

## TRYPTASE POLYCLONAL ANTIBODY AND PURIFICATION METHOD FOR USE IN HUMAN TRYPTASE IMMUNOASSAY

### FIELD OF THE INVENTION

The present invention relates to highly specific tryptase polyclonal antibodies, and a method to purify the antibodies. More specifically, the present invention relates to polyclonal antibodies which have the capacity to capture tryptase out of solution, a process to generate the antibodies, and enzyme-linked immunosorbent assays (ELISA) for human tryptase which utilize the antibodies.

### BIBLIOGRAPHY

Complete bibliographic citations to the non-patent references cited herein can be found in the Bibliography section, immediately preceding the claims.

### DESCRIPTION OF THE PRIOR ART

#### Mast Cells and Tryptase Release

In order to comprehend the unique sensitivity and specificity of the present immunoassay, some background information regarding human mast cells is in order. Mast cells are granulated, immunologically activatable cells which are positioned in various tissues throughout the body. Mast cells are found in large numbers in tissues at potential entry points of noxious substances. For instance, mast cells are abundant in the skin, the conjunctiva, the gut, and the lungs. Mast cells also are present, in more modest numbers, in the liver, kidney, and adrenals.

In humans, mast cells have a mean diameter of approximately 10  $\mu\text{m}$ . The most striking morphologic feature of mast cells are granules which constitute approximately fifty percent of the total cell volume. Mature mast cells also have nuclei which lack deeply divided lobes.

Several different nomenclatures are currently in use for describing particular types of mast cells. However, none of the nomenclature systems is optimal because they do not necessarily relate to putative functional differences between the cells. For instance, rodent mast cells are often referred to as either mucosal or connective, depending on their origin. These terms, however, imply that mast cell differences are determined by where the cell is found, which is inaccurate. Identical mast cells in rodents may be found in both mucosal and connective tissue sites.

In humans, perhaps the most useful nomenclature relates to the presence of chymase proteases within the granules of the mast cells. All granules of human mast cells contain histamine, tryptase, and heparin. A sub-population of human mast cells also contain chymase, carboxypeptidase, and a cathepsin G-like protease. Those mast cells which contain chymase are designated  $\text{MC}_{TC}$ , while those mast cells which do not contain chymase are designated  $\text{MC}_T$ . As used herein, the term "mast cell" or "MC," without further designation, shall refer to any and all types of mast cells, without limitation. Further clarification of these terms will be used where necessary.

$\text{MC}_T$  cells are the most predominant, but not the exclusive, type of mast cell found in the lungs and in the small intestine mucosa, while  $\text{MC}_{TC}$  cells predominant in the skin and intestinal submucosa. The relative abundance of the two types of MC's may, however, change with tissue inflammation or fibrosis.

Upon immunologic activation, MC's degranulate and release pre-formed mediators including histamine, proteoglycans such as heparin, and proteinases including tryptase. The degranulation process begins essentially immediately upon activation, and ends within minutes.

The release of histamine from MC's is the major initiating event of the allergic response. Within minutes of its release, cellular histamine is rapidly metabolized to either methylhistamine, methylimidazole acetic acid, or imidazole acetic acid. The extremely rapid metabolism of histamine makes it quite difficult to directly quantify histamine levels either in vivo or in vitro.

Simultaneously with the release of histamine, activation and degranulation of mast cells also results in the release of tryptase. Tryptase appears to account for about twenty percent of the entire protein content in a typical MC. Tryptase is released from MC's as a macromolecular protease-proteoglycan complex, in conjunction with heparin. Tryptase diffuses relatively slowly through tissues compared to histamine, presumably due to its association with the macromolecular proteoglycan complex.

A very important characteristic of tryptase is that it is virtually absent from any other type of cell. While negligible amounts have been measured in human basophils (0.04 pg/cell), Nilsson and Schwartz (1994), other cell types found in normal lung, skin, and bowel, as well as eosinophils, neutrophils, monocytes, and lymphocytes from peripheral blood all lack any detectable level of tryptase. Therefore, tryptase is an extremely discriminating marker of human mast cell degranulation. Sensitive quantitation of in vivo tryptase levels therefore may serve as an indicator of the extent of MC degranulation.

#### Tryptase

Tryptase itself is a tetrameric endoprotease of approximately 134 kDa. Each of the four subunits is approximately 31 to 34 kDa in size. Tryptase is a neutral, serine protease found exclusively in mast cell granules, as noted above. For a complete discussion regarding mast cell heterogeneity, structure, and mediators, see Nilsson and Schwartz (1994).

As noted in Schwartz (1995), human tryptase was first purified to apparent homogeneity from dispersed and enriched lung mast cells in 1981. However, further research has shown that there are at least two different types, or groups, of human tryptase. These tryptase isoforms are designated herein as  $\alpha$ -tryptase and  $\beta$ -tryptase.

Little and Johnson (1995) describe the isolation and characterization of two forms of human lung mast cell tryptase, which they designate high-HLT (high-molecular-mass human lung tryptase, 33 kDa) and low-HLT (low-molecular-mass human lung tryptase, 29 kDa). These two tryptase isoforms were found to be immunologically cross reactive. It also was found that these two isoforms of human lung tryptase cleave the natural substrates HMMK and VIP in a different manner and at different rates.

#### Disease States Associated with Mast Cell Degranulation

In addition to a wide range of allergic responses, including allergic rhinitis (Rasp and Hochstrasser, 1991) and suspected food allergies (Beyer et al., 1994; Ohtsuka et al., 1993), elevated tryptase levels are associated with a diverse range of human disease states and conditions, including interstitial cystitis (Sant and Theoharides, 1994; Niles et al., 1995), acute atopic dermatitis (Amon et al., 1994), inflammatory arthritis (Lavery and Lisse, 1994), temperature-induced nasal congestion (Johnston et al., 1993), sudden infant death syndrome (Platt et al., 1994), HIV type I infection (Hattori et al., 1989) and anaphylactoid shock reactions (Watkins and Wild, 1994). It must be noted that