APPENDIX C

PCR EVALUATION OF TRYPSINOGEN EXPRESSION

The polymerase chain reaction is a highly sensitive method for detecting the presence of a specific nucleic acid sequence. It can be used on RNA derived from a tissue to ascertain the presence of a sequence of interest, such as a trypsinogen mRNA.

PCR suffers from several drawbacks. It cannot accurately assay the quantity of a particular sequence. Its high sensitivity makes it vulnerable to false positives from contaminated samples. During the amplification process, highly identical sequences can recombine, producing artifacts. The error rate of PCR can be high, making it difficult to accurately assign the sequence of an amplified product to a particular allele. Therefore, PCR results must be interpreted with caution.

For this study, I have examined several tissues from the human and mouse to determine which trypsinogens were present.

C.1 METHODS AND RESULTS

Tissues were obtained as described in Sections C.2 and C.3. Total RNA was isolated with the guanidine thiocyanate protocol (Promega). Whole frozen tissues were ground under liquid nitrogen before RNA isolation. Frozen ground powder was added to denaturation solution and homogenized with a dounce. For peripheral blood cells, isolated cells were added directly to denaturation solution.

Purified total RNA samples were employed as templates for RT-PCR. First strand reverse synthesis was accomplished with a poly-T primer. The PCR primers used in this study are tabulated in Table 3.3. Nested PCR was required to obtain products from all tissues studied, except the pancreas. Initial amplification was performed with primers TRYA and TRYD, and subsequent amplification with either TRYB and TRYC or TRYF and TRYR. Each of these primers has a *Bam*HI 5' leader. None of the predicted trypsinogen PCR products has an internal *Bam*HI site. Twenty to thirty cycles were employed for each round of amplification, annealing at 55° for 30 seconds and extending at 72° for 50 to 80 seconds. PCR products were isolated, cleaved with *Bam*HI, and cloned into the *Bam*HI site of the m13mp9 vector. Individual clones were isolated and sequenced.

Table C.1. Number of cDNAs sequenced by PCR from each of several human tissues.

	T8	T4	T9	T6	Total
Pancreas	6	8	5	Ø	19
Cadaver Pancreas	Ø	15	2	Ø	17
Thymus	Ø	4	11	17	32
Fetal Thymus	1	10	8	Ø	19
Liver	Ø	Ø	11	Ø	11
Cadaver Liver	Ø	Ø	32	Ø	32
Fetal Liver	Ø	1	17	Ø	18
Fetal Spleen	Ø	Ø	2	Ø	2
Spleen	Ø	Ø	4	Ø	4
PBMC	Ø	2	3	Ø	5

Human tissues were obtained from four sources. Pancreas and liver samples were obtained from a 45-year-old male who died of arrhythmia 18 hours before the tissue was quick frozen in an ethanol and dry-ice slurry. Fetal liver, spleen, and thymus samples were obtained form an 84-day embryo. Tissue was immediately quick frozen. Peripheral blood mononuclear cells were isolated from a 24-year-old volunteer and purified on a Ficoll gradient. In addition to these samples, pancreas-, thymus-, liver-, and spleen-specific cDNA samples were obtained commercially (Clontech). The results are summarized in Table C.1.

Mouse thymus, liver, and spleen tissues were obtained from a freshly killed Balb/C mouse. Tissues were immediately quick frozen. The results are summarized in Table C.2.

C.2 DISCUSSION

All human trypsinogens with hypothetically-functional genomic sequences are expressed, as seen by PCR. This observation is consistent with the presence of all of them in the EST database (Table 3.7). The pancreas shows the broadest range of expression of the various isozymes. Human trypsinogen T9 is found by PCR in all tissues examined. However, human T9 also appeared inconsistently in negative control PCR reactions, indicating the possibility of contamination accounting for this observation. Human T6 was only observed in the thymus by PCR, but has been found in pancreatic cDNA libraries (Table 3.7). This raises the possibility that human T6 is somewhat thymus-specific. These data suggest that there is differential expression of trypsinogen isozymes, although little can be said with respect to

Table C.2. Number of cDNAs sequenced by PCR from each of several mouse tissues.

	T8	**T9**	**T10**	**T11**	**Total**
Thymus	10	11	Ø	Ø	21
Liver	14	13	Ø	Ø	27
Spleen	4	5	8	3	20

relaitve abundance in various tissues.

An inconsistent competitive advantage of human T6 during PCR could also account for this observation. Alternatively, the tissue sample employed for PCR may have come from an individual homozygous for a deletion of human T6.

Assuming that the PCR data from the mouse spleen represents pancreatic contamination, the mouse pancreas also demonstrates the broadest range of trypsinogen isozyme expression (Table C.2). Not all hypothetically-functional genomic sequences from the mouse are observed by PCR – only mouse T8, T9, T10, and T11. ESTs for mouse T7, T8, and T9 are present in the EST database (Table 3.8). Additionally, the sequence of the cloned mRNA for mouse T20 is present in Genbank. There is currently no concrete evidence for the expression of the hypothetically-functional sequences mouse T4, T5, T12, T15, and T16. It may be that they are expressed at very low levels, or not at all. Alternatively, any of the possible systematic errors of PCR or EST sequencing, discussed above, may account for the failure to detect their mRNAs.

ESTs for both the human and mouse “trypsinogen” T1 are present in the database, as discussed in Section 3.21.