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EFFECTS OF AMBIENT TEMPERATURES ON CLINICAL AND IMMUNE RESPONSES OF PIGS INFECTED WITH TRANSMISSIBLE GASTROENTERITIS VIRUS

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(Accepted 25 April 1979)

ABSTRACT

Shimizu, M. and Shimizu, Y., 1979. Effects of ambient temperatures on clinical and immune responses of pigs infected with transmissible gastroenteritis virus. Vet. Microbiol., 4: 109--116.

Two- to three-months-old pigs infected with transmissible gastroenteritis (TGE) virus showed no clinical response when housed at 30°C, but comparable infected pigs exposed to temperature changes from 30°C to 4°C following infection showed typical signs of TGE. Development of TGE-specific immune responses, as measured by blastogenic response of tissue lymphocytes, occurred at 3 days post-inoculation (DPI) in pigs held at 30°C, but not until 7 DPI in infected pigs held under the adverse conditions.

Immunosuppression with corticosteroids resulted in a fall in circulatory T cells, lowering of non-specific blastogenic response of circulatory lymphocytes, and clinical signs of disease when immunosuppressed pigs were infected with TGE virus and held at 30°C. It is suggested that clinical responses to TGE virus infection may be affected by the influence of ambient temperatures on the immune responses of pigs.

INTRODUCTION

Transmissible gastroenteritis (TGE) of pigs is a highly contagious, enteric, viral disease, affecting pigs of all ages but causing severe death losses only in piglets less than 2 weeks old. The disease, characterized by severe diarrhoea and vomition in piglets, is caused by a coronavirus (Tajima, 1970).

One of the interesting epizootiological features of TGE is its seasonal appearance. It is recognized that the majority of herd outbreaks of TGE occur during the colder months of the year (Ferris, 1973). In a previous report (Shimizu et al., 1978), the effects of ambient temperatures on infection of TGE in feeder pigs 2 to 3 months old were examined. It was shown that a high ambient temperature resulted in increased resistance of pigs to clinical disease, whilst a low ambient temperature, and temperature changes, caused a dramatic enhancement in clinical sequelae. These results might explain in part the seasonal occurrence of TGE outbreaks. However, the mechanism of
adverse effects of low temperature and temperature changes in induction of clinical disease in feeder pigs remains obscure.

The purpose of the present paper is to investigate the mechanism by which adverse ambient temperature enhances the severity of clinical disease following infection with TGE virus.

MATERIALS AND METHODS

Viruses. The virulent Shizuoka strain (Sasahara et al., 1958) of TGE virus was used for oral inoculation of pigs. The strain had been passaged 17 times in piglets and stored at -80°C in the form of a 10% suspension of infected small intestine.

The TO strain of an attenuated TGE virus was used for production of antigen to be used for lymphocyte stimulation in vitro. The strain had been serially passaged in pig kidney cell cultures (Harada et al., 1969), and was used at passage 168 in the present studies.

Vesicular stomatitis virus (New Jersey type) was used as challenge virus in an interferon (IF) assay.

Experimental pigs. A total of 28 Yorkshire pigs 2 to 3 months old, serologically negative for TGE antibody, were obtained from a farm where no outbreaks of TGE had been reported.

Experimental design. Two experiments were carried out. In the first experiment, effects of the ambient temperatures on induction of immune responses and on production of IF in the intestinal tract in pigs inoculated orally with TGE virus were investigated. Twenty-two pigs were kept at the high temperature (30 ± 2°C) for 4 days and then divided into two groups. Eleven pigs (Group 1) were transferred to the low temperature (4 ± 1°C) room. Nine of them were infected orally with 10^4 50% pig infectious doses (ID_{50}) of virulent TGE virus and kept at 4°C. The remaining two pigs kept at 4°C were not infected and served as controls. Eleven pigs (Group 2) were maintained at the high temperature throughout the experiment. Nine of these pigs were exposed orally to 10^4 ID_{50} of virulent TGE virus on the fourth day of the experiment, and the remaining two pigs served as uninfected controls.

Three pigs inoculated with virus in each group were sacrificed at 3, 5 and 7 days post-inoculation (DPI), respectively. All the control pigs were killed on the seventh day of the experiment. Lymphocytes were collected from Peyer's patches, mesenteric lymph nodes, spleen, and peripheral blood of these pigs, and tested for proliferative reactivity to TGE viral antigen in vitro (Shimizu and Shimizu, 1979a, 1979b).

IF activity of the intestinal contents and washings, obtained from all the pigs, was also examined.

In the second experiment, the influence of an immunosuppressive drug on the protective effect induced in pigs by high ambient temperatures was
investigated. Six pigs were kept at the high temperature. Three of them were
given dexamethasone (DM), a synthetic corticosteroid, subcutaneously in
doses of 5 mg/kg daily for 5 days, except on the third day when this dose of
the drug was administered intravenously. On the fifth day of the experiment,
all pigs were exposed orally to $10^4$ ID$_{50}$ of virulent TGE virus, and maintained
continuously at 30°C. The concentration of circulatory T lymphocytes and
non-specific blastogenesis of peripheral lymphocytes to phytohaemagglutinin-P
(PHA) were determined on the first and the fifth day of the experiment.

**Preparation of lymphocyte suspensions.** Lymphocyte suspensions to be used
in a lymphocyte proliferative assay were prepared by methods described
previously (Shimizu and Shimizu, 1979a, 1979b).

**TGE viral antigen.** TGE viral antigen used for lymphocyte stimulation in vitro
was prepared from pig kidney cell cultures infected with the attenuated TO
strain of TGE virus (Shimizu and Shimizu, 1979a, 1979b).

**Nonspecific mitogen.** PHA was purchased from the Difco Laboratories, Inc.
(Detroit, U.S.A.).

**Lymphocyte proliferative assay.** Lymphocyte proliferative reactivity to TGE
viral antigen and PHA was determined by measuring the amount of $[^3]$H-
thymidine incorporated into nucleoprotein of cells (Shimizu and Shimizu,
1979a, 1979b). The results were expressed as a stimulation index, which was
defined as the mean count per minute of cultures incubated with mitogen,
divided by the mean count per minute of cultures incubated with control
antigen.

**Detection of T lymphocytes.** The number of T lymphocytes was determined
by rosette-formation of cells with sheep erythrocytes. The details of the
rosette-formation test have been described elsewhere (Shimizu et al., 1976).

**Preparation of intestinal samples for IF and its assay.** The small intestine, 1 m
in length, was removed from all pigs in the first experiment and washed with
300 ml of phosphate buffered saline solution (PBSS). The intestinal contents
were mixed with the corresponding intestinal washings and centrifuged at
2000 $\times$ g for 30 min. Resulting supernatants were heat-inactivated at 56°C
for 30 min. Zinc acetate was added to the samples at a concentration of 0.02 M.
Precipitates formed were collected by centrifugation at 2000 $\times$ g for 1 h and
resuspended in 0.2 N solution of hydrochloric acid to 1/30 of the original
volume. After dialysis against several changes of a large volume of PBSS, the
solutions were tested for IF activity. Tubes containing pig testicle cell cultures
were treated overnight with 0.5 ml of serial dilutions of the intestinal samples,
using Eagle's minimal essential medium supplemented with 10% bovine serum.
After overnight incubation at 37°C, the samples were removed, and the cultures
were challenged with 100, 50% tissue culture infectious doses of vesicular stomatitis virus. The cultures were incubated at 37°C for 40 h. The samples which inhibited development of cytopathic effect in the assay were regarded as positive samples for IF.

RESULTS

Effects of ambient temperatures on early immune responses in pigs inoculated with TGE virus. Results of the first experiment are shown in Table I. All pigs inoculated with TGE virus immediately after transference to the low temperature room showed profuse diarrhoea and vomition after an incubation period of 2 days. On the other hand, pigs infected and held at 30°C showed no clinical signs of the disease.

Development of immune responses, as measured by blastogenic responses of lymphocytes from Peyer's patches and mesenteric lymph nodes, was first noted at 3 DPI in pigs held at 30°C, whilst infected pigs held at 4°C showed no comparable response until 7 DPI (convalescent stage).

In both groups of uninfected control pigs no blastogenic responses of lymphocytes were noted when these animals were killed on the seventh day of the experiment.

Effects of ambient temperatures on production of IF in the intestine of pigs inoculated with TGE virus. The intestinal samples obtained from all pigs gave negative results in the IF assay.

Effects of an immunosuppressive drug on infection with TGE virus. In comparison to untreated pigs (Table II), T lymphocytes of the peripheral blood of pigs treated with DM and held at 30°C were significantly reduced in number and reactivity to PHA by the fifth day of treatment.

Infection with TGE at this stage of apparent immunosuppression was associated with profuse diarrhoea which continued for 3–4 days after incubation periods of 1–2 days. Control pigs, not treated with DM, infected at the same time, and held at the same temperature (30°C), showed no clinical evidence of TGE infection, their faeces remaining normal throughout the experiment.

DISCUSSION

There are many reports dealing with effects of ambient temperatures on the pathogenesis of various virus diseases (Boring et al., 1956; Marshal, 1959; Carmichael et al., 1969; Lycke et al., 1971; Bell and Moore, 1974; Kiorpes and Yuill, 1975; Bell et al., 1977). Lower temperatures usually intensified the severity of the particular disease, whereas higher temperatures often resulted in an increased resistance to the clinical syndrome. In a previous report (Shimizu et al., 1978), it was shown that feeder pigs 2 to 3 months old, kept at a high
TABLE I

Effects of ambient temperatures on development of lymphocytes reactive to viral antigen in pigs inoculated with transmissible gastroenteritis virus

| Group | Ambient temperatures | Days post-inoculation | Clinical* signs | Stimulation index** |
|-------|-----------------------|-----------------------|-----------------|---------------------|
|       | Before inoculation    | After inoculation     |                 | Peyer's patches | Mesenteric lymph nodes | Spleen | Peripheral blood |
| 1     | 30°C, 4 days          | 4°C                   | 3               | +                  | 0.9 | 1.2 | 1.2 | 1.1 |
|       |                       |                       | 5               | +                  | 1.0 | 1.2 | 1.2 | 1.3 |
|       |                       |                       | 7               | +                  | 2.7 | 2.8 | 1.6 | 1.7 |
|       | control***            |                       |                 | -                  | 0.9 | 1.1 | 1.2 | 1.0 |
| 2     | 30°C, 4 days          | 30°C                  | 3               | -                  | 2.0 | 2.4 | 1.3 | 1.5 |
|       |                       |                       | 5               | -                  | 3.1 | 2.5 | 1.3 | 1.6 |
|       |                       |                       | 7               | -                  | 3.5 | 2.8 | 1.9 | 1.8 |
|       | control***            |                       |                 | -                  | 1.1 | 0.8 | 0.9 | 1.2 |

*Diarrhoea and soft faeces were regarded as positive clinical signs.

**Data are shown as average of the results obtained from three pigs of each experiment, except those of controls.

***Four control pigs of both groups were examined on the seventh day of the experiment.
Temperature, endured infection with TGE virus without showing clinical signs, and that both temperature change, and low temperature, exacerbated the infection in pigs. The change from the high to low temperature was considered to be an important factor in the induction of TGE in feeder pigs.

At present, however, the mechanism by which the adverse temperature enhances the severity of various virus diseases is not fully understood. One possible explanation for the mechanism might be that an elevated body temperature, induced by the high ambient temperature, enhances the resistance of animals to the infection. The elevated body temperature may directly inhibit intracellular replication of virus, or may induce abortive infection. In rabies virus infection of mice (Bell and Moore, 1974) and canine herpesvirus infection of puppies (Carmichael et al., 1969), resistance of animals to infection was associated with an elevated body temperature. It remains obscure, however, whether resistance induced by elevated body temperatures results from direct effects on viruses, or from indirect effects through protective responses of the host.

According to Furuuchi et al. (1975), the optimum temperature for growth of virulent TGE virus ranges from 37°C to 40°C. Bustad and Book (1975) have shown that the body temperature of pigs weighing 30–65 kg, and kept at 4.4°C, was about 39°C and was about 40°C in pigs held at 32.2°C. It seems unlikely, therefore, that pigs kept at 30°C showed subclinical disease as a result of direct effects of the elevated body temperature on TGE virus replication in vivo.

Based on observations that mice inoculated with Coxsackie B1 virus, and kept at 25°C, developed IF with reduced virus levels in their livers, compared

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### TABLE II

Effects of dexamethasone (DM) on infection with transmissible gastroenteritis virus

| Pig no. | DM treatment | Number of T lymphocytes in peripheral blood ($\times 10^6$/ml) | Responsiveness of peripheral blood lymphocytes to PHA* | Clinical signs*** |
|---------|--------------|---------------------------------------------------------------|------------------------------------------------------|------------------|
|         |              | Before treatment                                              | After treatment**                                    |                  |
|         |              |                                                               | Before treatment                                    |                  |
|         |              |                                                               | After treatment**                                   |                  |
| 1       | +            | 3.17                                                          | 0.73                                                 | 108              | 27               | +                        |
| 2       | +            | 4.65                                                          | 0.53                                                 | 177              | 29               | +                        |
| 3       | +            | 3.98                                                          | 0.81                                                 | 107              | 35               | +                        |
| 4       | -            | 4.51                                                          | 3.87                                                 | 134              | 197              | -                        |
| 5       | -            | 3.97                                                          | 4.25                                                 | 157              | 181              | -                        |
| 6       | -            | 4.48                                                          | 4.30                                                 | 129              | 115              | -                        |

All pigs were maintained at a high ambient temperature (30°C) throughout the experiment.

*Data are expressed as a stimulation index.

**After treatment data are recorded for the fifth day of treatment (day 0 of infection).

***Diarrhoea and soft faeces were regarded as positive clinical signs.
with the absence of IF and high levels of virus when kept at 4°C, Ruiz-Gomez and Sosa-Martinez (1965) suggested that ambient temperatures play an important role in virus infections by the regulation of IF production. In the present studies, however, IF was not detected in the intestinal contents and washings obtained from pigs with and without clinical signs of TGE, suggesting that the effects of ambient temperatures may not operate through regulation of IF production in this situation.

Results from the present studies suggest that the nonspecific cell-mediated immune response of pigs may be reduced at lower temperatures. The delay in initiation of the immune response in infected pigs held at 4°C, compared with that in comparable pigs held at 30°C, was in the order of 4 days. It will be noted that clinical disease in the pigs kept at 4°C resulted after an incubation period of 1–2 days. If suppression of cell-mediated responses is the cause of clinical disease in pigs held at 4°C, it would imply that such cell-mediated responses in pigs with subclinical disease held at 30°C, occur within 48 h. From the experiment with an immunosuppressive drug, there would seem no doubt that immunosuppression was associated with clinical disease even at 30°C, and the tentative conclusion is drawn that low ambient temperatures may interfere with the early initiation of local cell-mediated responses in the TGE model.

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