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Analysis on genetic diversity of *Theileria* parasites and host's immune response to the major piroplasm surface protein

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Benign *Theileria* parasites, which are referred to as *Theileria sergenti/buffeli/orientalis* complex, can be divided into at least four genotypes, I, C, B-1 and B-2, based on the nucleotide sequences of the major piroplasm surface protein (MPSP) genes. The majority of *T. sergenti*-infected calves in Japan harbor mixed parasite populations bearing different alleles of the MPSP genes. This group of parasites widely distributed in the world has not been characterized in detail. To confirm whether mixed population of parasites are commonly present in infected cattle in other countries, *Theileria* parasites isolated from different countries were characterized based on the MPSP genes.

The *Theileria* parasites in China, Taiwan, Korea and Italy contained mixed populations. However, the isolates of Thailand, U.S.A., and China, could not be classified into the known genotypes of *T. sergenti/buffeli/orientalis* complex. From the results of the sequence analysis of the MPSP and 18 S ribosomal RNA (ssrRNA) genes, these parasites were thought to be new parasite species or groups different from *T. sergenti/buffeli/orientalis* complex.

The *Theileria* species have been found in a variety of wild animals. To characterize the theilerial parasites of domestic and wild animals and to study on their genetic diversity, the sequence analysis of the MPSP and ssrRNA genes was carried out for the *Theileria* parasites of wild and domestic animals. It was shown that MPSP genes were highly diverse among *Theileria* species. From the result of phylogenetic

analysis based on the MPSP genes, *Theileria* parasites were divided into three groups. The first group contained the malignant *Theileria* species represented by *T. parva*. The second group was considered to be the benign *Theileria* group including *T. sergenti/buffeli/orientalis* complex. The third group consisted of *T. mutans*, *T. sp.* (Impala) and *T. sp.* (Kudu). In the wild animals, the genetic polymorphism of parasites were also detected on the nucleotide sequences of the ssrRNA genes, showing that the mixed populations of parasites bearing the different genotypes exist in an individual host.

It is known that MPSP is the most abundantly expressed molecule among proteins at the piroplasm stage and that it is the target molecule recognized by the host-immune system. Therefore, the diversity of MPSP can be related to the parasite evasion from host immunity. From this point of view, the cellular immune response against MPSP was characterized in order to confirm whether cellular immunity is specific to the MPSP-type. The cattle were immunized with MPSP (C type)-liposome, and the proliferative responses of lymphocyte to MPSP were detected from all the immunized animals. The responding cells were CD4<sup>+</sup>T cells which were restricted by MHC-class II. RT-PCR analysis revealed that proliferating T cells expressed relatively high levels of IFN  $\gamma$  mRNA, low levels of IL-2, IL-10, and TNF  $\alpha$  m RNAs. The MPSP-type specific T cell responses were observed in two animals. From the results of T cell epitope-mapping, T cell lines derived from

two animals responded to DTSKFTPTVAHRLKHAEDLF<sup>92</sup>, and that from another animal responded to

GTGKWDFVCNFKVTKVKFE<sup>182</sup>. In the regions of MPSP containing these epitopes, diversities were found among four types of the MPSP.

Gastric mucosal hyperplasia and hypergastrinemia in rats and mice heavily infected with *Taenia taeniaeformis* larvae

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Rats heavily infected with *Taenia taeniaeformis* larvae in the liver show a remarkable increase in their stomach weight and intragastric pH, hyperplasia of the gastric mucosa accompanied by mucous cell proliferation, decrease in number of chief cell and parietal cell and hypergastrinemia. These characteristics are similar to those of some human conditions such as Zollinger-Ellison syndrome, Menetrier's disease (MD), hypertrophic hypersecretory gastropathy. *T. taeniaeformis* /rat system can be a candidate model for such human gastrointestinal diseases and others with hypergastrinemia. However, there is not enough detailed report to make comparison with these diseases. Hence, the pathological changes of the gastric mucosae were examined in rats heavily infected with *T. taeniaeformis* larvae using light and electron microscope.

In the infected rats, gastric mucosal hyperplasia began to be observed at 56 days postinfection (DPI), the structural disturbance of zymogenic units in the corpus and mucous units in the antrum had increased with time. In the corpus, after 56 DPI, four types of alcian blue (AB) and/or Periodic acid Schiff (PAS) -positive mucous cells increased in their numbers around hyperplastic gastric glands. At 56 DPI, two types of those mucous cells were observed using electron microscope, one was type I cells, which

were rectangle in shape, whose cytoplasm was occupied by a few large secretory granules and a few small granules, and another was type II cells, which were square in shape, and its cytoplasm contained many secretory granules. At 70 DPI, along with type I and II, type III cells, which was square in shape and contained some secretory granules, were observed. Type IV cells, which were ovoid in shape containing some granules, were observed at 84 DPI. Contrary to these increases, zymogenic and parietal cells decreased in their numbers, which continued to 112 DPI. Apoptotic bodies also started to appear after 56 DPI at the corpus and the antrum and increased in their numbers with the time of the infection.

The infected rats showed increase in the serum concentration of alanine aminotransferase and aspartate aminotransferase, which suggested some damage to the host's liver. Decrease in the concentration of serum total protein was also observed, which was also known to occur in patients with MD. These histopathological and blood biochemical findings strongly suggested that *T. taeniaeformis* /rat system could become a candidate model for human gastrointestinal diseases such as Zollinger-Ellison syndrome, MD, hypertrophic hypersecretory gastropathy.

The gastric mucosal hyperplasia was observed ultrasonographically prior to the onset