

Continuing Search for the Etiology of Cat Scratch Disease

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Fifty cat scratch disease patients were tested for antibodies against candidate etiological agents, with inconclusive results. Future research should emphasize new viral isolation methods to discover the elusive agent.

The causative agent of cat scratch disease (CSD) is still unknown, although CSD has been extensively reported in the medical literature since 1950, with about 600 scientific articles describing it (see review articles, references 1, 10, 12, 16, 17). Numerous unsuccessful attempts have been made to discover the etiology (presumed by most investigators to be a virus), using traditional virological and bacteriological techniques. The few recent reports that candidate agents may have been found (2, 5, 7, 8, 14, 17) have not, to our knowledge, been confirmed by the authors themselves or by others. Further efforts must be made, since the disease is relatively common and is a significant cause of morbidity, loss of school or work time, surgical and medical care expense, and occasionally severe complications including encephalitis and even death. CSD may simulate other infectious diseases, leading to inappropriate therapy, or Hodgkin's disease or lymphosarcoma, resulting in psychological trauma to the patient and family. Definitive studies on the sources, transmission, and control or prevention of CSD will be hampered until the etiological agent is found.

We recently conducted an antibody survey of CSD patients for some possible etiological agents, including several agents other than the chlamydial group traditionally tested for, utilizing indirect immunofluorescence (IF) methods as well as the usual complement-fixation (CF) test. We wish to briefly record our results along with suggestions for future directions of research.

Serum samples from 50 patients with clinical, pathological, and/or epidemiological evidence of CSD were selected for study from the serum bank of the Viral and Rickettsial Disease Laboratory, California State Department of Health. It was not feasible to select serum samples from a "control group" which might have provided valid comparative results for this study. Our purpose was merely to see if some

previously untried serological tests might yield encouraging findings.

The standard microtiter CF test, using chlamydial group antigen, had been used initially to test the sera before they were stored in our 4°C serum bank. After selection of the sera for this study, the CF tests were repeated (J. Schachter), using the standard macro-titer method (9).

The microtiter IF test, as described by Wang and Grayston (15), was used at the G. W. Hooper Foundation Laboratory (J. Schachter) to test sera against various chlamydial group antigens as follows: (i) FPs 562 and FPs 545 strains of feline pneumonitis agent, the latter one known to be capable of infecting man; (ii) LGV 434 strain of lymphogranuloma venereum agent; and (iii) TW3 and ICCal³ strains of trachoma-inclusion conjunctivitis agent. Briefly, slides were prepared with infected and uninfected (control) yolk sac antigens and were acetone-fixed; then serial serum dilutions (beginning at 1:4) of test sera and positive and negative control sera were applied, followed by commercial anti-human gamma globulin conjugated with fluorescein isothiocyanate. The antibody titer was considered as the highest serum dilution yielding specific fluorescence (3+) of chlamydial elementary bodies. Paired sera (when available) were tested in parallel, to try to demonstrate antibody titer rises.

An indirect IF test was similarly used in the Viral and Rickettsial Disease Laboratory (J. Riggs) to test sera by screening them at a 1:4 dilution against the following antigens: (i) two strains (Theilen's FL-74, and Gardner-Arnstein) of feline leukemia virus grown in RD human cell culture line; and (ii) the F-10 strain of feline syncytium-forming virus grown in human diploid cell cultures. Acetone-fixed infected and normal (control) cell culture slide preparations were reacted with the test sera and with positive and negative control sera,

followed by anti-human gamma globulin conjugated with fluorescein isothiocyanate (prepared by Riggs). Many of the sera had been depleted by the previous tests and could not be included.

The results of the tests are summarized in Table 1. The chlamydial group CF test showed 7 out of 50 (14%) positive at 1:16 or greater and 13 out of 50 (26%) positive at 1:8 or greater, with a few titers as high as 1:128. Combined results of the IF tests for FP, LGV, and trachoma-inclusion conjunctivitis agents showed 34 out of 50 (68%) positive at 1:8 or greater, with titers varying up to 1:512. There were broad cross-reactions within this group. Only four patients' sera reacted with one antigen only. Patients with significant CF antibody levels were invariably also positive for the various antigens used in the IF tests. No significant changes were found in the CF or IF antibody titers of early and late sera in the 19 cases for which serum pairs were available. None of the 42 sera tested for feline leukemia virus or the seven sera tested for feline syncytium-forming virus by IF tests were positive.

In the absence of a valid age- and sex-matched control group, it is impossible to interpret with confidence the significance of the above findings. Traditionally, chlamydial group CF antibodies have been found in many, but not all, CSD patients, although significant increases in antibody titer during the course of illness have not been demonstrated. The problem of obtaining acute-phase sera early enough (i.e., before lymph node enlargement) is well recognized and adds to the difficulty of using serological methods for diagnosis. A similar situation exists with LGV, a disease of proven chlamydial etiology. Rising antibody titers are rarely encountered, but CF titers of $\geq 1:64$ are commonly accepted as laboratory support for the diagnosis of LGV. Thus, past experience

and results of the present study confirm the intriguing possibility, but continuing uncertainty, of an etiological relationship between a chlamydial agent(s) and CSD. The findings may only represent general experience of the population with a variety of antigenically related *Chlamydiae*. Our impression from previous studies is that the range and general levels of antibody found in the present study are to be expected in many population groups. Similar results have been obtained in serological surveys of sexually active young women, veterinarians, and people with occupational exposure to *Chlamydiae*-infected birds or mammals. The commonly occurring feline pneumonitis agent has been shown capable of infecting man (11). Perhaps our results, and those reported previously by others, simply reflect human infections with this or other chlamydial agents, and the antibody titers are unrelated to CSD. A larger serological survey with matched controls might further clarify this point. Antigens for various recently described feline herpesviruses should also be included in such a serosurvey (these were not available to us), since herpes-like particles have been reported associated with CSD (8). The cat, of course, may be only an incidental inoculator of the agent, or indeed may not be related to the disease at all, despite the almost universally accepted hypothesis.

Future search for the cause of CSD, however, would be better directed at attempts to isolate the agent, rather than at serological surveys. Lymph nodes biopsied early in the course of the disease should be studied by newer virological techniques: explant or trypsin-dispersed cell cultures; cell fusion and co-cultivation with a variety of cell types, including feline cells; electron microscopy and immune-electron microscopy; immunofluorescence methods, using con-

TABLE 1. Results of serological tests on cat scratch fever patients

Test procedure	No. of patients tested	No. of patients positive at end point antibody titer							
		$\leq 1:4$	1:8	1:16	1:32	1:64	1:128	1:256	1:512
Chlamydial group complement-fixation	50	37	6	4	0	2	1	0	0
Chlamydial group indirect immunofluorescence tests ^a	50	16	4	7	3	7	4	5	4
Feline leukemia virus indirect immunofluorescence tests ^b	42	0							
Feline syncytium-forming virus indirect immunofluorescence tests ^c	7	0							

^a Includes FPs 562 and FPs 545 strains of feline pneumonitis agent, LGV 434 strain of lymphogranuloma venereum agent, and TW3 and ICCa1³ strains of trachoma-inclusion conjunctivitis agent.

^b Includes Theilen's FL-74 and Gardner-Arnstein strains of feline leukemia virus.

^c F-10 strain.

valescent-phase human serum; and chemical treatment of cultured lymph nodes to attempt to activate latent agents (3, 4, 6, 13). Efforts along these lines are in progress in our laboratories.

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