Lymphoproliferative Responses in Pigs Infected with *Mycobacterium avium*

Akira IWAKIRI1), Miho TOSHIMASU1), De Long XU1), Toshiharu SHINJO1) and Yoshitaka GOTO1)

1)Department of Veterinary Microbiology, Faculty of Agriculture, Miyazaki University, 11 Gakuen Kibanadai Nishi, Miyazaki 889–2192, Japan

(Received 24 October 2000/Accepted 3 April 2001)

**ABSTRACT.** Naturally infected cases of swine mycobacteriosis were divided into two groups, localized infection (LI) and disseminated infection (DI). Lymphoproliferative response (LPR) was then examined to estimate their immunological states. Both control and LI groups showed strong response to Concanavalin A (Con A) and phytohemagglutinin (PHA) in the LPR, and lymphocytes recovered from the LI responded well to purified protein derived from *M. avium* (PPD). On the other hand, the DI group showed weak response to both Con A and PHA, despite their strong response to PPD stimulation. These data suggest that the low LPR to Con A and PHA observed in the DI groups was probably not due to the general unresponsiveness of T-cells.

**KEY WORDS:** lymphoproliferative response (LPR), *Mycobacterium avium*, swine.

*Mycobacterium avium* is widely distributed in the environment and is the causative agent of atypical mycobacteriosis in humans and animals [6, 8]. In swine, most of the lesions are formed in the mesenteric or mandibular lymph nodes, and the disseminated lesions are occasionally observed in the liver, spleen, and lungs [3, 5]. The organism apparently enters through the tonsils or the lining of the intestine, and then passes into the bloodstream through the portal circulation, subsequently causing miliary lesions in the liver and other organs [11]. Outbreaks of swine mycobacteriosis were detected at the Meat Inspection Centers of Japan. We found 13 cases of systemic infections among 1,588 pigs, which were diagnosed as having atypical mycobacteriosis. There is little knowledge about the immunopathological mechanism by which systemic infection occurs in only a few individuals and what factor(s) regulate the resistance or susceptibility to *M. avium* infection in pigs. It has generally been thought that suppression of cell-mediated immune function converts local tuberculous lesions into miliary tuberculosis, and that a decrease in the T lymphocyte population deteriorates the equilibrium of this disease. It has been reported that in vitro swine lymphocyte immunostimulation assay with purified protein derivative from *M. avium* (PPD) might be useful in the diagnosis of individuals with *M. avium* infection [1]. In the present study we examined the difference in cell-mediated immune responses in pigs with localized and systemic mycobacteriosis infections by evaluating the lymphoproliferative response (LPR) in order to certify the immune state.

All pigs used in this investigation were two- or three-way crossbreeds (LWD) raised on commercial and conventional farms, and which were 6 months of age and about 100 kg in weight. The discovery and examination of *M. avium*-infected individuals was performed at three Meat Inspection Centers in Miyazaki Prefecture. According to the severity of the disease, they were divided into two categories. The individuals in which tuberculous granuloma lesions were widely distributed in the liver and/or spleen were categorized as cases of disseminated infection (DI), whereas the pigs whose lesions were confined to the mesenteric lymph nodes (MLN) were defined as cases of localized infection (LI). Definite diagnosis of the diseases was performed by identifying the bacteria isolated from these lesions. Out of 26 naturally infected cases of swine mycobacteriosis, 38 strains of *M. avium* were isolated from MLN, livers and spleens. Ultimately, 11 cases of DI, 15 cases of LI and 8 non-infected healthy pigs were used in this study. Lymphoproliferation assay was performed as follows. Spleens and MLN were cut into small pieces and passed through a #200 stainless steel mesh (aperture size: 77 µm) for the preparation of single-cell suspensions. After washing, the number of cells was adjusted to a final concentration of 5.0 × 10^6 per ml in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). There was no significant difference among the three groups in the proportion of lymphocytes included in these cell preparations. Concanavalin A (Con A) and Phytohemagglutinin (PHA) were purchased from Sigma Chemical Company, St. Louis, Mo and used as non-specific T cell mitogens. PPD-avium (PPD) was purified from culture supernatant of *M. avium* (Tasaka strain) and used as antigen-specific mitogen. Triplicate cultures of 100 µl of cell suspensions were stimulated with 100 µl of mitogens (5 µg/ml of Con A, 10 µg/ml of PHA, 10 µg/ml of PPD) or medium alone. The mixtures were then incubated for 2 days at 37°C in a 5% CO₂-humidified atmosphere. They were then pulsed with 185 kBq of 3H-thymidine (DuPont, Wilmington, Boston, MA) for 16 hr before cell harvest. The retained radioactivity was then measured with a B-scintillation counter (MicroBeta Pharmacia Biotech, Upsala, Sweden). The results were in counts per minute (cpm), and expressed as a stimulation index (SI)=mean cpm of mitogen-stimulated culture/mean cpm of unstimulated lymphocyte culture. Statistical analysis of experimental groups was performed with the paired Student’s t-test.

Results are summarized in Fig. 1. Both spleen and MLN lymphocytes obtained from healthy control pigs responded
very well to Con A or PHA stimulation. The mean of SI in the LI group was almost the same as that of the normal group when they were stimulated with Con A and PHA, but in the DI group the means of SI were significantly lower than those of other groups (Con A; P<0.01, PHA; P<0.05). On the other hand, the lymphocytes recovered from LI and DI groups responded strongly to PPD stimulation and their SI means were significantly higher than that of the healthy control group (P<0.01). Especially the SI mean in the DI group was significantly higher (P<0.05) than that in the LI group. The majority of cases in the DI group showed only weak response to both Con A and PHA stimulation despite their strong response to PPD.

In this study, we compared the cell-mediated immune responses of LI and DI groups of swine naturally infected with *M. avium*. The LPR to nonspecific mitogens, Con A and PHA, in the DI group, turned out to be lower than those in the LI group. These findings showed for the first time that the cell-mediated immune response deteriorated in pigs with systematic *M. avium* infection, but the DI group showed strong response to PPD, which indicates that an induction of *M. avium*-specific T cell clones might occur in the DI group as well as in the LI group. This seems to be inconsistent with the above findings. Therefore, comparison of antigen-specific T cell subsets and their cytokine profiles in the DI and LI groups associated with the protective immunity should be necessary in order to understand the immunoregulatory mechanisms in swine mycobacteriosis. And immunological studies on the factors on the reduction of Con A-stimulated T cell responses in the DI group should be done.

It has been documented that chronic *M. avium* infection is often associated with the immunodeficiency of the host. In a mouse model, systemic *M. avium* infection caused damage to various organs in genetically susceptible mice due to an explosive increase in bacteria and numerous granuloma formations [4]. In human cases, functional defects in T cells and deficiency of interferon-γ (IFN-γ) were associated with impaired protective immunity to *M. avium* [2, 9]. In this study, the defects in T cell activation in the DI group might be the cause of disseminated mycobacteriosis. Alternatively systemic *M. avium* infection might cause functional defects

![Fig. 1. Lymphocyte blast transformation of spleen cells (A) and mesenteric lymph node cells (B) with mitogen (Con A, PHA and PPD). 1: normal swine, 2: localized mycobacteriosis swine, 3: disseminated mycobacteriosis swine.](image)
in the T cell population in pigs except for PPD-specific reactive T cells. It is known that a large amount of *M. avium* heat-killed cells or their lipid components impairs the capacity of human peripheral blood mononuclear cells or murine spleen lymphocytes to proliferate *in vitro* in response to both Con A and PPD [7, 10].

We are now investigating the role of antigen-reactive T-cell subsets and their IFN-γ producing ability in post-*M. avium* infection. In addition, T cell responsiveness in pigs experimentally infected with a large dose of *M. avium* organisms will remain a research subject.

ACKNOWLEDGMENTS. The authors acknowledge the advice of Associate Professor Hiroyuki Iwata of Yamaguchi University. This study was supported by a Grant-in-Aid for Scientific Research (11660298) from the Ministry of Education, Science Sports and Culture, Japan.

REFERENCES