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Received Sept. 5, 1967. P.S.E.B.M., 1968, Vol. 127.

Human Follicular Conjunctivitis Caused by Infection with a Psittacosis Agent* (32675)

J. SCHACHTER,† P. ARNSTEIN, C. R. DAWSON, L. HANNA, P. THYGESON, AND K. F. MEYER

George Williams Hooper Foundation, Francis I. Proctor Foundation and Department of Microbiology, University of California Medical Center, San Francisco, California 94122; and U. S. Department of Health, Education and Welfare, National Communicable Disease Center, Atlanta, Georgia 30333

The Bedsoniae (Chlamydiae or psittacosis-lymphogranuloma venereum-trachoma group of microorganisms) comprise a large number of parasites of both man and animals (1). It has been generally believed that within this group only the agents causing trachoma and inclusion conjunctivitis (TRIC) produce follicular conjunctivitis in man. When the lymphogranuloma venereum (LGV) agent infects the conjunctiva of man, the resulting conjunctivitis is nonfollicular. Infection of the human conjunctiva by psittacosis or ornithosis agents has not been reported. In subhuman primates, infection with TRIC agents produces an inclusion-positive follicular conjunctivitis. Psittacosis agents, on the other hand, are said to be incapable of producing conjunctivitis in the primate conjunctiva, although multiplication of the agent has been demonstrated (Thygeson, unpublished). This paper reports a case of human follicular conjunctivitis resulting from a laboratory infection with a Bedsonia apparently of psittacine origin.

The patient, a 27-year-old laboratory technician at the G.W. Hooper Foundation, was first seen June 8, 1966, after suffering for one week from foreign-body sensation, discharge, and redness in the left eye. On examination she had a small palpable left preauricular node, a slight ptosis in the left eye, conjunctival follicles in the lower fornix and on the upper tarsal plate, and moderate papillary hypertrophy. During the following week, discrete epithelial erosions on the cornea and small grey corneal infiltrates were observed.

On June 15, 1966, the patient was given 1 gm of tetracycline orally. This was continued daily for 2 months. The follicular conjunctivitis slowly subsided over the next month. She had epithelial keratitis and subepithelial infiltrates until late November, 1966.

Materials and Methods. Clinical specimens. The conjunctiva was anesthetized with 0.5% proparacaine topically. Specimens were obtained by scraping the conjunctival surface with a sterile platinum spatula. This material was spread on glass slides for cytologic studies or collected in antibotic broth for isolation attempts.

Cytology. Slides were air-dried and fixed in methanol for Giemsa stains. Immunofluorescent staining techniques were also applied to conjunctival smears. These techniques have been described elsewhere (2, 3).

Isolation attempts. Streptomycin-treated conjunctival scrapings were inoculated into the yolk sac (YS) of embryonated hens' eggs. Two different isolation techniques were performed by two different laboratories (JS and LH) in order to rule out any possibility of cross contamination. These techniques have been reported elsewhere (2, 4).

Serology. Complement fixation (CF) tests were performed as described by Meyer and

* This work was supported in part by U. S. Public Health Service grants AI-07698, AI-04406, NB-00604, NB-06207, and the Burroughs Wellcome Fund.

† Address: The George Williams Hooper Foundation, University of California Medical Center, San Francisco, California 94122.

Eddie (5), using a boiled and phenolized antigen prepared from the psittacosis isolate, 6BC.

Pathogenicity tests. Tissue culture monolayers were prepared with L929 and HeLa 229 cells. The cells were grown in medium 199 with 10% inactivated calf's serum. Monolayers were infected by incubation for 2 hours with 10% YS suspension. Cells were then washed with Hanks' balanced salt solution and incubated at 35°C in complete media.

Mice were inoculated intraperitoneally (IP) with 0.5 ml of serial tenfold dilutions of 10% YS suspension; intracerebrally (IC) with 0.025 ml; intranasally (IN) with 0.025 ml.

Several avian species were infected with the YS suspension(s). These included parakeets, turkey poults, and chickens. The host species tested were ascertained to be free of natural Bedsonia infection.

Attempts were made to infect the conjunctiva of subhuman primates. Three macaques *(Macaca mulatta)* and two langurs *(Presbytis entellus)* were infected by dropping a 50% YS suspension of the first egg passage of the isolated Bedsonia onto the conjunctiva of the right eye. The infected YS suspension had been stored at —70°C for 9 months. There was little change in egg infectivity during this time. The monkeys were examined at weekly intervals; conjunctival scrapings were taken for Giemsa staining, immunofluorescent tests, and reisolation attempts.

Results. Cytology. Immunofluorescent stains of the patient's conjunctival scrapings taken before and after the initiation of treatment were negative by the two techniques used. In both laboratories, the antisera had been prepared against TRIC agents. Giemsa stain revealed few inflammatory cells, primarily polymorphonuclear neutrophils. At no time were inclusions found.

Isolation. Results of isolation attempts made before the initiation of antibiotic therapy were positive in both laboratories. In one laboratory (JS), isolation attempts involved blind passage of yolk sacs 13 days post inoculation of the clinical specimen(s). In this laboratory all the eggs died between day 10 and day 12 of the first passage in two specimens collected before tetracycline treatment. The isolate was given the laboratory designation 197F.

The other laboratory (LH) followed a regimen of blind passage 7 days after inoculation of the clinical specimens. In this laboratory, egg deaths occurred on the third and fourth days of the second passage and elementary bodies were observed in the YS impression smears. The isolate was designated Cal-USA/Cal-29/0.

Results of isolation attempts were also positive 3 weeks after the initiation of tetracycline therapy, but further isolation attempts were negative.

The agent isolated was identified as a member of the Bedsonia group by the presence of a group CF antigen in infected YS suspensions. First passage YS material was inoculated into L and HeLa cells, and the monolayers were stained with iodine and with Giemsa at 16, 20, 24, 36, and 48 hours post infection. The inclusions formed did not take up the iodine stain and did not appear in a vacuole as do the inclusions formed by TRIC agents.

Serology. Serum specimens collected prior to the onset of symptoms did not fix complement with the psittacosis group antigen. A serum specimen collected at the initial examination was positive at a dilution of 1:4. The CF titer rose to 1:16 and 1:32 in 2 and 3 weeks, respectively. After tetracycline therapy the patient become seronegative.

Mice. Second YS passage material was inoculated into mice. The isolated Bedsonia was lethal for mice by i.c., i.n., and i.p. routes. Titers ranged from 10^4 to 10^5 LD_{50} per gram of yolk sac.

Avian hosts. A 10% suspension of third passage YS material of isolate 197F was inoculated intramuscularly into healthy mature parakeets. Eight inoculated parakeets were placed in contact as cage mates with three susceptible parakeets. The inoculated birds died 6–12 days postinfection and their postmortem appearance was typical of parakeet psittacosis; air-sac smears were positive for Bedsonia. The three contacts became ill a few days after the death of the inoculated birds and two died; these also had postmortem findings typical of parakeet psittacosis and air-sac smears were positive. Agent 197F was also lethal for young chicks and turkey poults, 6 and 8 days old, respectively.

Comparative studies. The likelihood that

isolate 197F was one of the laboratory strains that had been handled by the technician was explored. Comparative pathogenicity studies were made of the eight Bedsonia agents handled in the laboratory during the month before the patient's original symptoms. Agent 197F was virulent for mice by the i.p. route, whereas the agents of bovine enteritis, India Paddy Bird, and India Heron were not. Bedsoniae derived from a muskrat (M56), synovial tissue from a patient with Reiter's syndrome (25SM) and aspirate from an inguinal lymph node of a patient with clinical lymphogranuloma venereum (33L) were not regularly lethal for parakeets when injected intramuscularly, whereas 197F was. A Mexican parrot isolate (X460) was not regularly lethal for turkey poults, whereas 197F was. Agent 197F was identical in pathogenicity pattern with 51C, which was isolated 6 years ago from a naturally infected parakeet. Agents 197F and 51C were both lethal for mice by the i.c., i.p., and i.n. routes; for parakeets by the i.m. and i.c. routes; for finches, rice birds, turkey poults, and chicks by the im. route.

Monkeys. Infected monkeys developed a follicular conjunctivitis within 7 days after infection. Conjunctival scrapings taken at day 7 were negative for inclusions although there was a marked inflammatory cell response and many Leber cells were seen. Similar cytologic findings were made 14 days after infection. The follicular conjunctivitis that developed was exceedingly mild and only the use of an uninoculated eye as a control in the same animals made the reaction apparent. Six weeks after infection one of the four monkeys had developed a marked lymphoid response with follicles across the width of the upper conjunctival fornix. The other animals had minimal changes. Results of reisolation attempts were positive in all animals at week 2 and week 6. The eggs all died in the first passage, even though conjunctival scrapings during this period were inclusion-negative and showed only a minimal inflammatory response after the first week.

Discussion. It has been thought that the TRIC agents were the only Bedsonia agents capable of producing follicular conjunctivitis in man. In this study, a laboratory infection, probably with a parakeet Bedsonia, resulted in a follicular conjunctivitis. There were no systemic signs of disease in this patient and infection apparently was limited to ocular sites. The patient's CF antibody rose significantly from 0 to 1:32, although this titer dropped to zero after treatment with tetracycline. The initial clinical impression suggested an adenovirus infection. Cytologic studies did not shed any light and it was only after isolation of a Bedsonia that the true nature of the infection was revealed. The ease and rapidity with which the Bedsonia isolations were made was unusual, considering the negative cytologic studies with few inflammatory cells. Pathogenicity studies on the isolated agent indicated that it was of parakeet origin.

The disease in this patient was not identical with the clinical syndromes of trachoma or inclusion conjunctivitis. There was pronounced corneal involvement, but the conjunctival reaction was limited. Treatment was difficult and it was still possible to isolate Bedsonia 3 weeks after tetracycline administration began. Although the conjunctival reaction diminished, some corneal lesions were still evident 6 months after the infection was diagnosed.

It is worth noting that it was the patient's occupation that prompted the attempts to isolate Bedsonia from her conjunctiva. Indeed, the possibility that a psittacosis agent might have been involved induced the two laboratories to pursue isolation attempts independently, in order to rule out any possibility of cross contamination. An etiologic diagnosis was possible only because this patient worked in a laboratory with these agents and because such isolation studies could be performed. A similar disease picture, seen elsewhere, would probably not have been properly diagnosed, for there was neither precedent for such infection nor cytologic evidence (no inclusions found) of its nature.

In this case, there was probably direct contamination of the conjunctiva with the psittacosis agent during laboratory manipulation. However, direct inoculation of LGV agent has not produced follicular conjunctivitis in man or experimental animals. Previous attempts in this laboratory to produce follicular conjunctivitis in the subhuman primate by in-

oculation with psittacosis or ornithosis agents have failed. The agent isolated in this study did cause follicular conjunctivitis in *M. mulatta*.

Several Bedsoniae have been shown to cause follicular conjunctivitis in lower animals. The ocular diseases in the guinea pig (6) and the cat (7) have been studied and others will undoubtedly be found. Although this single case probably resulted from a laboratory infection, it is possible that infection with members of the Bedsoniae, other than TRIC agents, may cause follicular conjunctivitis in man. This study indicates that the tissue tropisms, formerly thought to be a major differential factor among the Bedsoniae, are not absolute. TRIC agents have generally been defined as being Bedsoniae which are sensitive to sulfonamides, produce an iodine-staining inclusion, do not kill mice (i.c., i.p., or i.n.) and are the only Bedsonia to cause follicular conjunctivitis in primates. However, human infection with TRIC agents is not limited to the conjunctiva (8). The agent isolated in this study differed from TRIC agents in being pathogenic for mice and other animals and growing in tissue culture without producing an iodine-staining inclusion. The latter characteristic has been used in attempts to divide the Bedsoniae into two groups (9). The morphologic and biochemical interpretations for differentiation may well be valid (10) but the ability to produce follicular conjunctivitis in man and the subhuman primates does not seem to be limited to TRIC agents. In the enormous array of parasites that the Bedsoniae represent, tissue tropisms apparently will not be valid taxonomic tools. A spectrum of pathogenic qualities will undoubtedly be found as more of these agents are studied.

Summary. A laboratory technician engaged in research on Bedsoniae developed a follicular conjunctivitis. A Bedsonia was isolated from conjunctival scrapings by two different laboratories. Experimental infection of subhuman primates resulted in a follicular conjunctivitis. The isolate was characterized as a psittacosis agent and clearly differentiated from the TRIC agents which had been thought to be the only Bedsoniae capable of producing follicular conjunctivitis in man. The validity of using tissue tropisms as taxonomic tools within the Bedsoniae is questioned.

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Received Sept. 5, 1967. P.S.E.B.M., 1968, Vol. 127.