cm $\times$ 7 cm) (0.1% SDS) according to the method of Laemmli³⁰. The gels were stained with Coomassie Brilliant Blue G-250.

METHODS. Purified U937 IRAP protein sample (35 μ g, $\sim$ 1.4 nmol) was denatured in 6 M guanidine-HCl, 250 mM Tris-HCl, and 50 mM EDTA, pH 8.5. One μ l of 2-mercaptoethanol (10% v/v) was added, and the solution was incubated at 37 °C for 30–40 min. One μ l of 4-vinyl-pyridine (Aldrich Chemical Co.) was then added and the solution incubated at room temperature for 3 h. Low-molecular weight reagents were removed by dialysis and the final volume of the reaction product was reduced to 50 μ l by Speed Vac centrifugation. This solution of S-pyridylethylated inhibitor protein was treated with 300 μ l of 10 mM Tris-HCl, pH 8.7, followed by 1 unit of reconstituted endoproteinase Lys C (Boehringer) and incubated at 37 °C for 12–14 h. The resulting peptides were resolved on a C18 reverse phase HPLC column (4.5 $\times$ 150 mm) using a linear gradient from 0 to 36% acetonitrile in 0.1% TFA over 52 min. The column was monitored at 206 nm and peptides were collected manually during peak elution. Solvent was removed by vacuum centrifugation (Savant). Automated Edman degradation identified one peptide as KIDVVPIE. Six batches of oligonucleotides (each batch contained 32 degenerate oligonucleotides) were synthesized on an Applied Biosystems 380B DNA synthesizer using the solid-phase phosphoramidite cyanoethyl coupling approach³¹. After end-labelling each batch of oligonucleotides with [γ -³²P]ATP, each batch was hybridized overnight to a northern blot of stimulated U937 poly(A⁺) RNA at 42 °C in 6X SSC-1% Denhardt's-0.5% SDS-100 μ g ml⁻¹ single-strand salmon sperm DNA and washed with 6X SSC-0.5% SDS at 45 °C (ref. 32). One of the batches of labelled oligonucleotides produced a strong signal at 2 kilobases (kb) (5'-GGGACGACGTCATCTT-3') and was used to screen a λ gt10 cDNA library (Clontech HL1036A) derived from U937 poly(A⁺) RNA which had been isolated from PMA-differentiated U937 cells. Eight positives were plaque-purified from 200,000 clones plated, phage DNA prepared and digested with *Eco*RI (refs 33, 34). These eight clones all contained a 1.7-kb insert, several of which were subcloned into the *Eco*RI site of pGEM2. Enzymatic sequencing was carried out using a Sequenase version 2 kit (US Biochemical). Oligonucleotide primers flanking the cloning site of λ gt10 were used to determine the limits of the insert clone in λ gt10 IRAP P5. DNA sequencing reactions were electrophoresed on thermostated gels (55 °C) measuring 20 cm wide $\times$ 104 cm long³⁵.

Using degenerate oligonucleotides based on one of the IRAP peptides, KIDVVP, we plaque-purified several clones from a

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TABLE 1 Purification of U937 IRAP

No.	Fraction	Volume (ml)	Protein (mg)	Specific activity (inhibitory units mg ⁻¹)	Purification (×)
1	Culture supernatant	7,400	1,590		
2	Ultrafiltration	34	877	0.323	1.0
3	Superose 12 FPLC-1	1.0	62.3	0.310	0.96
4	TSK Bio-Sil 125 HPLC-1	0.14	1.8	163	504
5	TSK Bio-Sil 125 HPLC-2	8.5	1.1	482	1,493
6	C4 RP HPLC	dry	0.07	3,222	9,975

U937 culture supernatants were produced, pooled and then subjected to five consecutive fractionation procedures. At each step, the volumes and protein contents were measured and the specific activities were measured in a 1A5 bioassay as previously described^{6,13}. Specific activities per mg protein were calculated such that one inhibitory unit (IU) gave 50% inhibition of one half-maximal thymocyte unit of rIL-1 β (ref. 29) in the 1A5 assay.

METHODS. U937 cell cultures were obtained from the American Type Culture Collection, Rockville, Md. Cells were cultured at 37 °C for 48 h at 5×10^5 cells ml⁻¹ in RPMI 1640 medium (GIBCO), 7% fetal bovine serum (FBS, GIBCO), 2 mM L-glutamine, 100 U ml⁻¹ penicillin, 100 μ g ml⁻¹ streptomycin, 20 mM HEPES buffer, and 100 nM PMA (Sigma). After differentiation, the medium was removed and the cells, now adherent, were gently washed once with a small volume of Dulbecco's PBS. Cells were then cultured for an additional 48 h in RPMI 1640 containing 1% low-endotoxin FBS (HyClone), L-glutamine, penicillin, streptomycin, and rhGM-CSF (75 U ml⁻¹; Amgen). Cell-free supernatants were collected, pooled and frozen at -20 °C. After thawing, phenylmethylsulphonyl fluoride was added to 0.2 mM final concentration. The solutions were concentrated by ultrafiltration with YM-5 and YM-10 filters (Amicon). Protein measurements were made with ISS Protein Gold (Enprotech) or the Bradford assay (Bio-Rad Kit) using BSA as the standard protein. Superose 12 FPLC-1: in 17 repetitive cycles, 2-ml aliquots of Fraction 2 concentrate were injected onto a Superose 12 prep grade FPLC column (1.6 $\times$ 50 cm) equilibrated with 0.1 M potassium phosphate, 1 M NaCl, pH 6.0. Fractions of 1 ml were collected at a flow rate of 1 ml min⁻¹. Appropriate fractions were collected and individually concentrated by Centricon-10 units (Amicon). After analysis by SDS-PAGE and bioassay, appropriate fraction concentrates were pooled and further concentrated to 1 ml by Speed Vac centrifugation (Savant). TSK Bio-Sil 125 HPLC-1: in 20 repetitive cycles, 50 μ l of fraction 3 were injected onto a TSK-Bio-Sil-125 HPLC column (7.5 $\times$ 600 mm), using the same buffer system as described above. Fractions of 200 μ l were collected. Appropriate fractions were pooled and concentrated by Centricon-10 filter units and finally by Speed Vac centrifugation as described previously. TSK Bio-Sil 125 HPLC-2: in seven repetitive cycles, 20 μ l of fraction 4 were injected onto the same column as described above. Active fractions were pooled to give a final volume of 8.5 ml. C4 reverse phase HPLC: appropriate fractions from the TSK Bio-Sil 125 run (fraction 5) were injected directly onto a C4 reverse phase column (4.6 $\times$ 150 mm). Proteins which remained bound to the matrix following an extensive wash with 0.1% trifluoroacetic acid (TFA) were desorbed using a linear gradient from 28 to 32% acetonitrile in 0.1% TFA over a period of 44 min at a flow rate of 1 ml min⁻¹ (room temperature). Representative aliquots of each fraction or pool were removed, taken to complete dryness by Speed Vac centrifugation, and then re-solubilized in tissue culture medium in preparation for assay.

and 69 conservative amino-acid substitutions for a total shared similarity of 72%. The motifs TTATTTAT or ATTTA, which may be associated with messenger RNA stability, found in the 3'-untranslated region of IL-1 α and IL-1 β and of other mRNAs specifying inflammatory mediators^{16,17} were not found in the nucleotide sequence of the IRAP cDNA.

Expression of the cDNA in *E. coli* was carried out by placing the cDNA coding for mature IRAP, beginning at position 84, behind the *trp* promoter in an *E. coli* expression vector¹⁸. We used synthetic oligonucleotides to supply IRAP sequence from positions 84-214 and to add an initiation codon to the cDNA. On induction by tryptophan starvation, a 22K protein (apparent M_r by SDS-PAGE) was synthesized that did not appear in extracts from *E. coli* cells carrying the expression vector without the IRAP cDNA. When the *E. coli* extracts were assayed in either the 1A5 assay or the IL-1 RBA, the extracts containing the 22K protein were active. The recombinant IRAP (rIRAP) protein was easily extracted in high yield from the *E. coli* pellets

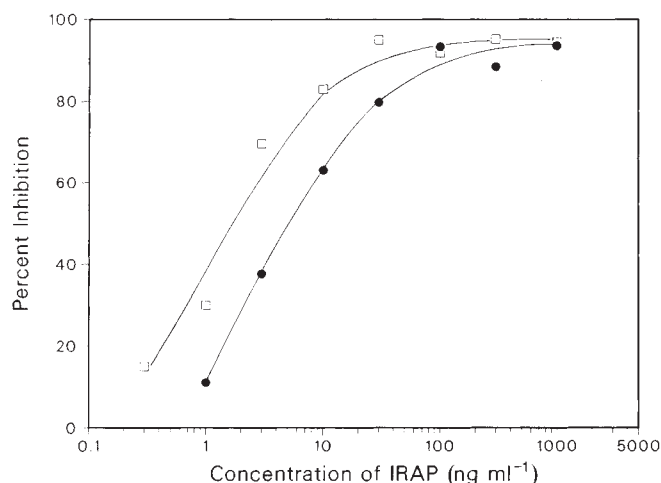
by mild hypotonic lysis to provide a protein at a purity greater than 80%. Homogeneous rIRAP was subsequently obtained by QAE Sepharose and TSK DEAE 5PW anion exchange chromatography steps, followed by size exclusion chromatography on a TSK Bio-Sil-125 HPLC column. A single spot having the coordinates of pI 6.0 and M_r 22K was observed (by two-dimensional PAGE). Edman degradation of the purified intact rIRAP gave the sequence NH₂-MRPSGRKSSKM, corresponding to that expected for IRAP preceded by a methionine.

rIRAP characterization

The bioactivity of rIRAP was measured in assays¹⁹ of IL-1-augmented human endothelial cell adhesiveness for human neutrophils and eosinophils. As shown in Fig. 3, rIRAP inhibited IL-1-induced adhesion for neutrophils and endothelial cells in a concentration-dependent fashion. With a specific activity of IL-1 β of 2×10^7 U mg⁻¹ as a base (murine thymocyte assay half-maximal units), rIRAP had specific activities (one inhibi-

FIG. 3 Dose-response inhibition of IL-1-induced endothelial cell-leukocyte adhesion by rIRAP.

METHODS. Purified human blood neutrophils³⁶ (—□—), or eosinophils³⁷ (—●—) were labelled with ⁵¹Cr and suspended in Hanks balanced salt solution containing 1 mg ml⁻¹ human serum albumin. Human umbilical vein endothelial cells (HUVEC) were cultured in Costar 48-well plates³⁸ and stimulated with rhIL-1 β (4 U ml⁻¹) for 4 h in the presence or absence of rIRAP. The HUVEC cultures were washed three times in culture medium (37 °C) to remove excess IL-1/rIRAP; leukocyte suspensions (neutrophil/eosinophil) were added to each well in a volume of 0.3 ml containing 4×10^5 cells and incubated for 30 min at 37 °C. Unattached leukocytes were gently washed off the plates using culture medium (37 °C); the remaining radiolabelled leukocytes adherent to the HUVECs were dissolved in SDS in 0.1 M NaOH and counted in a gamma counter to assess the degree of adhesion. The mean neutrophil adhesion in the absence of IL-1 was $8.12 \pm 0.84\%$ of total cells added and increased to $57.34 \pm 4.86\%$ after incubation of HUVEC with rhIL-1 β (4 U ml⁻¹); the mean eosinophil adhesion in the absence of IL-1 was $9.48 \pm 1.83\%$ of total cells added and increased to $48.24 \pm 2.45\%$ after incubation of HUVECs with rhIL-1 β . As shown in the graph, rIRAP caused a dose-related inhibition of neutrophil and eosinophil adhesion to HUVEC stimulated with IL-1.



tory unit (IU) gives 50% inhibition of one unit of IL-1) of 2.2×10^6 IU mg^{-1} and 7.5×10^5 IU mg^{-1} for inhibition of neutrophil and eosinophil adhesion, respectively. These differences may reflect different levels or types of IL-1-induced endothelial cell adhesion molecules responsible for binding to these two granulocyte populations.

The bioactivities of rIRAP in the 1A5 assay and murine thymocyte IL-1 co-mitogenesis assay²⁰ were also measured. In the 1A5 assay, rIRAP had a specific activity of $2.9 \pm 0.7 \times 10^4$ IU mg^{-1} compared with recombinant human IL-1 β (rhIL-1 β) and comparable activity against rhIL-1 α . In the thymocyte assay, however, rIRAP had a specific activity of $8.8 \pm 1.6 \times 10^5$ IU mg^{-1} . No inhibition by rIRAP of IL-2-induced thymocyte proliferation was seen. The specific activities of rIRAP were

comparable with those obtained with native IRAP against either rhIL-1 α or rhIL-1 β and complete inhibition of activity was seen at concentrations 10-fold above the respective IC_{50} values. The differences in rIRAP specific activities between the two assays may reflect differences in the numbers of occupied IL-1 receptors on 1A5 cells or thymocytes required for initiation of IL-2 production or IL-2 receptor expression.

Having established the activity of rIRAP in several *in vitro* assays, it was important to determine whether this protein inhibited IL-1-induced responses *in vivo*. To test this concept we assessed the effects of rIRAP on IL-1-induced corticosterone induction²¹ and neutrophilia²² in the mouse. The induction of raised plasma corticosterone levels and neutrophilia by intraperitoneal (i.p.) or intravenous administration of IL-1 in various species, including the mouse, has been well documented^{21,22}. We found that subcutaneous (s.c.) injection of rhIL-1 α in the mouse hind paw elicited dose-dependent increases in serum corticosterone levels, which peaked around 3 h and coincided with a profound blood neutrophilia; the suboptimal dose of 10^4 U rhIL-1 α /rhIL-1 β per mouse was selected to test the potential inhibitory activity of rIRAP on these two parameters 3 h after injection. Co-injection of rIRAP (300 μg per mouse) with rhIL-1 α s.c. in the hind paw completely abrogated the increase in serum corticosterone levels seen in mice treated with rhIL-1 α alone (Fig. 4a); maximum inhibition occurred between 30–300 μg rIRAP per mouse, whereas 50% suppression of corticosterone release (IC_{50}) was observed around 0.5 μg rIRAP per mouse (Fig. 4a). The approximate specific activity of rIRAP was 1.2×10^7 IU mg^{-1} in this assay. Similar effects were observed with rhIL-1 β , where doses of 0.3–300 μg rIRAP per mouse suppressed corticosterone production by 61–96%. Neutrophilia remained unaffected by rIRAP treatment (0.3–300 μg per mouse). Co-administration of rIRAP and rhIL-1 α at the same site was not essential for activity as a similar inhibitory effect was seen after i.p. injection of rIRAP in mice receiving s.c. rhIL-1 α (Fig. 4b).

The apparent selective suppression of corticosterone release induced by rhIL-1 α , compared with neutrophilia, by rIRAP treatment suggests a possible predilection of rIRAP for the IL-1 receptors in the pituitary and brain²³, as opposed to those required for IL-1-induced neutrophilia. Recent evidence for the existence of two distinct classes of IL-1 receptors supports this possibility. A high-affinity 87–100K IL-1 receptor has been identified on T lymphocytes and fibroblasts, in contrast to a 66–80K IL-1 receptor on murine bone marrow granulocytes, pre-B cells and macrophages, which has a slightly lower affinity^{24,25}. The K_D of murine pituitary and brain IL-1 receptors was found to be the same as for murine EL-4 6.1 thymoma cells²³, suggesting that the IL-1 receptors responsible for ACTH/corticosterone induction are of the T cell/fibroblast form. In order to investigate whether the differential *in vivo* activities of rIRAP could be related to selective inhibition of the T cell/fibroblast form of IL-1 receptor, we performed IL-1 receptor competition studies with rIRAP. The competition curves in Fig. 5 show that whereas rhIL-1 α and rhIL-1 β competed for the binding of radiolabelled rhIL-1 α to YT cells (bearing the T-cell/fibroblast form of IL-1 receptor) as well as to murine bone marrow cells, rIRAP only competed for IL-1 binding on YT cells. Thus, the lack of effect of rIRAP on IL-1 induced neutrophilia *in vivo* may reflect its inability to block the IL-1 receptors on bone marrow cells which include the precursor cells for peripheral blood neutrophils²⁶. Further support for the possibility that rIRAP may selectively bind to one class of IL-1 receptor was recently provided by the demonstration that IL-1ra blocked IL-1 binding to EL-4 thymoma cells, but not to 70Z/3 pre-B cells⁸. In addition, we found that rIRAP did not block IL-1 binding to 70Z/3 cells (Fig. 5 legend). Further studies are required to clarify the possible selectivity of IRAP IL-1 receptor interaction and its relationship to selective inhibition of IL-1 activities *in vivo*.

When the equilibrium binding constants for ^{125}I -rhIL-1 α or

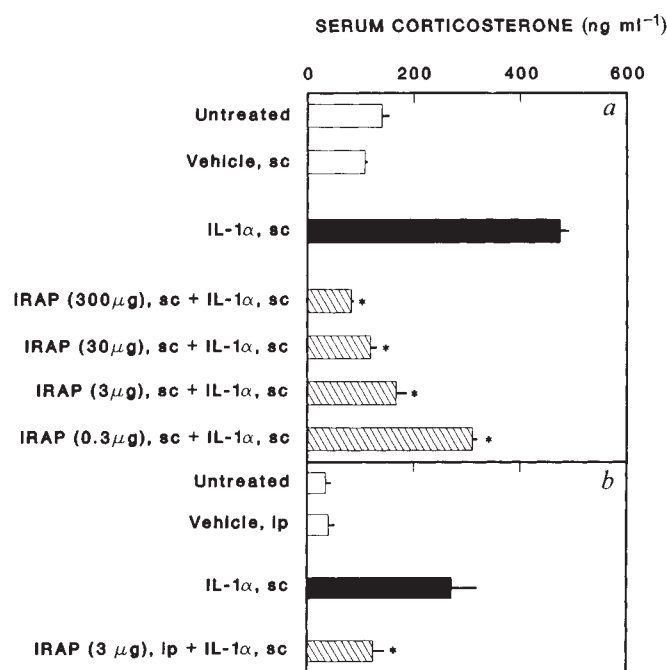


FIG. 4 a, Dose-related inhibition by rIRAP administered s.c. in the mouse hind paw of serum corticosterone levels induced by rhIL-1 α (10^4 U per mouse, s.c.). Dose-response studies indicated that 10^4 U rhIL-1 α (0.5 μg , 2×10^7 IU mg^{-1} specific activity determined in the murine thymocyte proliferation assay) induced suboptimal stimulation of corticosterone (63% of the level obtained with 10^5 U rhIL-1 α) and neutrophilia. Maximal corticosterone levels were attained between 2–3 h after s.c. injection of rhIL-1 α (10^4 U per mouse), with levels falling significantly by 4 h. Control, vehicle-injected mice showed no significant increase of corticosterone levels compared with non-injected normal mice at the three timepoints studied. The ratio of neutrophils to mononuclear leukocytes increased from a mean of 42:58 for control/vehicle-injected mice to 93:7 and 84:16 at 3 and 4 h respectively after rhIL-1 α injection. Neutrophil to mononuclear leukocyte ratios were unaffected by rIRAP treatment; rIRAP (0.3–300 μg per mouse) administered alone failed to alter normal ratios of corticosterone/neutrophil to mononuclear leukocytes or normal levels of corticosterone.

METHODS. All solutions were prepared in vehicle consisting of pyrogen-free sterile saline (0.9%) containing glycerol (10%) and injected s.c. in the plantar aspect of the mouse hind paw in a volume of 25 μl using a sterile 1 ml plastic syringe with a 27.5G stainless steel needle. The rhIL-1 α was obtained from Dainippon Pharmaceuticals Ltd. Endotoxin levels of rIRAP were determined to be less than 0.3 ng per mg of pure rIRAP. Male C3H/HeJ mice (20 gm body weight, Jackson Laboratories) were used throughout ($n=5$ mice per group); sera were assayed in duplicate for corticosterone using a radioimmunoassay kit (ICN Biomedicals). Blood smears were prepared from tail-vein puncture, fixed and stained using Diff-Quik (American Scientific); differential counts were performed recording the percentages of neutrophils and mononuclear leukocytes. b, Inhibition of rhIL-1 α -induced corticosterone production in the mouse by i.p. rIRAP. rIRAP (3 μg per mouse) was injected i.p. 30 min before rhIL-1 α (10^4 U per mouse, s.c.). * $P < 0.01$, treatment groups compared with groups treated with IL-1.

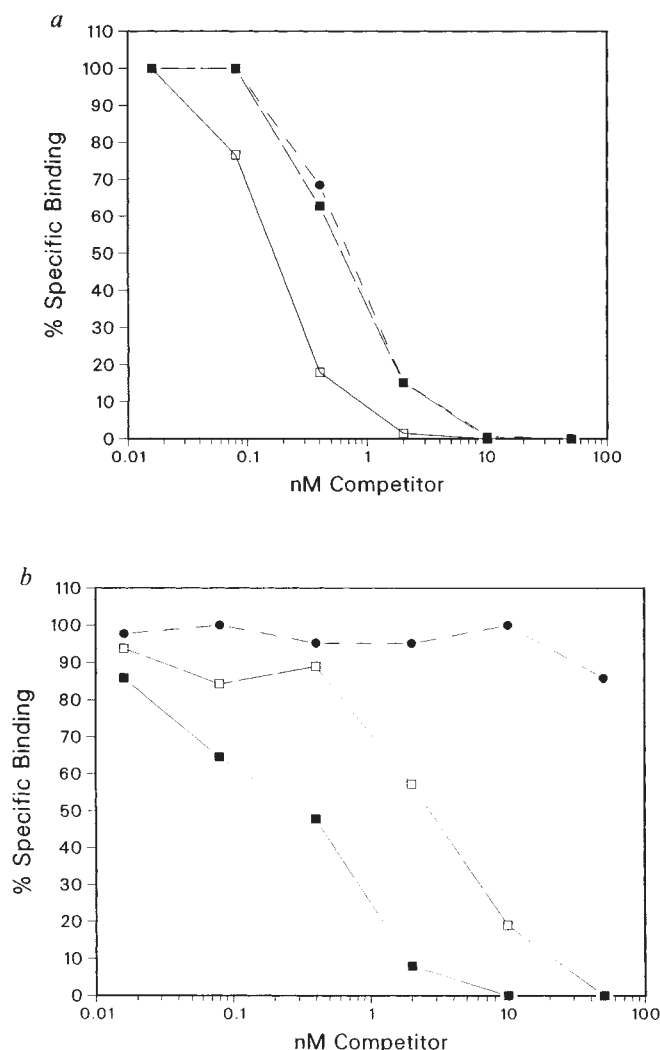


FIG. 5 Competition of IRAP for ^{125}I -IL-1 binding to IL-1 receptors. The per cent specific binding of ^{125}I -rhIL-1 α to a, YT-NCI cells and b, C3H/HeJ mouse bone marrow cells²⁶ in the presence of increasing concentrations of unlabelled competitors is shown. ■, Unlabelled rhIL-1 α ; □, unlabelled rhIL-1 β ; ●, unlabelled IRAP. Similar results were seen using ^{125}I -rhIL-1 β , when the K_i values for rhIL-1 α , native IRAP and rIRAP binding to YT cells compared with ^{125}I -rhIL-1 β were 0.081, 0.29 and 0.23 nM, respectively. The specificity of IRAP competition was tested using a TNF-receptor positive human osteosarcoma cell line, G-292. Excess unlabelled rIRAP (20-fold molar excess) did not inhibit ^{125}I -rTNF from binding in a competition assay.

METHODS. Radiolabelling of rhIL-1 α and rhIL-1 β was performed as described previously^{39,40}. Receptor-binding assays were conducted in 96-well microtitre plates and contained 10^5 YT-NCI or 2.5×10^6 bone marrow cells, 250 pM ^{125}I -rhIL-1 α or 50 pM ^{125}I -rhIL-1 β and 0.016 nM to 50 nM unlabelled competitor proteins. Non-specifically bound counts were determined in the presence of 1,000-fold excess unlabelled ligand. The cells were incubated at 22 °C for 1 h and unbound radioactivity was removed by filtration. TNF-binding assays were done under conditions comparable to the IL-1 RBA. The IC_{50} of unlabelled competitor was calculated with a computer program using probit-log transformation to linearize competition curve data. K_i values were derived from IC_{50} values by the Cheng-Prusoff relationship⁴¹. To verify the applicability of this approach, Hill analysis of the data was performed by linear regression of the $\log [\% \text{ bound}/(100 - \% \text{ bound})]$ against $\log$ (inhibitor concentration) curve⁴¹. The slopes of the resulting lines were near 1, indicating that the conditions for valid application of the Cheng-Prusoff formula (single-site binding with no cooperativity) are met. In competition-binding experiments using the murine 70Z/3 pre-B cell line²⁵, unlabelled rhIL-1 α , rhIL-1 β and rIRAP affected ^{125}I -rhIL-1 α binding with results virtually identical to those shown with bone marrow cells.

^{125}I -rhIL-1 β were determined for cells expressing the T-cell form of the IL-1 receptor, such as YT cells, the K_D values were 0.074 nM and 0.050 nM respectively. Competition studies using radiolabelled IL-1 α and IL-1 β binding to YT cells were performed using unlabelled rIRAP or native IRAP at various concentrations. A concentration-dependent inhibition of specific radiolabelled IL-1 binding was seen, with K_i values for both IRAP proteins in the 0.2–0.3 nM range, nearly identical to each other and about 3–4-fold higher than for unlabelled IL-1 β (Fig. 5 legend). No inhibition by rIRAP of labelled TNF binding to its receptor was seen. Thus, rIRAP is a specific, high-affinity competitor of IL-1 binding to the T-cell/fibroblast form of IL-1 receptor and is indistinguishable from native IRAP in this regard. The concept of selective IL-1 receptor antagonism by IRAP raises several intriguing questions concerning IL-1 and IRAP binding to the two classes of IL-1 receptor. Distinct IL-1 receptor binding and triggering domains may exist on IL-1 α/β , but perhaps IRAP lacks the triggering domain. As IL-1 α/β and IRAP are structurally homologous and compete for one class of IL-1 receptor, they probably share a common domain responsible for receptor binding. The failure of IRAP to bind to IL-1 receptors on bone marrow and pre-B cells may indicate that distinct domains are responsible for binding of IL-1 α/β to the two classes of IL-1 receptors and that IRAP lacks one of these domains.

Evidence for the *in vivo* activity of recombinant IL-1ra (refs 8, 9), which seems to be identical to rIRAP, has been described in the rabbit²⁷; intravenous infusion of IL-1i (IL-1ra, 1–4 mg kg⁻¹) with rhIL-1 β (15 μg kg⁻¹) inhibited IL-1-induced hypotension and the induction of acute phase proteins. Monoclonal antibody specific for the murine T-cell/fibroblast IL-1 receptor suppressed responses elicited by IL-1 (acute inflammation; IL-6 production) in addition to lipopolysaccharide-induced acute inflammation and acute bacteraemia in which IL-1 seems to have an important role²⁸. These observations, together with the *in vitro* and *in vivo* data presented above, strongly suggest that antagonism of IL-1 binding to IL-1 receptors *in vivo* results in inhibition of a broad spectrum of pathophysiological responses associated with host defence and inflammation. Naturally induced IRAP may be a negative IL-1 feedback inhibitor whose role *in vivo* may be to regulate certain IL-1 functions. The exciting possibility that rIRAP may be a useful therapeutic agent to enhance the feedback mechanism for the treatment of inflammatory diseases remains to be tested. □

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LETTERS TO NATURE

Blue stragglers at the centre of the post-core-collapse globular cluster NGC6397

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THE nearby globular cluster NGC6397, 2.2 kpc from us, is an evolved cluster which has passed through core collapse^{1–3}; its core region is expanding after an earlier dynamical phase of contraction. Its proximity means that observations of it with CCD (charge-coupled device) detectors in arcsecond-seeing conditions simulate Hubble Space Telescope observations of more-distant objects, such as the archetypal post-core-collapse globular cluster M15, and are capable of testing the predictions of some recent models^{4,5} of post-collapse cluster dynamics. We have discovered in the central region of NGC6397 a population of 'blue stragglers'⁶, stars that are too hot for their luminosity in relation to subgiant stars. Five of these blue stragglers are within 0.07 pc of the centre of the cluster, and are brighter than the rest by one magnitude. We suggest that the blue stragglers are either coalesced stars resulting from stellar collisions expected to occur especially during core-collapse^{7,8}, or belong to a population of primordial close binaries that could provide the energy for the expansion of the core⁴.

Our observations were obtained at ESO (La Silla) on 19–20 March 1988 with a double-density RCA CCD at the 1.5-m Danish Telescope. The exposures were 60 s and 20 s and the FWHM of star profiles was 1.07'' and 0.95'' in the *B* and *V* passbands respectively. We used the same photometric package for stellar and surface photometry as in ref. 9, where details of the photometric technique can be found. Figure 1a shows an image of a 2' × 2' square field centred on NGC6397 in the *V* passband. Figure 1b shows contours of the 14'' × 14'' central region where several stars of the central clump¹ are well resolved with round images. Numbers correspond to blue straggler stars (BSs) in Table 1 and will be explained below. We used the equibarycentric method^{1,9} for resolved stars with *V* < 16 to determine the dynamical centre of NGC6397 and we found it less than 1'' away from star 1.

A power law can be fitted to the central part of the brightness profile of NGC6397, which is the signature of the post-core-collapse phase^{2,3}. CCD surface photometry of globular clusters is limited by sampling errors due to the statistical fluctuations of the bright end of the luminosity function. We used the star-stripping technique⁹ to reduce them when measuring the slope of the power law, subtracting numerically with Daophot bright stars which are resolved in the whole field studied. Fitting between 2.5'' and 1'' the *B* profile obtained after subtracting 29 red giants, we obtain a slope value of −0.60 with an error estimate of 0.1. This slope value is steeper than that obtained without star stripping, as there are no bright stars near the very centre of the cluster (the sky background was found to be <1 ADU per pixel on the *B* 1-min exposure and thus negligible). The

slope of the power law is similar to that found for M15 and NGC6624¹⁰, and is what is expected if there is a dynamically significant number of stars in the cluster with a mass 1.9 times larger than that of the luminous stars¹¹.

Figure 2a shows the colour-magnitude diagram (CMD) for 1,899 stars in the 150'' × 240'' field centred on NGC6397. A sequence of stars brighter and bluer than the turnoff is present at the location of the BSs. The BSs are on the extension of the main sequence, having the photometric properties of stars with a larger mass than that of the turnoff.

Thirty stars are found in the BSs region: *B* − *V* < 0.51 and 16.35 < *V* < 14.15. From examination of the CMD of ref. 12, we can estimate the number of field stars expected in our CMD. Normalizing to the field stars brighter than *V* = 16 and located to the red side of the giant branch (where they are more numerous), we find that we should not expect more than two

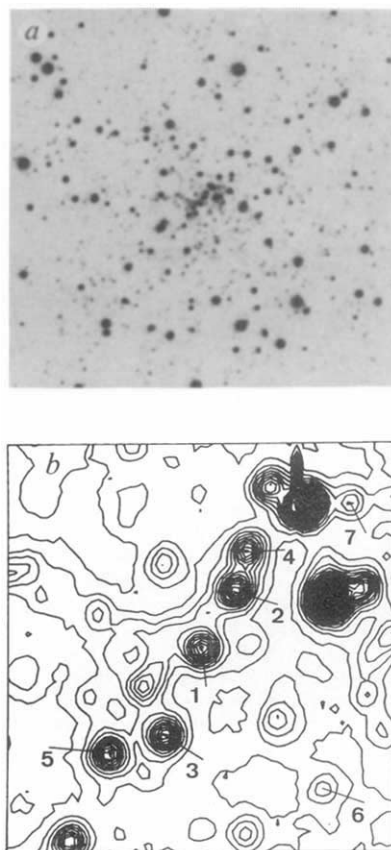


FIG. 1 a, The central region of NGC6397 in a 2' × 2' field in the *V* passband. FWHM of star profiles is 0.95''. North is up and east is to the left. There are no red giants at the centre and the central clump of stars is resolved into faint stars. b, Contours for the 14'' × 14'' central region in the *V* passband. One step is 60 ADU. Well resolved and measured BSs are numbered as in Table 1. The dynamical centre of the cluster is less than 1'' away from star 1.