Rapid and Specific Conversion of Precursor Interleukin 1β (IL-1β) to an Active IL-1 Species by Human Mast Cell Chymase

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Summary

Secretory granules of human dermal mast cells contain a chymotrypsin-like serine proteinase called chymase. In this study, we demonstrate that the inactive cytokine, 31 kD interleukin 1β (IL-1β), can be converted rapidly to an 18 kD biologically active species by human mast cell chymase. The product formed is three amino acids longer at the amino terminus than the mature IL-1β produced by peripheral blood mononuclear cells and has comparable biological activity. Because chymase is a secretory granule constituent, it is likely to be released into the surrounding tissue when mast cells degranulate. It is also known that non-bone marrow derived cells resident in skin (keratinocytes, fibroblasts) produce but do not process 31 kD IL-1β. In this context, chymase may be a potent activator of locally produced 31 kD IL-1β. Mast cells lie in close apposition to blood vessels in dermis; therefore, chymase mediated conversion of 31 kD IL-1β might be expected to have a critical role in the initiation of the inflammatory response in skin.

It has recently become clear that many important cytokines can be released from cells as biologically inactive precursor molecules. TGF-β and IL-1β both require post translational proteolytic processing to attain activity in vitro (1, 2). Biologically inactive precursor 31 kD IL-1β can be converted to an active 17 kD (NH_2-terminal Ala 117) molecule by a convertase enzyme recently isolated from monocytes (3, 4). Paradoxically, many cells that make 31 kD IL-1β do not contain such convertase activity and are unable to generate an active species of IL-1β (5, 6). We have recently demonstrated that 31 kD IL-β produced by human epidermal keratinocytes can be cleaved by exogenous chymotrypsin into biologically active 18 kD IL-1β species (NH_2-terminal Val 114) (5). Based on these and other data, we postulated that the processing of 31 kD IL-1β produced by non-bone marrow derived cells occurs extracellularly.

Human mast cell chymase is a chymotrypsin-like proteinase stored within the secretory granules of a subset of human mast cells termed MC_{TC}; this mast cell designation signifies the presence of two different serine proteinases referred to as human chymase (C) and tryptase (T). MC_{TC} mast cells are the predominant mast cell type found in skin and gut submucosal tissues whereas MC_T mast cells containing only tryptase predominate in lung alveoli and gut mucosa. Mast cell degranulation is the initial event in IgE-mediated inflammatory responses and has been implicated in various skin diseases (7). More recently, mast cell degranulation has been cited as an early event in cutaneous inflammatory reactions (8), in part because of release of preformed TNF-α, an important regulator in endothelial adhesion molecule expression. Because mast cell degranulation can be induced by diverse stimuli mast cell mediators may have a wider general role in acute and chronic inflammatory responses (8).

MC_{TC} type mast cells reside in several distinct tissues, including the dermis (9). As such, they are in close proximity to many cells capable of producing (but not processing) 31 kD IL-1β. Demonstration of the processing of 31 kD-1β by mast cell chymase provides a pathway for the activation of this material. Since IL-1 (like TNF-α) is also a potent regulator of adhesion molecule expression by endothelial cells, this model would provide an important mechanism whereby mast cell products could initiate an immune or inflammatory response.

Material and Methods

Purification of Mast Cell Chymase. Human chymase was purified from human skin as previously described (10, 11). Skin was extracted first in low and then in high ionic strength solutions. The latter solution solubilizes chymase and tryptase. The extract was

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dialyzed to reduce the ionic strength to 0.4 M NaCl and then chromatographed on heparin-Sepharose (12). Tryptase and chymase bind to the column and were eluted in a single step with 2 M NaCl. The 2 M NaCl eluate was then chromatographed on soybean trypsin inhibitor-Sepharose. Chymase but not tryptase binds to this resin. Chymase was eluted from the column using 1.0 mM HCl.

Interleukin 1. Recombinant "mature" or 17-kD human IL-1β encoded by the latter half of the IL-1β cDNA (codons 117-269) was synthesized in a prokaryotic expression vector under control of a temperature sensitive repressor (13). The protein was extracted from Escherichia coli and purified to homogeneity by sequential sulfopropyl sephadex, DEAE, and Procion Red column chromatography (Bethesda Research Laboratories, Gaithersburg, MD) as described (13). Expression of recombinant "precursor", 31-kD or "pro-IL-1β" involved the use of a full length (codons 1-269) IL-1β cDNA using the same temperature sensitive repressor (14). After lysis of bacteria, 31-kD IL-1β was purified using sequential Q Sepharose, Procion Red, and Phenyl-Sepharose CL-4B chromatography as previously described (14).

IL-1 Biological Assay. The assay to detect IL-1 activity utilizes the T cell clone D10.G41 and the clonotype-specific mAb 3D3 and has been described in detail (15, 16). 6 h before harvest (^{3}H)-thymidine (1 μCi per well; 1 Ci = 37 GBq) was added. Triplicate cultures were harvested on an automated sample harvester (Cambridge Technologies, Inc., Boston, MA) and radioactivity was measured with an LKB scintillation spectrometer (LKB Instruments, Inc., Gaithersburg, MD).

Immunoblotting. Western blot analysis was performed as described previously in detail (5). Samples were loaded on to a 13% polyacrylamide gel containing 0.1% SDS under reducing conditions. After electrophoresis, proteins were transferred onto nitrocellulose membranes (Bio-Rad Laboratories, Richmond, CA) in a transblot cell apparatus (Bio-Rad Laboratories). The blot was blocked with dry milk and then incubated for 1 h with monoclonal anti-IL-1 antibody (M_3; reference 5) diluted in 0.05% Tween 20 10 mM tris-buffer (pH 8.0) (TBST). After washing in TBST, alkaline phosphatase-conjugated goat anti-mouse IgG antibody (Promega Biotec, Madison, WI) was added and the blot was developed and photographed.

Amino Acid Sequencing. SDS-PAGE electrophoresis was performed as in Fig. 2. Protein was transferred onto PVDF membranes (Immobilon) (17) and subjected to amino acid sequence determination using an Applied Biosystems 470A gas phase sequencer (Applied Biosystems, Foster City, CA).

Results

Purified mast cell chymase migrated as a single protein species of 30 kD on a SDS-PAGE gel (Fig. 1) and maintained significant enzymatic activity in vitro on chymotryptic substrates (18). The broadness of the band is thought to be due to variable glycosylation. We next determined whether this enzymatically active, highly purified mast cell chymase from human skin mast cells could cleave highly purified recombinant human 31 kD IL-1β. IL-1β was identified by Western blot using an antibody to a carboxy-terminal peptide common to all forms of IL-1β as previously described (5). Fig. 2 indicates that cleavage of the precursor molecule by mast cell chymase occurs almost instantaneously and is essentially complete by 30 min. Formal analysis of the kinetics of the reaction are complicated by the appearance of multiple intermediate forms of immunoreactive IL-1β. At the conclusion of the assay, a homogeneous immunoreactive band is present at 18 kD. This species of IL-1β is resistant to further cleavage, even if the reaction is allowed to proceed for several hours. It can be appreciated that several immunoreactive IL-1β species of intermediate mol wt are generated by chymase cleavage, and analysis of the amino acid sequence of 31 kD IL-1β indicates that several putative chymotrypsin cleavage sites exist between the NH_2-terminal of 31 kD IL-1β and the NH_2-terminal Ala 117 of the mature molecule (19).

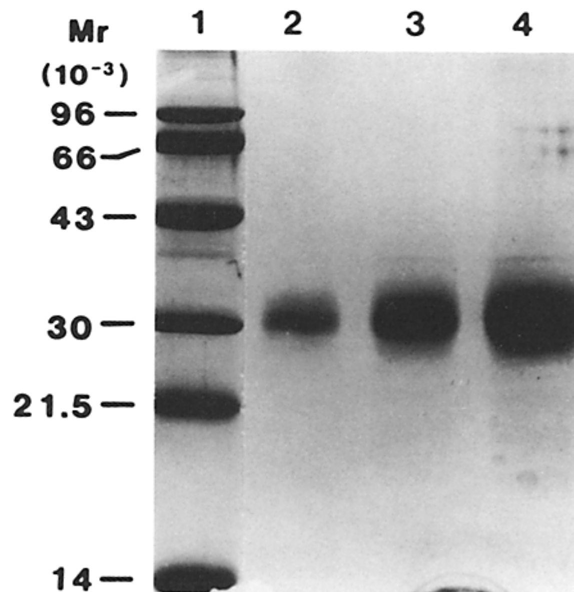

Figure 1. SDS-PAGE analysis of purified human chymase. Lanes *2*, *3*, and *4* contain 1.5, 2.5, and 5.0 μg chymase respectively. One major band is resolved migrating at an apparent Mr of 30,000. In analyses of other preparations, it was shown that the protein in this band represents material that can be labeled with radioactive DFP, an inhibitor of serine proteinases. The broadness of the band is due to glycosylation.

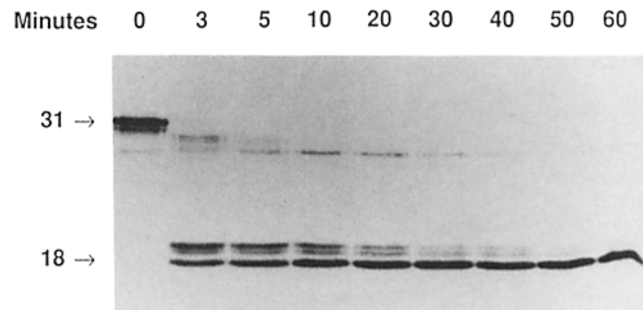

Figure 2. Western blot analysis of cleavage products of 31 kD IL-1β generated by mast cell chymase. Lane *1*, 31 kD recombinant IL-1β; lanes *2–8*, 31 kD IL-1β incubated with mast cell chymase for the indicated number of minutes. 31 kD IL-1β (10 μg) and mast cell chymase (100 pg) were incubated at 37°C for the indicated times. The reaction was stopped by boiling in sample buffer. Western blotting was performed as described in detail in reference 4. Samples were electrophoresed on a 13% SDS-PAGE gel and transferred to nitrocellulose using a transblot cell. M3 mAb to IL-1β was used for blotting.

	110					115					120
Pro-IL-1β	Asn	Glu	Ala	Tyr	Val	His	Asp	*Ala	Pro	Val	Arg
18 kD Protein	---	---	---	---	†Val	His	Asp	Ala	Pro	Val	Arg
					125					130	
Pro-IL-1β	Ser	Leu	Asn	Cys	Thr	Leu	Arg	Asp	Ser	Gln	Gln
18 kD Protein	Ser	Leu	Asn	(---)	Thr	Leu	Arg	Asp	Ser	Gln	Gln
				135					140		
Pro-IL-1β	Lys	Ser	Leu	Val	Met	Ser	Gly	Pro	Tyr	Glu	Leu
18 kD Protein	Lys	Ser	Leu	Val	(---)	(---)	(---)	(---)	(---)	(---)	(---)

* NH_2 terminal of "mature" IL-1β.
† NH_2 terminal of 18 kD protein.

Figure 3. NH_2 terminal sequence analysis of the final cleavage product (18-kD protein) of 31 kD IL-1β mast cell chymase. The sequence obtained is on the line below the amino acid sequence deduced from the cDNA sequence of full length 31 kD IL-1β. Identity is observed through 20 cycles.

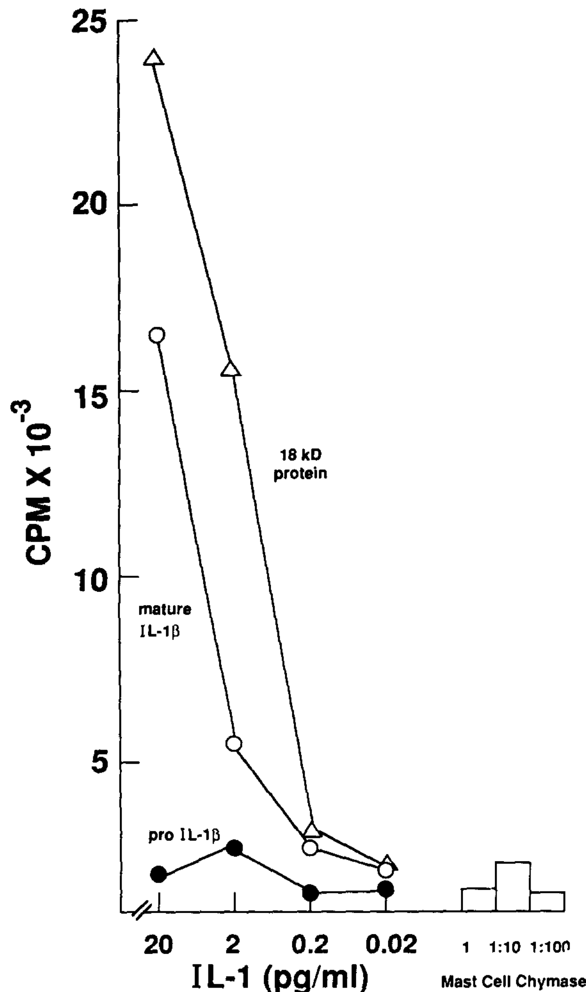

Figure 4. Chymase cleavage of 31 kD IL-1β generates a species of IL-1β with activity comparable to 17 kD recombinant IL-1β. Samples were mixed at various dilutions with 2 × 10^4 D10.G4.1 cells (American Type Culture Collection, Rockville, MD) and 3D3 antibody in triplicate cultures as described previously (5). Incorporation of ^3H thymidine into cellular DNA was assessed at 72 h by scintillation spectrometry.

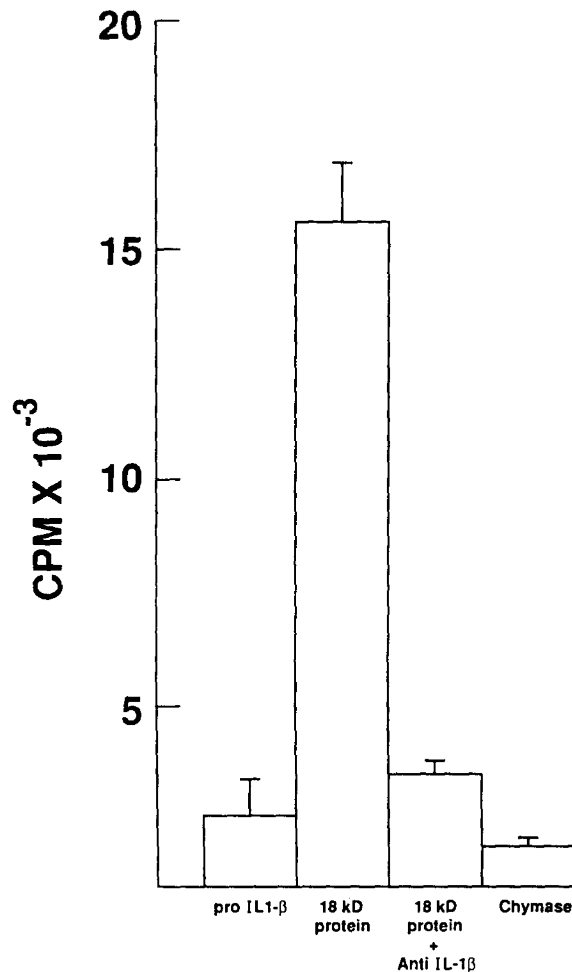

Figure 5. IL-1 activity of the 18-kD protein can be neutralized using antibodies to rIL-1β. A neutralizing polyclonal rabbit antiserum to IL-1β was used at a 1:200 dilution. While 2 pg/ml of pro–IL-1β had no activity in this assay, the same amount of chymase digested pro–IL-1 had significant activity. All activity was blocked upon the inclusion of anti–IL-1β.

The putative chymotryptic cleavage site nearest to the N-terminal aspect of mature 17 kD IL-1β appears to be Tyr 113-Val 114. Since conversion of 31 kD IL-1β to the 18 kD species was complete by 60 min, peptide sequencing was performed on this product. Fig. 3 indicates that a single predominant sequence was obtained consistent with an 18-kD fragment of IL-1β whose NH_2-terminus matched Val 114 of the full length precursor. Identity between the 18 kD fragment and the sequence of 31 kD IL-1β distal to Tyr 113 was found through the first 20 cycles. No other sequences consistent with IL-1β fragments of different mol wt were identified. Therefore, it appears that Tyr 113-Val 114 is indeed the most distal site susceptible to chymase cleavage.

We next asked whether this 18-kD fragment of IL-1β had significant IL-1 biological activity, using the IL-4–producing D10.G4.1 T helper cell clone as our assay (5, 16). Results of a representative experiment are shown in Fig. 4. Mast cell

chymase had no IL-1–like activity in the D10 assay. Precursor 31 kD IL-1β had no demonstrable activity in the assay at concentrations of between .02 and 20 pg/ml, as has been demonstrated by us and others previously (5, 14). However, significant biological activity could be demonstrated with concentrations of the 18-kD chymase generated IL-1β fragment as low as 2 pg/ml; this activity was comparable to the activity of authentic 17 kD mature IL-1β, in the same assay. Therefore, we conclude that the 18-kD protein (N-terminal Val 114) generated by the cleavage of 31 kD IL-1β by mast cell chymase is no less biologically active than authentic recombinant 17 kD IL-1β (NH_2-terminal Ala 117). Finally, we asked whether the IL-1 activity of the 18-kD protein could be neutralized by a polyclonal antibody to IL-1β. Fig. 5 shows that the 18-kD protein (but not 31 kD IL-1β or mast cell chymase) has significant activity in the D10 assay. This activity was neutralized completely by blocking antibodies to IL-1β.

Discussion

These data indicate clearly that human dermal mast cell chymase can generate a stable product from inactive 31 kD IL-1β that has biological activity comparable to that of recombinant 17 kD IL-1β. The potential importance of this interaction relates to the fact that the monocyte convertase enzyme cannot be found in cells resident to skin, such as fibroblasts and keratinocytes (5, 6). Both of these important cellular constituents of skin have been shown to produce mRNA and 31-kD protein identical to IL-1β and without IL-1 biological activity. Thus, proteolytic enzymes released by cells into the extracellular space might be expected to cleave 31 kD IL-1β released from such cells.

Activity of cleavage products of IL-1β diminishes rapidly as the cleavage site moves towards the NH_2 terminus of the precursor; chymotrypsin cleavage generates a molecule three amino acids longer than mature IL-1β with comparable biological activity. In contrast, elastase and trypsin cleavage generate molecules 13 and 41 amino acids longer than mature IL-1β, respectively (14); however, both molecules have less than 2% of the biological activity of mature IL-1β. We have found cathepsin G, a neutrophil protease with chymotryptic specificity, inefficient at cleaving 31-kD IL-1β into a biologically active species (our unpublished data). Thus, it would appear that the number of biologically relevant proteases capable of converting 31 kD IL-1β into an active species is limited.

While some 31 kD IL-1β can be released by uninjured cells, the majority remains in a cell associated compartment. Under what conditions might mast cell chymase encounter 31 kD IL-1β? Injury (mechanical, thermal, radiant, or inflammatory) to skin can damage or even disrupt cells, thus releasing intracellular 31 kD IL-1β. Injury is a well known stimulus to mast cell degranulation, and it would be predicted that such a combination of events would lead to generation of active IL-1β via chymase induced cleavage of 31 kD IL-1β. In turn, this would lead to a series of events including induction of endothelial adhesion molecules (thus trapping leukocytes), and the simultaneous production of IL-1 inducible cytokines with chemotactic and leukocyte activating properties by cells resident to skin (reviewed in reference 21).

The chymase generated 18 kD IL-1β may be a contributing cytokine in the observed expression of endothelial adhesion molecules after mast cell degranulation (10). ELAM-1 was recently identified as a relatively skin specific endothelial cell receptor for a ligand on memory T cells (22), providing an important mechanism for recruiting such cells to sites of cutaneous injury and antigenic challenge. Another role for mast chymase may be to cleave preformed or induced 31 kD IL-1β produced by mast cells themselves. While attractive, this latter possibility remains speculative, and human dermal mast cells are currently being analyzed for 31 kD IL-1β production. In summary, we and others have recently demonstrated the production of 31 kD IL-1β by cells that are apparently unable to process this molecule into a biologically active form (5). Identification of proteases, such as mast cell chymase, that can generate active IL-1β from the inactive precursor may help define how adjacent cells and tissues can function as a whole to induce a complex biological phenomenon such as inflammation.

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