Brief Definitive Report

CHARACTERIZATION OF A MONOCLONAL ANTIBODY DIRECTED AGAINST THE BIOLOGICALLY ACTIVE SITE OF HUMAN INTERLEUKIN 1

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Lymphokines like IL-1 are of multicellular origin, and through their multifaceted regulatory actions they affect a variety of different target cells during host response to infections. IL-1 at the site of inflammation activates lymphocytes, granulocytes, and fibroblasts (1). Moreover, IL-1 also may act as mediator of the acute-phase response, promote catabolism of structural protein and matrix, and regulate the febrile response (1). To further elucidate these multiple biological effects on different tissues and to investigate whether they are regulated by a single entity or a family of related molecules, it is necessary to analyze the sequence of the IL-1 molecule and to develop antibodies directed against IL-1. The sequence of murine IL-1 recently has been discovered (2), and cloning of two distinct human IL-1 cDNAs has also been reported (3, 4). However, to date, only conventional antisera directed against IL-1 (1, 5) are available. In this study, an mAb that binds different IL-1 species and blocks the biological effects of IL-1 was developed and characterized.

Materials and Methods

Preparation of IL-1 and Bioassays. IL-1 was prepared from human adherent cells as described (6). Concentrated samples (100 μl) were subjected to HPLC using Bio-Sil TSK 125 (Bio-Rad, Richmond, CA) size-exclusion chromatography or chromatofocusing on Mono P (Pharmacia Fine Chemicals, Upsala, Sweden) as described (6).

For the detection of IL-1 activity, the thymocyte costimulator assay or the fibroblast (CRL 1445) proliferation assay were used (7). IL-2 activity was measured using an IL-2-dependent cytotoxic T cell line (CTLL) (7), and IL-3 activity was evaluated using an IL-3-dependent cell line (32 DCL) (8).

Immunization and Cell Fusion Procedures. BALB/c mice were injected with six doses of partially purified (TSK 125 pool) IL-1 (40,000 U/ml) in complete Freund's adjuvant at 2 wk intervals over a period of 3 mo. 3 d after the last immunization, splenocytes (1.5×10^8) from an immunized mouse that exhibited plasma anti-IL-1 activity were fused with 1.5×10^8 plasmocytoma cells (P_3-X63-A_2 8-653, SP3) (9). Subsequently, the cells were resuspended in HAT selection medium, and 10^6 cells/well were plated into 96-well tissue culture plates. After 2 wk, cell cultures that produced IL-1 activity–inhibiting supernatants were expanded and cloned by a limiting-dilution method. Subsequently, the clones were

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retested for inhibiting activity and injected i.p. into BALB/c mice that had been primed with 0.5 ml of 2, 6, 10, 14-tetramethylpentadecane (Pristane; Aldrich Chemical Co. Milwaukee, WI).

Preparation and Purification of mAb. Each mAb was precipitated from clarified ascites fluid by the gradual addition of saturated ammonium sulfate to 45% saturation. Precipitated proteins were dissolved and dialyzed against several changes of PBS. IgG mAb were purified further by affinity chromatography using goat anti-mouse IgG (Nordic Immunology, Tilburg, The Netherlands) coupled to CNBr-activated Sepharose 4 B (9). The Ig class was determined by a dot immunobinding assay using anti-mouse Ig antisera (10).

Preparation of an Immunoaffinity Gel for IL-1. Monoclonal anti-IL-1 (40 μg) was coupled to (2 ml) CNBr-activated Sepharose 4 B as described (9), and incubated 30 min at 37°C with different preparations of IL-1. Subsequently, the residual IL-1 activity in the supernatant was measured in the thymocyte assay.

Immune Precipitation of Biosynthetically Labeled IL-1. 2×10^7 peripheral blood mononuclear cells (PBMNC) were incubated with LPS (10 μg/ml, from *E. coli*; Difco Laboratories, Detroit, MI) in serum-free Eagle's MEM. After 2 h incubation, the supernatant was replaced by medium containing ^{14}C-labeled amino acid mixture (12.5 μCi/ml), and incubation was continued for further 24 h. Subsequently, intracellular lysate as well as extracellular supernatant IL-1 was immunoprecipitated as described (11). Proteins were eluted by boiling the samples in electrophoresis sample buffer and applied to 15% SDS-PAGE (12). The gel was screened for radioactivity by using a TLC scanner (Berthold, Vienna, Austria) (13).

Results

Hybridoma supernatants were assayed for anti-IL-1 activity by testing their capacity to block IL-1-mediated thymocyte proliferation. After recloning, supernatants obtained from nine clones inhibited IL-1 activity (data not shown). Two of these clones, AK 7 and AK 17, were reexpanded and selected for ascites production. Both ascitic antibodies were of the IgG2a class.

IL-1 antibodies purified by affinity chromatography suppressed both IL-1-mediated thymocyte and fibroblast proliferation (Fig. 1). In contrast, nonspecific IgG or SP3 cell supernatant did not inhibit the effect of IL-1 on thymocytes or

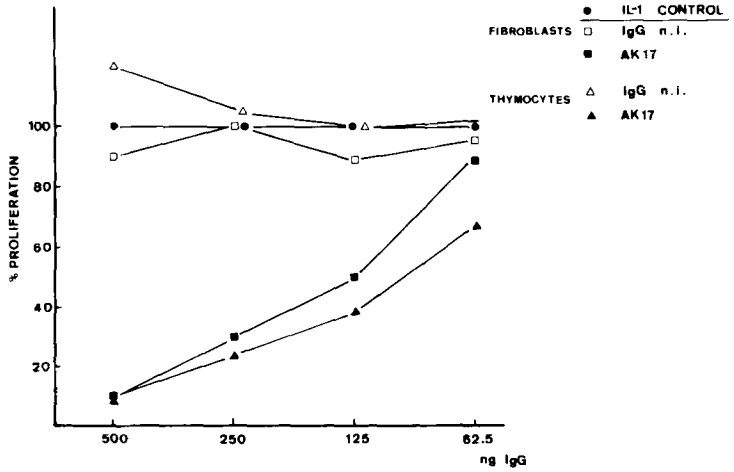

FIGURE 1. Effect of anti-IL-1 IgG on IL-1 activities. Serial triplicate dilutions of monoclonal anti-IL-1 IgG2a (AK 17) purified by affinity chromatography, and nonimmune murine IgG2a (*IgG n.i.*) were tested in microtiter plates for their capacity to inhibit IL-1 (50 U/ml) -mediated thymocyte or fibroblast proliferation. Results are expressed as percentage of IL-1 activity inhibited by the antibody.

fibroblasts. The inhibitory effect of the antibody was dose related, and 0.5–1 μg/ml anti-IL-1 IgG sufficed to completely inhibit the activity of 50 U/ml of partially purified IL-1.

To confirm the specificity of anti-IL-1 IgG, its effect on epidermal cell thymocyte-activating factor (ETAF), IL-2, and IL-3 activity was tested. ETAF was derived from a human squamous cell carcinoma cell line (7), IL-2 was prepared from human PBL as described (14), and IL-3 was obtained from the murine monomyelocytic WEHI 3 cell line (8). Monoclonal anti-IL-1 significantly inhibited the thymocyte proliferation in response to ETAF, which further supports previous data (7) showing that ETAF and IL-1 are closely related moieties sharing similar antigenic structures. In contrast, anti-IL-1 IgG failed to inhibit the IL-2-dependent proliferation of CTLL cells, nor did the antibody block the growth of the IL-3-dependent 32 DCL cells (Table I).

The efficiency of anti-IL-1 IgG binding to IL-1 was tested by the capacity of anti-IL-1 IgG to precipitate biosynthetically radiolabeled IL-1. Human mononuclear cells were stimulated with LPS and incubated for 24 h with ^{14}C-labeled amino acids. Subsequently, extracellular (supernatant) as well as intracellular (cell lysate) IL-1 were immunoprecipitated with anti-IL-1 IgG (AK 17), and the precipitates were analyzed by SDS-PAGE. Immune IgG precipitated three radiolabeled proteins with molecular masses of 33, 17, and 4 kD, which correspond exactly to the molecular masses of the different species of human IL-1 and were present in supernatant as well as lysate of mononuclear cells (Fig. 2).

Because monoclonal anti-IL-1 has been shown to be capable of precipitating IL-1, we wished to determine whether the antibody (AK 17) would facilitate the purification of IL-1 by preparing an immunoadsorbent column. IL-1 (95%) bound to the immunoaffinity gel and was not released by washing with PBS. To elute the specifically bound material, a 3 M KSCN solution was used. The yield of IL-1 after immunoaffinity chromatography was ~20%. The final product had a specific activity of ~10^7 U/mg protein. Upon HPLC gel filtration, immunoaffinity-purified IL-1 consistently eluted as a single peak of activity with a molecular mass of ~4 kD (Fig. 3).

Discussion

This paper outlines the development of mAb that appears to be directed against IL-1 and ETAF of human origin. The antibody neutralizes the biological activities of IL-1, as shown by its ability to block IL-1-mediated thymocyte as well as fibroblast proliferation. Moreover, anti-IL-1 also binds to IL-1, evidencing the adjacency of the epitope recognized by anti-IL-1 to the active site on the IL-

TABLE I

Specificity of Anti-IL-1 Neutralization Assay

Factor*	Cell source	Nonimmune IgG (μg/ml)			Ak 17 anti-IL-1 IgG (μg/ml)		
		1	0.5	0.25	1	0.5	0.25
IL-1 (100 U/ml)	Adherent cells	17,653	15,316	18,253	1,095	2,760	5,122
ETAF (50 U/ml)	SCC cells	5,724	6,002	5,934	803	1,978	4,024
IL-2 (100 U/ml)	Nonadherent cells	25,834	27,037	26,854	25,543	26,894	27,105
IL-3 (100 U/ml)	WEHI 3 cells	13,741	13,053	12,983	13,657	12,573	12,881

Results are expressed as mean cpm of triplicate cultures.

* Factors were partially purified by HPLC gel filtration on TSK 125.

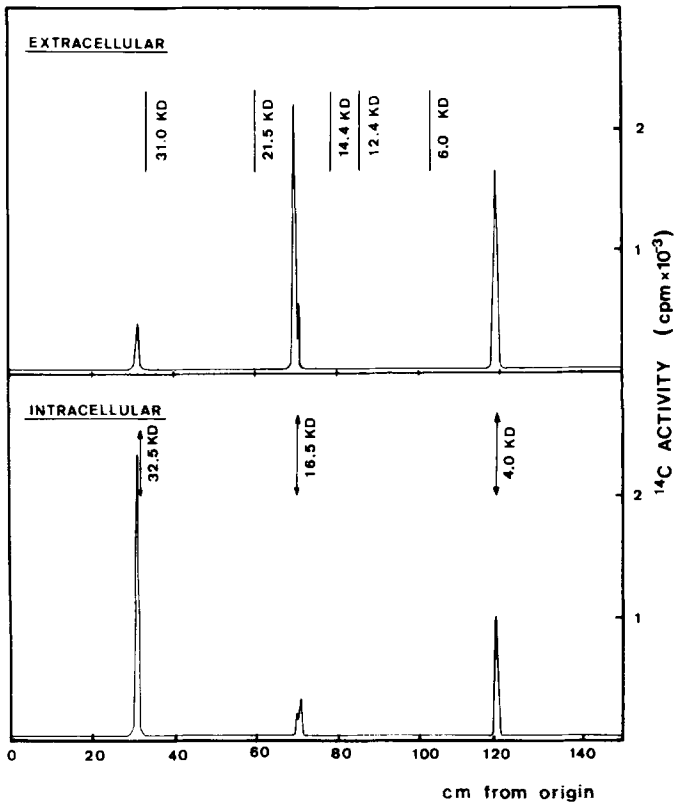

FIGURE 2. SDS-PAGE of in vivo–labeled IL-1. Immunoprecipitation of biosynthetically labeled IL-1. Autoradiogram of labeled proteins remaining in supernatant (*A*) or cell lysate (*B*) after precipitation with anti-IL-1 IgG2a (AK 17) The gel was screened for radioactivity by using a TLC scanner as described in Materials and Methods.

1 molecule. Since anti-IL-1 IgG also was capable of neutralizing ETAF activity, the hypothesis is further supported that ETAF and IL 1 are closely related moieties, which share similar antigenic structures, though they are released by different cell types. The inhibitory effect of the antibody was not cytotoxic, since it did not inhibit the proliferation of several cell lines tested (data not shown), and it did not interfere with IL-2 or IL-3 activity.

Although the molecular structure of human IL-1 is not yet fully understood, the different molecular mass species of IL-1 may be proteolytic cleavage products of a high molecular mass precursor IL-1 molecule (4). Upon immunoprecipitation of biosynthetically radiolabeled IL-1, the antibody reacted with all three of the different species of IL-1. In addition, immunoprecipitation of intracellular IL-1 yielded large amounts of the 33 kD IL-1, whereas if extracellular IL-1 was precipitated with anti-IL-1 IgG, only minute amounts of high molecular mass IL-1 were detected. In the supernatant, proteolytically cleaved 17 kD and 4 kD IL-1 molecules were found in larger amounts. This again supports the concept that human IL-1 is synthesized as a higher molecular mass precursor, which is subsequently enzymatically converted to the low molecular mass forms, as has been proposed (2).

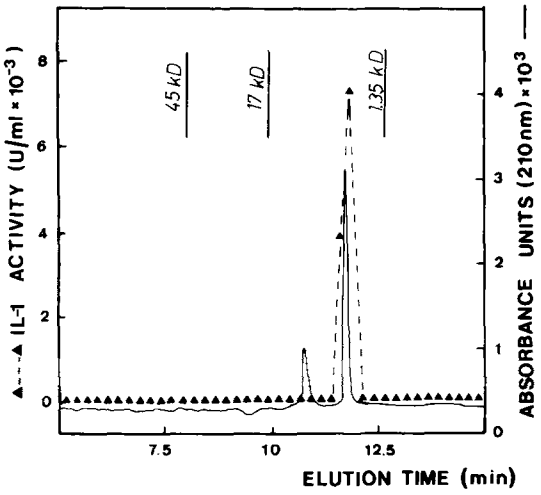

FIGURE 3. HPLC gel filtration (Bio-Sil TSK 125) of human mononuclear cell-derived IL-1 purified by immunoaffinity chromatography using anti-IL-1 IgG (Ak 17). IL-1 activity was measured using the thymocyte assay, and results are expressed in U/ml IL-1 activity. In addition, the column eluent was monitored on a Uvicon 720 LC UV-visible wavelength detector.

It is especially noteworthy that immunoaffinity-purified IL-1 exhibited a molecular mass of ~4 kD. Therefore, monoclonal anti-IL-1 IgG appears to react with a biologically active low molecular mass breakdown product of the IL-1 molecule. Biologically active 4 kD IL-1 molecules have also been detected in vivo (1, 15), suggesting that the active site is located in the part of the IL-1 molecule that apparently is recognized by the monoclonal anti-IL-1 IgG.

Since anti-IL-1 IgG reacts with the active site of IL-1 molecule, it may be useful to localize the amino acid sequence within the protein that are responsible for the biological activity. Moreover, the antibody may help to determine whether both of the recently described (4) human IL-1s, IL-1α and IL-1β, which are different in their amino acid sequences share a common area responsible for their biological functions. This reagent could also be helpful in determining whether the multiplicity of the biological effects of IL-1 are due to the same moiety. Because there is some homology between IL-1α and IL-1β, and murine IL-1 (4), it may also be that the active site is identical in human and murine IL-1. This is also supported by our finding that anti-human IL-1 IgG blocks the biological effect of murine IL-1 (our unpublished observations). In vivo, anti-IL-1 IgG may also prove useful for the investigation of the role of IL-1 during the pathogenesis of immunological as well as inflammatory diseases.

Summary

Human IL-1 was successfully used to produce an anti-IL-1 mAb. Anti-IL-1 (IgG2a) blocked IL-1-mediated thymocyte and fibroblast proliferation, but did not interfere with the biological effects of other lymphokines, such as IL-2 or IL-3. The antibody immunoprecipitated biosynthetically radiolabeled 33, 17, and 4 kD IL-1. An immunoadsorbent column yielded 20% of initial activity, and upon HPLC size-exclusion chromatography, affinity-purified IL-1 had a molec-

ular mass of ~4 kD. These results provide first evidence of a monoclonal anti-IL-1 that reacts with different species of IL-1 and apparently binds to an epitope close to the active site of IL-1. Thus, anti-IL-1 IgG may be very helpful for further investigations of the molecular as well as biological characteristics of IL-1 and related mediators.

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