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Abstracts

Eosinophil Proteins*

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Human eosinophil granule major basic protein homolog: biochemical, genetic and immunochemical aspects

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The eosinophil granule major basic protein (MBP) is the predominant proteinaceous molecule of the eosinophil granule and comprises the granule core. Human MBP is a 13·8 kDa protein with a calculated isoelectric point of 11·4. The cDNA sequence predicts initial translation of a 25·2 kDa pro-form containing a highly acidic proportion. MBP functions as a cytotoxin and a cellular agonist. The MBP pro-form is expressed in extravillous trophoblasts (placental X cells) and circulates in the blood of pregnant women as a complex with pregnancy associated plasma protein-A (PAPP-A).

In a search for novel molecules expressed by eosinophils, umbilical cord blood mononuclear cells (UCMC) were stimulated with IL-5 and a cDNA library prepared. Large scale cDNA sequencing of 8146 clones provided a picture (Transcript Image) of IL-5 stimulated UCMC gene expression. Table 1 shows the percentage abundance of the 23 most prevalent transcripts. Among these, one cDNA sequence specified the existence of a protein homologous to MBP (and referred to here as the MBP homolog or MBPH). The deduced amino acid sequence of prepro-MBPH showed that the molecule is 64% homologous to that of prepro-MBP. In keeping with the cDNA structure of MBP, the homolog possesses an N-terminal signal sequence, a highly acidic pro-portion and a basic MBP portion. Remarkably, the theoretical pI of MBPH (pI 8·7) is over 100-fold less basic than MBP (pI 11·4). Both MBP and MBPH mRNAs are expressed by bone marrow, whereas only MBP mRNA is expressed by placenta; neither molecule is expressed in peripheral blood leukocytes, presumably because of their terminal differentiation. Using a specific monoclonal antibody (J191-12H11), MBPH was identified and subsequently isolated from lysates of eosinophil granules. Analysis of disulfide and sulfhydryl groups showed five reactive cysteines in MBP and six in MBPH; both molecules contained two disulfide bonds. Extinction coefficients for MBP and MBPH were exceedingly high compared to other proteins at 3·67 and 3·32 (mg $ml^{-1} cm^{-1}$), respectively. Like MBP, MBPH is cytostimulatory causing histamine release from peripheral blood basophils, IL-8 release from neutrophils, and superoxide anion production by neutrophils. However, MBPH is significantly less potent than MBP in its ability to stimulate LTC_4 release from basophils.

The amino acid sequences of MBP and MBPH along with the sequences of two forms of guinea pig MBP, mouse MBP and rat MBP are shown in Fig. 1. The sequence similarities among these molecules suggest a gene duplication event occurring early during mammalian evolution because guinea pigs have two pro-MBP genes. However, both forms of guinea pig MBP have a characteristically high pI in contrast to MBPH. Therefore, MBPH may be evolving toward a new function or drifting away from a previous function.

A panel of monoclonal antibodies to MBPH has been raised and their cross-reactivities with MBP and MBPH tested. These results indicate that most monoclonal

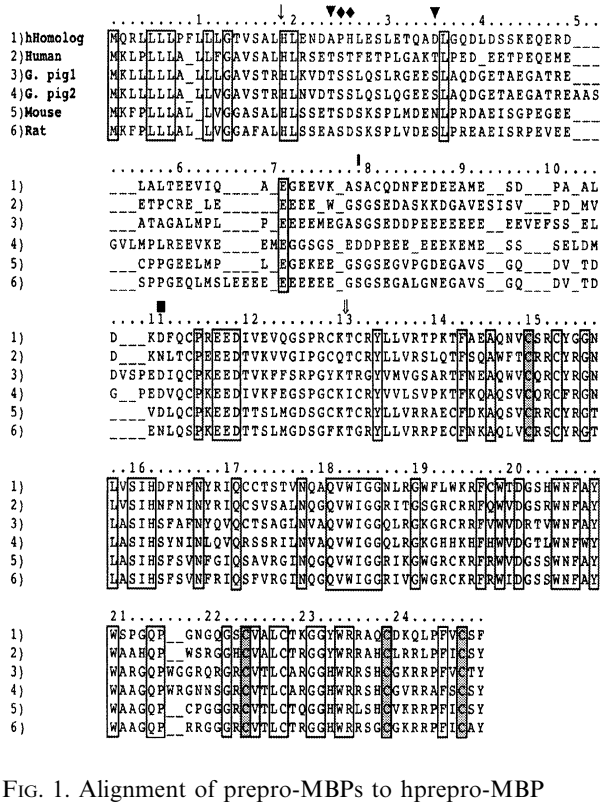

FIG. 1. Alignment of prepro-MBPs to hprepro-MBP homolog. Identical amino acids among the prepro-MBPs are enclosed in boxes. Half-cystines as determined for hMBP are shaded with gray. Partially substituted O-linked (▼), fully substituted O-linked (◆), N-linked (■) and glycosaminoglycan (■) glycosylation sites are shown. The start of pro-MBP (↓) and MBP (⇓) are also marked. Used with permission from ref. 2.

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TABLE 1. Twenty three most abundant transcripts of IL-5-differentiated UCC

Description of GenBankTM sequence*	percentage abundance†
Eosinophil major basic protein mRNA	8·12
Eosinophil Charcot-Leyden crystal protein mRNA	2·98
UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferase mRNA	1·31
Eosinophil-derived neurotoxin mRNA	1·26
MBP homolog mRNA	1·14
Secretory granule proteoglycan core gene	1·09
Elongation factor EF-1-gene	1·06
Eosinophil peroxidase gene	0·95
Osteopontin mRNA	0·92
Ferritin light subunit mRNA	0·89
Cathepsin B mRNA	0·83
Transcription factor Sp1 mRNA	0·78
CpG island DNA genomic *Mse*I fragment	0·72
Interferon-inducible mRNA	0·66
HLA-DR antigen-chain mRNA	0·54
Cathepsin D mRNA	0·50
Cytoplasmic-actin gene	0·49
3-Hydroxy-3-methylglutaryl coenzyme A synthase mRNA	0·47
NMB mRNA	0·47
-Interferon-inducible protein mRNA	0·40
Glyceraldehyde-3-phosphate dehydrogenase gene	0·40
Ribosomal protein S3a gene	0·40
Ia-associated invariant-chain gene	0·40

*All sequences listed are of human origin.
†Percentage abundance among 8146 total clonses sequenced.
Used with permission from ref.2.

antibodies do not distinguish MBP from MBPH. Selection of specific monoclonal antibodies to MBPH has permitted establishment of a specific immunoassay for MBPH. In this assay it is critical that MBPH be reduced and alkylated in order to detect maximal reactivity. This behavior of MBPH parallels that of MBP. Immunofluorescence examination of MBPH suggests that it is present in eosinophils and basophils. By immunoelectron microscopy MBPH has been localized to the eosinophil granule. Finally, genomic clones for MBPH have been identified and sequenced and chromosomal localization is underway.

References

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The role of eosinophils and their ribonucleases in respiratory viral infections

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The eosinophil ribonucleases, eosinophil-derived neurotoxin (EDN) and eosinophil cationic protein (ECP) are among the major secretory effector proteins of human eosinophilic leukocytes, cells whose roles in host defense remain poorly understood. Eosinophils have been implicated in the pathophysiological process of wheezing in patients with respiratory viral infections leading us to question the role of eosinophil-derived ribonucleases in viral infections. Our recent studies show that eosinophils are recruited to, and degranulate in, the lung parenchyma of infants infected with respiratory syncytial virus (RSV), a single stranded RNA pathogen in the paramyxovirus family (1). These