Hypergammaglobulinemia in Swine Infected with African Swine Fever Virus (34794)

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(Introduced by M. M. Mayer)

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African swine fever (ASF) is a highly contagious, fatal, peracute, viral disease of swine with mortality reaching nearly 100%. Several species of wild pigs, namely, wart hogs, bush pigs, and giant forest hogs are natural reservoirs of this virus, without manifesting clinical signs of the disease. The virus was successfully propagated in swine bone marrow and buffy-coat cell cultures (1), and several virus isolates were attenuated by passaging the virus in various stable cell lines as well as in buffy-coat and bone marrow cultures (2—7). By serial passage in cell cultures, some ASF virus isolates have been sufficiently modified to produce nonfatal infections in domestic swine (6, 8, 9).

In a previous communication, it was reported that swine surviving inoculation with well-attenuated virus and subsequent inoculation with virulent virus formed moderate levels of complement-fixing (CF) and agar-gel diffusion precipitating (AGDP) antibody (10). Neutralizing antibody has not yet been demonstrated with classical techniques (10—12).

Swine inoculated with partially attenuated virus frequently acquired chronic infection with recurring fever. They developed high titers of CF and AGDP antibody, presumably in response to the replication of the virus. Moreover, the chronic nature of the infection suggested that it might result in development of hypergammaglobulinemia. This has been observed in other chronic viral infections in animals, viz., Aleutian disease of mink (13), equine infectious anemia (14, 15) and hemolytic anemia of NZB/Bl mice transferred to Swice mice (16, 17) and lymphocytic choriomeningitis (LCM) in mice (18).

The paucity of information concerning this particular aspect and the need to correlate immunologic and clinical manifestations of ASF prompted this study.

Materials and Methods. Two groups of 15 and 4 Tamworth swine, approximately 2½ months old, were inoculated with the Lisbon '60[1] isolate of ASF virus of the 89th and 83rd swine bone-marrow-culture passages, respectively. The 83rd-passage virus had become attenuated for swine to a much greater degree than had the 89th passage. The animals in both groups were inoculated intramuscularly (IM) with 1 ml undiluted tissue culture virus (10^7 $TCID_{50}$) of the respective passage levels. The presence of virus in sera and tissue suspensions was determined by the hemadsorption reaction (1). The gamma globulin (GG) content of the serum was assayed in a series of samples collected from all the pigs and is expressed as grams of protein/100 ml. The percentage of GG content in the serum was calculated from the results of electrophoresis on cellulose acetate strips. The total serum protein was determined by the biuret method (19). For reference purposes, the mean GG content plus or minus 2 standard deviations obtained from 48 normal swine sera was 0.870 ± 0.472 g/100 ml. The mean albumin/globulin (A/G) ratio plus or minus 2 standard deviations obtained from 35 normal swine sera was 1.2 ± 0.2. Complement-fixing and AGDP antibodies were determined as previously described (11).

Results. The group of 15 swine, inoculated with virus of the 89th passage, experienced recurring fever at irregular intervals, and many animals developed chronic respira-

[1] Supplied by Dr. Armenio Eduardo Franca de Silva, Portugal, as 81st bone marrow passage.

TABLE I. Results of Attempted Virus Isolation from Various Tissues Using the Hemadsorption Test in Buffy-Coat Cultures.

	Tissues used for virus isolation								
	Lymph nodes								
Killed (DPI)	Mandibular	Mesenteric	Inguinal	Iliac	Gastro-hepatic	Spleen	Liver	Kidney	Lung
35	1/2[a]	0/2	2/2	1/2	1/2	1/2	0/2	0/2	1/2
49	0/2	0/2	1/2	1/2	0/2	0/2	0/2	0/2	0/2
63	0/2	0/2	1/2	0/2	2/2	0/2	0/2	0/2	1/2
71	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
77	0/4	0/4	0/4	2/4	1/4	0/4	0/4	0/4	0/4
89	0/1	1/1	1/1	1/1	0/1	0/1	0/1	0/1	0/1
91	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

DPI = days postinoculation.

[a] Number of pigs positive/number of pigs examined.

Note: The end result of hemadsorption tests was based on the outcome of the 3rd subpassage.

tory disease signs (coughing and dyspnea) after 35 days postinoculation (DPI). Blood samples were obtained from all the animals at 7-day intervals, and viremia could be demonstrated at various times up to 29 DPI. To determine the persistence of virus in the tissues, three groups of two swine were killed 35, 49, and 63 DPI, respectively. For the same purpose, one, four, and three swine were killed 71, 77, and 91 DPI, respectively. One pig died with extensive pneumonia and ASF lesions 86 DPI. With the exception of one and two pigs killed 71 and 91 DPI, respectively virus was isolated from some tissues of all the swine (Table I). All the swine had relative and absolute hypergammaglobulinemia and an absolute increase in total serum protein. The results summarized in Fig. 1 show that the GG level of all pigs increased from two to eight times over the original levels after 35

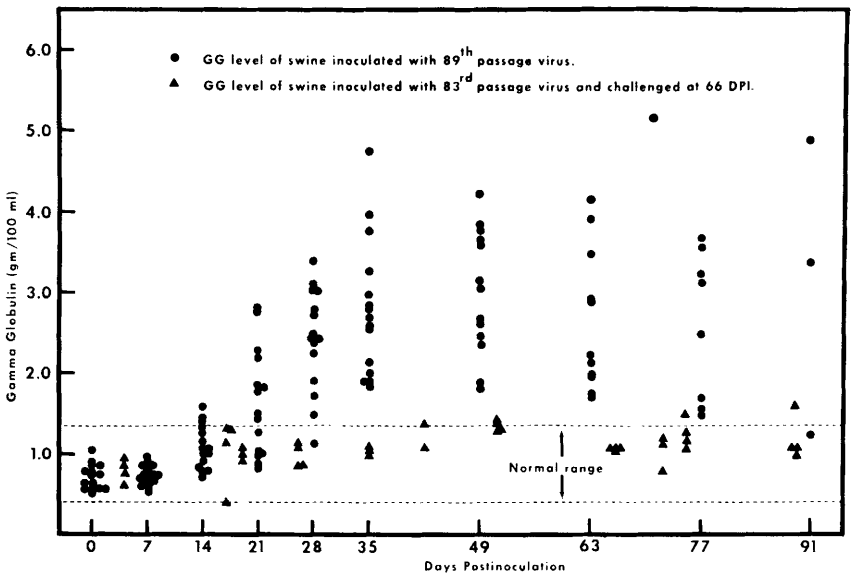

FIG. 1. Distribution of gamma globulin levels in two groups of swine inoculated with different tissue culture passage levels of African swine fever virus.

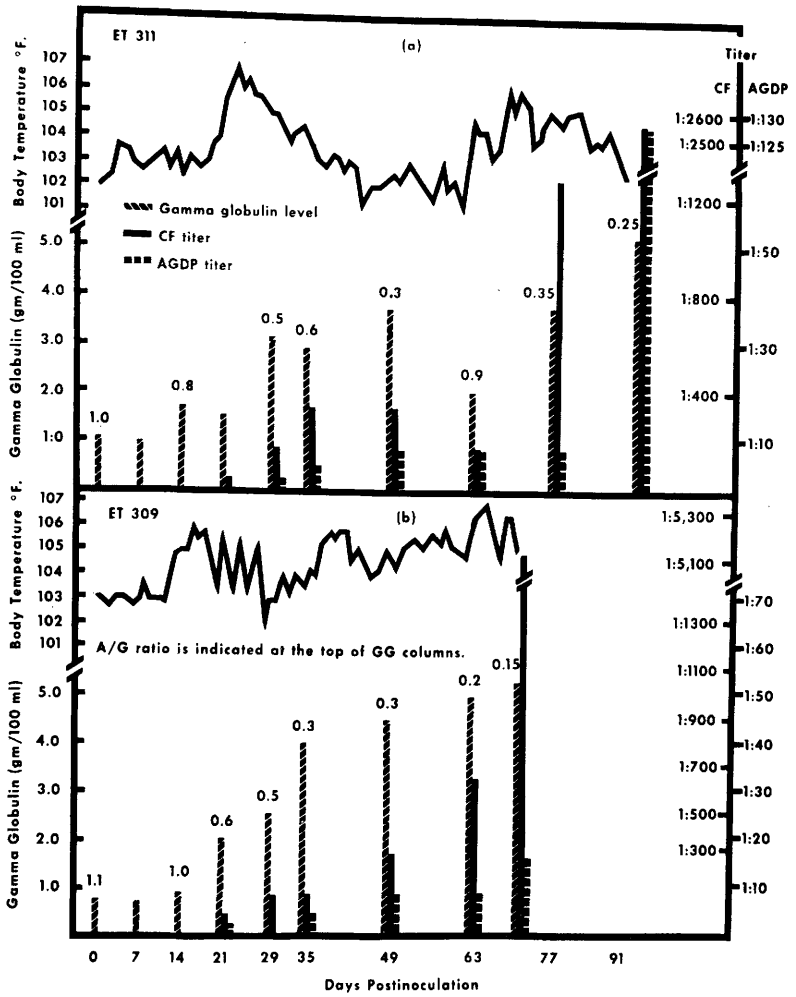

FIG. 2. Variations of GG levels and antibody titers parallel each other and are related to variations of fever responses: (a) decrease or increase of GG levels and antibody titers occurred after the resolution or recurrence of fever responses; (b) GG levels and antibody titers steadily increased with persistent fever response.

DPI. Moreover, a parallel was observed in variation of the GG levels and recurring fever. The GG level increased significantly within 7 days after onset of recurring fever, but decreased after the fever subsided. When fever persisted, however, a continuous elevation of the GG level was observed. The A/G ratio dropped markedly when the GG concentration rose to a high level. The minimal A/G ratio recorded was 0.15 in one pig when the GG level was 5.2 g/100 ml. Complement-fixing and AGDP antibody levels varying from 1:320 to 1:640 and from 1:8 to 1:16, respectively, developed within 71 DPI. Ninety-one DPI, however, two pigs had high CF (both 1:2560) and AGDP (1:40 and 1:128) antibody titers, respectively. The variation in the antibody titers closely paralleled that of the GG levels. The typical examples reflecting these observations are summarized in Fig. 2.

The group of four swine, inoculated with virus of the 83rd passage, experienced a 1- to 2-day fever of 105°–106°F, 3–4 DPI. No further rises in temperature were observed. Viremia appeared within 4 DPI and persisted in two pigs up to 35 and 42 DPI, respectively, even though there was no febrile response

in this period. The immunity of all 4 swine was challenged with virulent virus 66 DPI, but no febrile response occurred and viremia could not be demonstrated. Twenty-two days later, at termination of the experiment, virus could not be recovered from the spleen. The GG levels of sera before and after initial inoculation were all within normal range. Likewise, after challenge inoculation 66 DPI, the GG levels of all but one pig remained within the normal upper limit. This particular animal had a level of 0.24 g/100 ml above this limit (Fig. 1). All animals had low CF and AGDP antibody titers varying from 1:40 to 1:160 and from 1:2 to 1:4, respectively.

Apparently, the differences in host responses (clinical signs, antibody titers, and GG levels) between the 15-swine and 4-swine groups are due to the difference in attenuation of the passage levels of virus administered.

Discussion. The present study indicates that chronic ASF virus infection in swine becomes the fifth known example of hypergammaglobulinemia in animals induced by a viral infection. Chronic infection with ASF virus has some clinical similarities to the aforementioned four diseases, viz: persistent viral infection, poor neutralizing antibody production, if any, and hypergammaglobulinemia. This appears to be the first instance of hypergammaglobulinemia associated with a viral disease of swine.

Hypergammaglobulinemia in ASF may reflect a continuous antigenic stimulation by persistent viral infection, since virus was isolated from organs of a majority of chronically infected swine and correlated with elevated levels of GG and antibody, whereas pigs having low levels of GG and antibody were apparently free from virus.

As to cause(s) of a persistent viral infection with circulating antibody, the following possibilities—individually or combined—may be considered: (1) viral particles in the host cells inaccessible to the neutralizing effect of circulating antibody; (2) poor quality of neutralizing antibody produced; and (3) incomplete neutralizing effect of antibody due to heterogeneity of antigenic structures existing in mixed viral populations. Conversely, challenge-resistant swine, with low CF and AGDP antibody levels, were free from virus. The reason for this inconsistency may be related to the viral antigenic structure rather than the immunocompetency of the swine. It is also possible that modified procedures may reveal neutralizing antibodies previously undetectable in such swine.

Summary. Marked hypergammaglobulinemia appeared in swine which became carriers after inoculation with moderately attenuated African swine fever virus. It may have been induced by persistent viral infection. Swine given highly attenuated virus had normal gamma globulin levels.

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