Cloning of the Pig Counterpart of a Gene Involved in Resistance to Bacterial Infection

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Summary and Implications
The pig gene corresponding to a mouse protein known to cause susceptibility to infection by several different bacteria (NRAMP1) was cloned and the entire protein coding region sequenced. The pig protein encoded within this gene is highly similar to the mouse and human NRAMP1. A preliminary expression profile of pig NRAMP1 indicates it is expressed in spleen, a rich source of immune cells, and may be expressed in other tissues at low levels. Taken together, these data strongly indicate that the newly cloned gene has a similar physiological function in pigs to that seen for mouse NRAMP1. With this new information, the association of NRAMP1 to Salmonella infection in pigs can be tested.

Introduction
Salmonellosis is a very costly disease to pork producers; in Iowa, salmonellosis is second only to swine dysentery in losses. One possibility for low-cost control of Salmonellosis is the identification and multiplication of naturally resistant animals. Such animals may be in the pig population currently produced in the United States, or resistant animals may exist in the exotic Chinese breeds already known for their Escherichia coli resistance. To develop markers to select for general bacterial disease in pigs, a gene related to resistance to Salmonella and Listeria in rodents were proposed to be cloned, sequenced, and to have polymorphism analysis performed. This gene, NRAMP1, which stands for Natural Resistance Associated Macrophage Protein, was first isolated in mouse as a strong candidate for the Bcg/Ity/Lsh locus which conferred genetic resistance/susceptibility of inbred mouse strains to infection by Mycobacteria, Salmonella, and Leishmania (Vidal et al., 1993). More recently, transgenic mice homozygous for engineered mutations in NRAMP1 show very similar phenotypic effects on natural resistance to intracellular pathogens, proving the critical role of NRAMP1 protein in pre-immune macrophage function (Vidal et al., 1995).

Materials and Methods
PCR reactions used to clone pig NRAMP1: Using human, mouse and sheep sequences, primers were designed to amplify a short section of the NRAMP1 gene. These primers were used in a PCR reaction using as template an aliquot of a CLONTECH spleen library phage (Isola et al., 1991). A specific band was observed, cloned, and sequenced. This PCR product was highly similar to human NRAMP1, indicating the primers were specific. These primers then were used to screen the spleen cDNA library using serial dilution of phage particles. Two individual cDNA clones were identified and sequenced. Expression analysis: The above primers were also used to amplify NRAMP1 mRNA sequences from spleen, brain, hypothalamus, uterus, testis, skeletal muscle, and heart pig mRNA converted to cDNA using reverse transcriptase. PCR reactions were run on an agarose gel to determine relative amounts of NRAMP1 sequences amplified.

Results and Discussion
We have successfully cloned and sequenced a full-length cDNA for NRAMP1 (there now being two different NRAMP genes reported in both mouse and humans). Figure 1 reports a summary of the sequencing of pig NRAMP1. Analysis of the new NRAMP1 sequence identified a 538 amino acid protein. The derived pig protein sequence had much higher identity to NRAMP1 from humans, cattle, and mice (87%, 88%, and 85%, respectively) than to NRAMP2 from humans or mice (64% and 62%, respectively). Therefore, the new sequence was designated pig NRAMP1. In the screening process we used to isolate the NRAMP1 gene, we also have seen evidence for the existence of pig NRAMP2 gene, and will be investigating this observation in the future. The specific glycine residue at position 169 which is mutated to D169 in susceptible mouse strains is conserved in pig NRAMP1 as it is in humans, cattle, and sheep. The proposed glycosylation sites at S323 and T337 are conserved, although the proposed protein kinase C phosphorylation site at S529HK is not (Vidal et al., 1993).

Preliminary analysis of the expression of NRAMP1 in pig tissues was also performed. Messenger RNA from a number of pig tissues was isolated and used in RT-PCR reaction with the NRAMP1 specific primers. Strong amplification was seen only in spleen, indicating expression of NRAMP1. A low but detectable level of expression was observed in muscle, uterus, and heart, and no expression was observed in brain, hypothalamus and testis. This pattern is consistent with that observed for mouse NRAMP1 (Vidal et al., 1993).

In summary, we have cloned and performed preliminary analysis of the expression pattern of a new pig cDNA, that for NRAMP1. As the protein encoded by this gene controls susceptibility to bacterial infections in several strains of mice, pig NRAMP1 is of interest in the search for genetic resistance to Salmonella in pigs. As this structurally similar gene appears to also be expressed in a manner similar to the mouse gene, it is likely to perform a similar function in pigs.

Acknowledgments
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References


Figure 1. Nucleotide sequence of pig Natural Resistance Associated Macrophage Protein (NRAMP1). Top line is the DNA sequence, while the next line is the amino acid translation of the open reading frame starting at nucleotide 65.
GAATTCCGCAGAGAGCCACCCAGCACACCACTCACACAGAGTAGCATCTGAGTCTGTGGT
CCTCATGACAGGTGACACAGACCCCCCAAAGCAGAGCAGAACCCAATATGGCTCCATCTC
M  T  G  D  T  D  P  P  K  Q  S  R  T  Q  Y  G  S  I  S
CAGCTCACCGGCCAGAGGGCCACAGGCAGAATCCTCAGCTGACCCCGGGGAGAAGAGTAGT
G P G F L M S I A Y L D P G N I Q S D L
TCAGGTCGTCGGTGTGCTGGTGTGCCATCTGGTGTGGCTGGTGTGGCCAGCGGTGT
Q  Q A G A V A G F K L L N V L L W A T V L
GGGTTTCGTCGCGGACGACCTTCTGCGCCCCCGTGGGCGTGTTGACAGCGGACTCGG
G L L C Q R L A A R L G V V T G K D L G
TGAGATCTGCACATCTCTACTCATACGTTAAGTCTCCTCCCCGCACCCTCCTCTGGCTGA
E I C H L Y P K V R T L L W L N M E
GCTAGCATGGCGGTGGTGCACATCGGAAGATCCTGCGACAGGCTATGGCCATCTCATCT
L A I V G S D M Q E V I G T A I A F N L
GCTCTACGTCAGAAAGGGCGCCAGCAGGCTACCTGCTGAGGCTCCTGACTACGGGAC
L S A G R I P L W G G V L I T V V D T F
CTTCTTCCTCCTACTCAAATATACCCAGGCTGGAAGCTGGGACCTTGTCTCGATG
F T G C A T T A T G G C T T C A C T C A C T C A G T A T G A G T G C
I A I M A F T G F G Y E Y V V A R P A Q G
AGACATCTTCTAGGCGGTCTCCTGCCTCCTGGCTGGCAGCCGCGAGCTGCT
A L R G L F L P S C S G C G Q P E L L
CCAGGCGGCTGCGTGGTGGCTGGCCATCATCAGCGCGCGCGCGGAGCTGCTCAGGG
Q A V G I V G A I I M P H N I Y L H S A
CCGTGCAATGCTGAGATGGAGTAAAGCGGACGCGCCGCGGGAGGACATCGCGAGAC
L V K S R E V D R T R R E D I R E A M
GTACTCTCCTGATGTAATCACCACATCAGCCTCCGTGGTTCCTTCCTTCATCAACATCTCG
Y F L I E S T I A L F V S F F I N L F V
CATGCGTCTGAGTGCGCTGAGGAGTAAAGCGGACGCGCCGCGGGAGGACATCGCGAGAC
M A V F G Q A F Y Q Q T N Q A A F N I C
TGCCACAGGACGCCTCACAAGCTCAGGGAGATCGCTCACTCCGCGCGCGCGCGCGCTCA
1140 V D F Y Q Q G V I L G C L F G P A A L Y
CATTTCGGGCTGGTCTCTGCGCTGGGAGAACAAATGCTCCACCATGAGCCCGCATTACCC
1200 I W A V G L L A A G Q S S T H T G T Y A
GGGCAAAATTGTGATGGAGGGCTCCTGAACTGCGGCGTCGCGCTTCCGCCCGCTCCT
1260 G Q F V M E G F L K L R W S R P F A R L L
GCTACCTCCTTCCTGCCAATCTGCGCCGCCCGCCCTGCTTGGGAGCCCTACGGGAC
L T R S C A I L P A L L L V A V F K R L Q
GGACCTGCAAGGCTAAATGATCGCTCAAGGCTGAAAGCCCGTCCGCTGCTTCCCTCC
1380 D L S L N D L L N V L Q S L L L P F A
GGTGCTGCCACATCTCACCCTACCCACACGATCCCGCCGCTCCATCCAGGAGTTGCGCAGG
1440 V L P I L T F T S M P A L M Q E F A S G
CCGGGACGCCAATGCTCATCCTTCCATCCGCTACGCTGCTGTGGCCCTAACACCTTTTA
1500 R V N K V I T S S I M L L V C A I N F Y
CTTCTGGCTGAGCTCACTGCTGCCAGCCCTGCCGCCCTGCACTGCTGCGTGCTACGAGC
1560 F L V S Y L P S L P H P A Y F G L V A L
ACTTGCCGTCATCTCCGCGGCTTACACCCACCATCCTGCGAGGCGTCTATCCGCCGA
1620 L A V I Y L G T T Y L V W T C L I A N
TGAGACATTCTTTGCGTCACAGTGCTCCATCAAACACTTTCTGGACGTTGGGCTTGGGAAT
1680 G A T L L V H S H G Y O G L L E ' 538
GGAGCGGAGAGAGAAAGAAGACCTCAGGGGATGACCTCCCGCCGAGGGGGCTGAGTGGG
1740 AATGGCTGTGGGCGGATGTCGCTGAGGGCTGAGGGGACGGGAGGGCGTGGTG
1800 AAGCAGCAAGTGACGGCCAGGCTGCGGTTGAGTGGCAGGCGATCGCTGTGGG
1860 GCAGTTTCTCAGTTGACAAAGTATATCTCAACCCCTCAGCTGATCTGCTCTATCCAC
1920 ATGGGCAACCAACCCTCTTGAACATGGATGAAAGCAGTAATACAGAGACAGACTGCGC
1980 AGACAAAAA

1995