

THE VIRUS OF INFECTIOUS FELINE AGRANULOCYTOSIS*

II. IMMUNOLOGICAL RELATION TO OTHER VIRUSES

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Infectious feline agranulocytosis (1-3) is a disease entity in which the tissues, fluids, and excretory products yield a characteristic virus that is highly infectious by a wide variety of routes for members of the cat family, as the experiments in the preceding paper have shown. The immunological relation of this newly described virus to other viruses is considered in the present paper.

Tests for Active Immunity

It was necessary to learn first whether cats that recover from either the spontaneous or experimental infection (irrespective of the route of inoculation) are solidly resistant to reinfection by massive doses of virus, as measured by the absence of the accepted clinical, hematological, or pathological evidence of disease. In an experiment to decide the point, cats known to have had the typical disease in from a few days to many months previously were tested by the parenteral injection of virus for their capacity to resist reinfection.

Thirteen cats, which were known to have had either the spontaneously or the experimentally induced disease in from 4 to 288 days previously, were tested for immunity by the intraperitoneal injection of a single massive dose of virus, consisting of from 3 to 5 ml. of hepatic tissue suspension.

All of the animals proved refractory to reinfection.

It is apparent from the results of this experiment that recovery from infectious feline agranulocytosis is followed by complete resistance to reinfection. Furthermore, this evidence was confirmed repeatedly under natural conditions, for the disease has never been observed to recur in cats returned to the animal house after recovery. Susceptible cats, on the other hand, regularly develop the disease spontaneously shortly after admission to the same quarters.

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Susceptibility of Normal and Immune Cats to Other Agents

Experiments were undertaken in an attempt to demonstrate a relationship between the virus of infectious feline agranulocytosis and other agents. These experiments fall into three groups. Group A, consisting of three experiments, employed normal cats in two experiments and immune cats in the third. The animals in these experiments received a test dose of an agent conceivably related to the virus of feline agranulocytosis that was followed 3 weeks later by a challenge dose of the feline agent.

As the peripheral blood picture in hog cholera (15) is suggestive of that of feline agranulocytosis (the pathological changes in the various organs were not reported), hog cholera virus was employed in the first experiment to determine whether clinical symptoms and changes in the blood and bone marrow would result in cats infected by this virus.

5 ml. of whole blood containing hog cholera virus were inoculated intraperitoneally into 5 of 6 cats that had been in isolation for from 25 to 71 days. None of these animals showed any clinical or hematological evidence of infection. However, when these 6 animals were injected with a challenge dose of feline agranulocytosis virus 21 days later, the control and 3 of the 5 test animals developed typical agranulocytosis.

From the results of this first experiment, it was concluded that the cat is refractory to infection by hog cholera virus, and that there is no apparent relationship between the viruses of hog cholera and feline agranulocytosis.

Fox encephalitis virus was used for the next two experiments. The only reasons for selecting this virus were the slightly suggestive resemblances between the clinical and pathological pictures of the two diseases, and because in so far as we know, the blood picture of fox encephalitis has not been studied. Accordingly, Experiments 2 and 3 were planned to test normal cats and cats immune to infection by the virus of feline agranulocytosis for susceptibility to infection by the virus of fox encephalitis.

Each of the 4 test animals in each experiment was inoculated under light ether anesthesia with a 2 per cent virus suspension, 0.8 ml. intracisternally, and 4 ml. intraperitoneally. As controls, single animals were maintained under identical conditions but were not injected.

None of these animals yielded any evidence to suggest that fox encephalitis virus was pathogenic for the cat, or that it had any effect on the peripheral blood picture of this animal. Moreover, these results were substantiated further when a challenge dose of virus was given to each animal, for none of the immune animals showed any evidence of feline agranulocytosis infection, whereas 4 of the 4 normal cats developed the clinical disease.

These two experiments convince us that cats are insusceptible to infection by the virus of fox encephalitis, that this virus does not alter the peripheral blood

picture, and that there is no apparent immunologic relationship between the two viruses.

The experiments in group B were designed to learn whether the cat is susceptible to infection by any one of the five viruses: equine encephalomyelitis (Western type), vesicular stomatitis (Indiana type), lymphocytic choriomeningitis (W. S. strain), B virus infection, and herpes (HF strain).

For each experiment a single virus in 10 per cent suspension was employed for the inoculation of 2 cats, 0.25 ml. intracerebrally and 1 ml. intraperitoneally.

Of the 10 cats injected, only a single animal, injected with vesicular stomatitis virus, developed signs of disease as shown by a bilateral paralysis involving both hind limbs. Attempts to recover the virus of lymphocytic choriomeningitis virus from the spleens of cats that had received the virus intraperitoneally and intracerebrally were unsuccessful.

The results of this second group of experiments make it apparent that of the viruses, when tested by us,—the Western type of equine encephalomyelitis, vesicular stomatitis, lymphocytic choriomeningitis, B virus infection, or herpes—none is pathogenic for the cat.

Studies on the Identity of the Causal Agents of Infectious Feline Agranulocytosis, Malignant Panleucopenia of Cats, and Infectious Feline Enteritis

When the first paper by Hammon and Enders was published (4), it was obvious that they were working with a disease identical or closely related to the disease which we had described (1). It seemed desirable, therefore, to make comparative studies of the infective agents of these two feline maladies (16). Drs. Enders and Hammon kindly supplied immune serum and glycerinated tissues, consisting of the spleen, lymph node, and bone marrow from one animal, and splenic tissue from another. These materials were used in two experiments.

The first experiment was designed to show whether tissues from cases diagnosed as malignant panleucopenia contain an agent that would give rise to a disease clinically and hematologically identical with feline agranulocytosis.

The supernatant fluid of a 10 per cent suspension, prepared from representative portions of the glycerinated tissues supplied by Drs. Enders and Hammon, was used in 4 ml. amounts for the inoculation of 9 cats. Of these 9 animals, 3 were normal and 6 had recovered from feline agranulocytosis.

All 3 of the normal animals developed typical feline agranulocytosis in from 6 to 8 days after injection, whereas the 6 agranulocytosis-immune animals showed no evidence to suggest either illness or an altered blood picture.

This first experiment makes it evident that tissues removed from cats with malignant panleucopenia contain an infectious agent that gives rise to a disease

with clinical and hematological features indistinguishable from those of feline agranulocytosis. Added evidence to support the identity of the two agents is the refractoriness of agranulocytosis-immune cats to infection by the agent of panleucopenia.

Further evidence to support these findings was sought in the next experiment in which panleucopenia-immune serum was tested for its protective effect against infection by agranulocytosis virus.

Each of 3 normal cats was injected intraperitoneally with 5 ml. of the test serum, and within a few minutes 4 ml. of a suspension of feline agranulocytosis virus was injected subcutaneously. Each of 11 cats injected intraperitoneally with an identical amount of the same preparation of virus served as a control. (Because we wanted cats with the disease for other purposes we made the control group unusually large.)

None of the test animals contracted the disease, whereas the typical disease developed in 10 of the 11 animals serving as a control.

It was concluded that the results of these two experiments establish the identity of the two viruses.

Infectious feline enteritis is a second disease that somewhat resembles feline agranulocytosis in its clinical picture, but published descriptions characterize it as a severe enteritis. Moreover, the blood picture in feline enteritis has not been described. Accordingly, it seemed desirable to make comparative studies of this disease and feline agranulocytosis. As a strain of the virus of feline enteritis was not available, we sought tissues from cats with an illness diagnosed as feline enteritis by qualified veterinarians. Of three requests made to leading schools of veterinary medicine, two yielded tissues for study. The procedure followed and the results obtained were identical with those described in the study of the agent of panleucopenia.

It was found that both samples of tissue yielded an infectious agent that is identical with the virus of feline agranulocytosis. We feel that these studies are inconclusive, however, because the peripheral blood picture of the source animals was not studied. It is impossible to say positively, therefore, that the cases, which were diagnosed clinically as feline enteritis, were not feline agranulocytosis.

DISCUSSION

The data presented in the present paper and in that preceding it show the virus of infectious feline agranulocytosis to be the causal agent of a highly infectious disease of cats. To promote consideration of the significance of our observations, some of the results of these studies will be briefly stated.

The feline malady is characterized by an extreme granulocytopenia, marked relative lymphocytosis, less pronounced leucopenia, hypoplasia with the absence of differentiation of the myeloid cells of the bone marrow, prolifera-

tion of the reticuloendothelial cells of the lymph nodes and spleen, and intranuclear inclusion bodies in the cells of the gastrointestinal mucosa, lymph nodes, and bronchial mucosa. The high infectivity of the virus for the cat is manifest when it is inoculated by the oral, intragastric, intranasal, cutaneous, subcutaneous, intraperitoneal, and intravenous routes, but its pathogenicity is limited to feline hosts. The virus is widely distributed in the host's tissues and fluids, for it is readily recovered at the height of disease from the blood, liver, spleen, lungs, nasal mucosa and turbinates, nasal secretions, intestinal mucosa, feces, and urine. All strains of the virus that have been tested are immunologically identical.

The complete avirulence of the virus of feline agranulocytosis for any species other than the cat seems to distinguish this agent from the viruses whose pathogenicity for other species is well established. Our inability to infect white mice, guinea pigs, rabbits, ground squirrels (*Citellus richardsonii* Sabine), and the chorio-allantoic membrane of the developing chick confirms and extends susceptibility tests with animal species other than the cat, as reported by Hammon and Enders (4), and by Kikuth, *et al.* (6). On the basis of species susceptibility, therefore, the virus appears to be distinct from the etiological agents of lymphocytic choriomeningitis, influenza, Rift Valley fever, louping ill, canine distemper, fox encephalitis, mouse encephalomyelitis (Theiler), the pneumonia carried by normal mice, as described by Horsfall and Hahn (12), infectious ectromelia, vesicular stomatitis, equine encephalomyelitis, St. Louis encephalitis, the pox group, and the meningopneumonitis-psittacosis-lymphogranuloma venereum group. These viruses are further distinguished from the virus of infectious feline agranulocytosis by distinctive differences in the pathological findings that result from infection. Moreover, further evidence for our belief in the singleness of identity of the virus of feline agranulocytosis was our inability to establish clinical infections in the highly susceptible cat by the inoculation of viruses that readily infect a variety of laboratory animals. Thus, we found the cat to be clinically refractory to infection by the viruses of hog cholera, lymphocytic choriomeningitis, fox encephalitis, vesicular stomatitis, the Western type of equine encephalomyelitis, herpes, and B virus infection.

Because the disease is extremely contagious, Hammon and Enders (4), and Kikuth, Gönner, and Schweickert (6), were unable to prevent animals from contracting the disease spontaneously (except in two experiments reported by Hammon and Enders¹). We have had similar results when cats were introduced into our usual animal quarters. In the light of these ex-

¹ Hammon and Enders (4) reported two experiments in which they used two widely separated farms to maintain 7 cats in 2 groups without evidence of disease for 21 and 27 days, respectively. Following the period of isolation, 3 of the 4 cats, which were inoculated with filtrates, gave unequivocal evidence of the disease.

periences, therefore, it is worthwhile emphasizing that all of the cats employed in the present experiments were kept under rigid isolation for from 12 to 92 days before being used. During this time each cat was observed daily for clinical signs of disease, and its hematological status was followed by bidaily total cell and differential studies of the white blood cells. Thus, we were enabled to eliminate immediately any group in which a single member showed overt signs of illness. Our results, therefore, are based on the use of cats that were kept in isolation for a period that greatly exceeded the incubation period of the disease. Moreover, each group of animals under study was controlled further by the inclusion of 1 or more normal cats, which were not injected.

In order to understand the epizootology of any infectious disease, it is important to know the routes whereby the etiological agent can enter and leave the body of its host. In the present study, our experimental data suggest the gastrointestinal and respiratory routes as natural portals for the virus to infect the cat, and the cutaneous route as a possibility. The proven susceptibility of the cat to virus introduced intranasally and the extraordinary communicability of the disease make it apparent that the disease can be transmitted naturally by the respiratory route. On the other hand, the ready susceptibility of the cat to virus introduced by mouth or stomach tube, the pathological changes in the epithelial cells of the intestinal mucosa, and the massive amounts of virus excreted in the feces suggest contaminated food as a natural source of infection. Of significance too, perhaps, were repeated observations that administration of the virus by the oral or intragastric route resulted in an incubation period as short, or shorter, than by any other route. Moreover, the presence of virus in the urine of infected cats increases the probability that contaminated food acts to spread the disease. Most likely both are natural routes. Although the cutaneous route is a possibility, as has been shown by the demonstrated presence of virus in the blood stream and the infectivity of virus inoculated by the cutaneous route, it seems unnecessary to assume that the virus is spread by a biting arthropod. Our knowledge can be summarized, therefore, by stating that the natural vehicle for the spread of the disease could be nasal droplets, contaminated food, or contaminated arthropods.

As with each new infectious agent, it is difficult to learn whether the disease under investigation, or its causative agent, has been described previously. Such is the case with the virus of infectious feline agranulocytosis. It early became apparent from a survey of the literature related to epizootic diseases of cats, however, that a feline disease with the distinctively characteristic hematological and pathological findings of feline agranulocytosis had not been reported. Soon after our note (1), Hammon and Enders described a disease (4) (which they named malignant panleucopenia in a later publication (5)) that was proven by immunological studies (16) to be caused by a virus identical with the virus under investigation. Moreover, Kikuth, Gönner, and Schweickert (6) have described what appears to be the same disease in

Germany, and which they named "infectious aleucocytosis of cats." Thus, it appears that a single disease has been given three names as the result of studies carried out in three widely separated laboratories. Moreover, the problem was recently complicated further by the recovery of 2 strains of this same virus from cases diagnosed as feline enteritis by two highly competent veterinarians. These last results suggest two possibilities: either the virus under investigation is identical with that of feline enteritis, or the clinical designation "feline enteritis" may be employed loosely to cover a variety of feline maladies, which have in common involvement of the gastrointestinal tract. If the first possibility be right, then it is remarkable that the extensive blood changes and the formation of inclusion bodies, which are known to occur regularly in feline agranulocytosis, had not been discovered by earlier workers (17). On the other hand, it is not surprising that a variety of feline illnesses should be caused by filterable agents, or that these illnesses should be accompanied by signs and symptoms related to the gastrointestinal tract, for it is well known that human maladies of virus etiology without inappetance, nausea, vomiting, or diarrhea are rare indeed. Therefore, it becomes apparent that the mere presence of signs or symptoms related to the gastrointestinal tract are not adequate evidence for a specific diagnosis. It remains a question, therefore, as to how this clinical entity should be designated. The infectious nature of the disease, the strict limitation of its host range to cats, and a cytological picture of the bone marrow and blood that is indistinguishable from that of human agranulocytosis, suggested the name "infectious feline agranulocytosis." Certainly if agranulocytosis is a satisfactory name for the human syndrome, then the feline disease should be so designated. Malignant panleucopenia, on the other hand, is not as descriptive and is misleading, for it implies an essentially fatal disease in which all the white cells of the blood are involved equally. That the marked leucopenia and neutropenia are accompanied by a relative lymphocytosis was shown previously (3); and that the disease is not uniformly fatal was established in the same investigation. Moreover, these earlier findings are substantiated by the results of the present study in which fully as many of the cats tested (over 400) were shown to have an acquired immunity as to be susceptible. Thus, it would seem that "malignant panleucopenia" is ill suited as a name for this disease.

The status of the name "feline enteritis," on the other hand, is not readily disposed of, for feline enteritis is a disease that is accepted by veterinarians as a clinical entity caused by a filterable virus. Moreover, two competent diagnosticians found the disease under investigation to be indistinguishable from illnesses that they considered clinically to be feline enteritis. When it is realized, however, that the term feline enteritis is often used in veterinarian circles to cover any infectious malady with clinical signs pointing to the gastrointestinal tract, and that the hematological aspects of feline enteritis have never been investigated, it is difficult to know whether "feline enteritis" refers

to a single specific disease which has not been studied hematologically, or whether the term is used as a clinical designation for a variety of maladies that are included under a single name. It seems advisable, therefore, to retain and to perpetuate the name "infectious feline agranulocytosis," and to encourage veterinarians to use blood studies for the separation of the entity from other feline maladies.

SUMMARY

The infection of cats by the virus of infectious feline agranulocytosis is followed by the production of specific neutralizing and protective antibodies, and recovery from the disease is associated with the development of solid immunity to reinfection. From the evidence presented it is obvious that the virus is not related to the viruses of hog cholera, lymphocytic choriomeningitis, fox encephalitis, vesicular stomatitis, the Western type of equine encephalomyelitis, herpes, and B virus infection.

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