THE BETA FORM IS THE DOMINANT INTERLEUKIN 1

RELEASED BY MURINE PERITONEAL MACROPHAGES

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Using highly specific polyclonal antisera raised against recombinant murine IL-1 α and β, we performed solid-phase immunoabsorption studies on supernates of resident and adjuvant-elicited CBA/J mouse peritoneal macrophages. Antibody specificity was established by reciprocal absorption studies and Western blot analysis. Supernates obtained from macrophages cultured for 18 hr in the presence of 1μg/ml lipopolysaccharide (LPS) were subjected to immunoabsorption. Approximately 78-90% of the released bioactive material was IL-1 and about 80% of this could be attributed to IL-1 β. Analogous to that reported for human monocytes, these data suggest that IL-1 β is the predominant released form of IL-1.
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Interleukin 1 (IL-1) is produced primarily by mononuclear phagocytes and

is synthesized as a 31-33 kd cytoplasmic precursor, then is processed and

secreted as a 17 kd molecule (1,2). Numerous functional activities have been

ascribed to IL-1. These are generally growth promoting, proinflammatory and

involve a variety of target tissues (3-5). Based on molecular cloning

studies, two forms of IL-1 activity, known as alpha and beta, have been

described in both humans and mice (6,7). Interestingly, there is only 22-26%

homology between the α and β forms, but there is high degree of

homology (>60%) between mouse and human α or β forms. Oppenheim et

al. reported that the β form is the major secreted form of human

monocytes (8). However, little is known regarding the relative secretion of

α and β forms in mature murine macrophages. In the present study, we

approached this question using immunoaffinity columns with IgG isolated from

highly specific polyclonal antisera against murine IL-1 α and β.

MATERIALS AND METHODS

Animals. Female, CBA/J (Jackson Laboratories, Bar Harbor, ME)) mice were maintained under specific pathogen-free conditions and provided with food and water *ad libitum*.

Macrophage isolation and culture. Peritoneal exudate cells were obtained by aseptic peritoneal lavage at 10-14 days after intraperitoneal injection of 0.5 ml of complete Freund's adjuvant (CFA) emulsified in an equal volume of normal saline (Sigma Chemicals, St. Louis, MO). Resident peritoneal cells were obtained from uninjected, normal mice. The cells were washed by centrifugation then suspended in RPMI (GIBCO, Grand Island NY) containing 10% fetal bovine serum (FBS) (Hazelton, Lenexa, KS), 2 mM glutamine and 100 U penicillin/100 μg/ml streptomycin. Cell concentrations were adjusted to achieve optimal cell densities for monokine production, usually 0.5 to 1 X 10^6/ml. Macrophages were isolated by adherence to 35 mm culture dishes (Corning Glass Works, Corning, NY) for 1 hr at 37°C in a 5% CO_2, humidified atmosphere. The nonadherent cells were removed by two vigorous washings with 1 ml of warm RPMI. The resident monolayers were >95% and elicited monolayers were >90% macrophages based upon nonspecific esterase and morphologic criteria. The macrophage monolayers were then overlaid with RPMI-FBS containing 1 μg/ml of LPS (*Escherichia coli* 0111:B4)(Sigma, St. Louis, MO);controls contained no LPS. Supernates were collected 18 hr after LPS stimulation, centrifuged at 500g for 10 min to remove particulates, then frozen at -20°C before monokine assay.

Antisera preparation. Anti-IL-1 alpha and anti-IL-1 beta were produced in goat and rabbit, respectively. Twenty to 40 micrograms of recombinant murine IL-1 α and β (Pfizer Pharmaceuticals, Groton, CT). were admininstered in multiple intradermal sites with Freund's complete adjuvant followed by a equivalent boost 2 weeks later. The resulting antisera reacted with recombinant material and both 17-18 kd and 33 kd species in Western blot analysis of macrophage lysates. Specificity of these antisera was confirmed by both competitive inhibiton and reciprocal absorption studies.

Preparation of immunoaffinity columns. Immunoglobulin G was purified from anti-IL-1 α and β antisera using protein A agarose (Affigel A, Biorad, Richmond, CA). Corresponding control IgG preparations were isolated from nonimmune sera. The individual preparation were then bound covalently to CNBr activated Sepharose 4B (Sigma) according to described methods (9). Approximately 8-10 mg of IgG was bound per 1 ml gel. Following blocking of unbound sites with 0.2 M Tris-buffer (pH 8.0), 2.5 ml of gel was packed into 5 ml columns and washed extensively with PBS prior to use. Columns were operated at a flow rate of about 0.2 ml/min and were regenerated with 0.2 M glycine buffer (pH 2.5). As supernates contained FBS, this acted as a source of carrier protein to monitor column fractions. Protein containing fractions were pooled and adjusted to equivalent concentrations with PBS before assay.

Interleukin 1 assay. IL-1 was assayed by the standard thymocyte comitogen assay as modified from the procedure of Mizel *et al.* (10) and Koopman *et al.* (11). Briefly, single cell suspensions of thymocytes were prepared from 5-7 week old CBA/J mice. The cells were washed and suspended to 5 X 10^6/ml of RPMI-FBS. The thymocytes (5 X 10^6) were cultured in 0.2 ml in 96-well flat bottom culture plates (Corning) in the presence of 1μg/ml of purified phytohemagglutinin (PHA) (Wellcome Research Laboratories, Beckenham, England) and serial $\log_2$ dilutions of test supernate for 72 hr. The culture wells were each pulsed with 0.5μCi of ^{3}H-methyl-thymidine (ICN, Irvine, CA) for the final 14 hr of culture. The cells were then harvested onto glass filters and uptake was quantitated by scintillation spectrophotometry. IL-1 activity was quantitated by calculation of 1/2 maximal units. Recombinant murine IL-1α and β standards were used as positive controls; approximately 50-100 pg were equivalent to a 1/2 maximal unit.

Statistics. The Student's t-test was used to determine differences between control and experimental groups. Values of p>0.05 were considered not significant.

RESULTS

Specificity of immunoaffinity columns. In order to test specificity of the immunoabsorbents we passed 150 ng of rIL-1 α and β through the prepared immunoaffinity columns with bound anti-IL-1 α or anti-IL-1 β antibodies.   As shown in Figure 1, there was total removal of the respective activities but no loss of activity when passed through reciprocally or when passed through control IgG columns. The recombinant material was dissolved in PBS with 0.5% bovine albumin added as a carrier protein, allowing for adjustment of column fractions to equivalent protein concentrations. Specificity has also been demonstrated in Western blot assays which showed no cross-reactivity of these antibodies and no reactivity with an unrelated monokine tumor necrosis factor (data not shown).

Determination of IL-1 α and β content of macrophage supernates. Using the immunoaffinity columns, we determined the relative distribution of IL-1 α and β in supernates of resident and elicited macrophage populations.   Supernates collected 18 hrs after LPS stimulation were passed in parallel or serially through control or antibody columns. Appropriate fractions were identified by detection of carrier protein, pooled, then adjusted to similar protein concentrations before IL-1 assay.   Table 1 shows the IL-1 activity remaining in supernates following column passage.
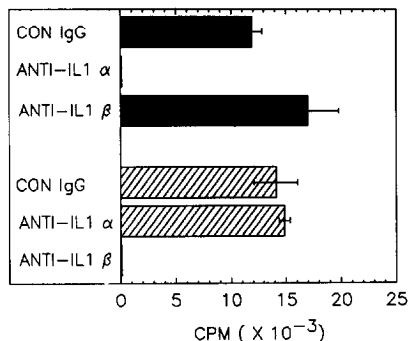

Figure 1. Specificity of anti-IL-1 α and β immunoaffinity columns. Immunoaffinity columns were prepared as described in the Materials and Methods. Recombinant murine IL-1 α and β (150 ng) were passed through respective and reciprocal columns then the effluent was assayed for IL-1 activity.   Solid bars, IL-1α activity; hatched bars, IL-1β activity. Bars are mean cpm $\pm$ S.D.

<u>Table 1.</u> Distribution of IL-1α and β in resident and elicited macrophage
supernates as determined by specific immunoabsorption

Column specificity	IL-1 bioactivity following column passage (CPM ± SD)			
	Resident*		CFA-elicited	
NG IgG**	3707±906		15967±4507	
IL1α	2900±1521	(22%)***	13716±5628	(14%)
NR IgG	3692±1520		17481±7271	
IL1β	958±811	(74%)	7655±1681	(56%)
NG+NR IgG	4615±2469		13765±2627	
IL1α+β	1022±587	(78%)	1195±1018	(90%)

  *Values represent means of supernate dilutions of comparable activities
   derived from 3 separate experiments.
 **NR=nonimmune rabbit, NG=nonimmune goat
***Values in parentheses represent the percentage of activity removed by
   column passage as compared to the nonimmune controls.

Interleukin 1 α was detected in lower amounts among both resident and CFA
elicited cells.   In contrast, IL-1 β was found in both the resident and
elicited macrophage cultures as the dominant form, being produced at 3-4 fold
higher levels.   These studies also showed that 78-90% of the supernate
activity was IL-1 and not another material with similar biologic effect.

DISCUSSION

    The above immunoabsorption studies yielded a number of novel findings.
Both resident and elicited macrophages secreted detectable IL-1 α and
β which accounted for up to 90% of the supernate bioactivity. Interleukin
1β was the dominant secreted form at 18 hrs, being produced in 3-4 fold
greater amounts than IL-1 α.   This is in complete agreement with that
reported for human monocytes in which IL-1 α and β were measured by
radioimmunoassay (12).   Since our conclusions are based on biologic activity
it was important to establish that the two forms of IL-1 had similar
bioactivity and stability.   Extensive studies of recombinant IL-1 α and
β revealed virtually identical dose response curves, no synergism and
retention of 50-90% of biologic activity after boiling for 30 minutes (data
not shown). The similar dose-response relationships and lack of synergism is

consistent with reports that both forms bind to the same receptor (13-15).

Since our study was limited to an 18 hr period we cannot rule out the

possibility that the alpha form is released at a later time. However, based

on our findings, it is tempting to speculate that the beta form of IL-1 is

the primary released form, whereas the alpha form may remain largely

cell-associated, possibly as a membrane protein as previously reported (16).

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