

DARROW
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A PROPOSAL FOR LABORATORY INVESTIGATION OF
KAPOSI SARCOMA/OPPORTUNISTIC INFECTIONS
AND "LYMPHADENOPATHY SYNDROME" PATIENTS
TO SEARCH FOR AN HYPOTHESIZED MICROBIAL
AGENT CAUSING IMMUNODEPRESSION

I. INTRODUCTION

Since the beginning of CDC's involvement in investigation of the national outbreak of Kaposi sarcoma and opportunistic infections (KS-OI), CID has performed over 6,000 laboratory tests on specimens from approximately 400 patients or matched controls. Such a great expenditure of time and effort was considered necessary because of the potential public health importance of the outbreak, and the unknown cause of these diseases.

Preliminary evaluation of information and specimens obtained from case and control patients enrolled in the case-control study, and from case patients not enrolled in the case-control study, suggests the following outbreak scenario. The epidemic began in 1979 or 1980 in homosexual men and male drug addicts in New York City, Los Angeles, San Francisco, and perhaps other American cities, including Atlanta. None of the affected men have had underlying disease, their past medical histories being similar to those with similar lifestyles. The "outcomes" believed to be part of the same general problem include Kaposi sarcoma, Pneumocystis carinii pneumonia, and various other "opportunistic" infections, including atypical mycobacterioses, toxoplasmosis, candidiasis, cryptococcosis, PML, progressive herpes simplex disease, and others. It is thought that the "lymphadenopathy syndrome", a poorly defined condition predominantly identified in homosexual men and characterized by malaise, fever, lymphadenopathy, and weight loss may also be a part of the same problem, either as a prodromal condition, or as another manifestation. Furthermore, it is hypothesized that immunodepression underlies all of these conditions: although evidence is incomplete, and patients have not been found to be immunodepressed before illness onset, it is clear that at initial evaluation most patients have immunologic abnormalities not normally associated with the specific conditions for which they present. These abnormalities are predominantly related to defective cell-mediated immunity, including inversion of the helper:suppressor T-cell ratio to reflect an excess of T-suppressor cells. It is therefore hypothesized that some "event" has resulted in an outbreak of homotypic immunodepression which, in conjunction with microbial or other agents to which the case patients are intensely and repeatedly exposed by virtue of their lifestyles, results in the recognized outcomes: Kaposi sarcoma, Pneumocystic carinii pneumonia, and other opportunistic infections.

An early contender for that event was drugs, especially nitrite inhalants, used or abused by virtually all of the cases in the case-control study. CID and NIOSH are currently conducting nitrite exposure studies to determine whether sparged butyl nitrite may cause immunologic abnormalities in inbred laboratory mice. While the question is still open, two lines of evidence suggest that nitrites may not be the sole answer: recently reported male and female heterosexual cases generally deny exposure to nitrites; and preliminary data from the case-control study has been insufficient to implicate nitrites as other than a lifestyle-associated confounder.

While the issue of nitrites is being pursued intensively, it is essential that CDC explore other possible causes of immunodepression in these patients. In developing hypotheses, several questions should be borne in mind. Why did the outbreak arise only in homosexual men and abusers of intravenous drugs?

Why not in promiscuous heterosexuals, including prostitutes? Why not homosexual women? What factors exclusively associated with the lifestyles of homosexual men and intravenous drug abusers might provide clues to the etiology? And most importantly, are there known medical conditions which exclusively or disproportionately affect homosexual men and intravenous drug abusers that could serve as disease models?

In the last decade, virologists have documented that two viral diseases transmitted both venereally and intravenously are more incident and prevalent in homosexual men and intravenous drug abusers: hepatitis B and cytomegalovirus. In hypothesizing an infectious cause of immunodepression in the outbreak under discussion, it may be helpful to reflect upon at the following aspects of these two conditions. First, in both diseases a prolonged viremic phase associated with demonstrably infectious particles may occur. Secondly, excretion of infectious particles in both the semen and colon may occur. These two facts alone explain why the diseases are so highly incident in the populations under consideration: drug abusers are the major subpopulation exposed intravenously to unscreened, unsanitary, and potentially infectious human blood; and homosexual men are the major subpopulation repeatedly and continuously exposed to potentially infectious colonic material, and to potentially infectious semen, under circumstances that may routinely permit exposure to the intravascular space (via abrasions, strictures, and other lesions).

In searching for an infectious cause of immunodepression preceding KS-OI, this model suggests that we look closely at blood (cells and serum), and also stool and semen.

The following proposal takes these observations into consideration, and suggests in addition that other sites of possible excretion of a microbial agent be examined (Table).

II. METHODS

Specimens will be obtained from 15 living patients, 5 with KS, 5 with PCP, and 5 with the lymphadenopathy syndrome, according to the following criteria: good documentation of disease, good general clinical and laboratory work-up, availability, and desire to cooperate on the part of the patient and physician.

A CDC physician will evaluate each candidate to determine that patient's suitability for inclusion in the study. After obtaining informed consent of the patient and approval of the patient's primary physician, the investigator will complete an epidemiologic questionnaire similar or identical to that used in the case-control study. Thereupon, the investigator will obtain the following laboratory specimens according to an established protocol (Appendix A): throat swabbing in transport medium, stool, urine sample (100 cc), semen sample, serum sample (40 cc in red-topped tubes) and uncoagulated whole blood sample (80 cc in tubes with added sodium heparin without preservative). The specimens will be immediately placed on dry ice or in liquid nitrogen at the examination site and will be stored under the same conditions until such time as they are tested at CDC.

At CDC, aliquots of all starting specimens will be banked at -70C. Specimens will be tested according to the scheme outlined in the table. Briefly, the following studies will be performed: serology--EBV-EA, EBNA, VCA-IgG, VCA-IgM; CMV-CF, IHA; HSV type 1-CF, IHA; HSV type 2-CF, IHA; Adenovirus group CF; BK virus-HI; JC virus-HI; SV-40-neutralization; hepatitis A virus-RIA; hepatitis B virus-anti-surface antigen, anti-core antigen, and

anti-e antigen; and attempts at isolation in PMK, HEK, HELF, and E6 Vero cells. In cell culture attempts, the cells and supernatant from all except the challenge-negative tubes will be separated, stored separately, and saved for possible future study. Indicators of viral replication or infection will include CPE, interference with echovirus 11 challenge, indirect immunofluorescence with a convalescent pool (described below) and a goat or rabbit anti-human conjugate, EM, and development of transforming foci. Additional studies to detect a virus will include assay for hepatitis B surface antigen, EM of urine, IEM of stool with the convalescent pool, attempts to transform cord blood leukocytes, and the following studies of the WBC buffy coat: inoculation onto HEK and HEL cells, attempt to establish a culture, inoculation with at least one continuous B-cell and T-cell line, and FA study of acetone fixed smears for VCA, EBNA, and unspecified antigen to be detected by the convalescent pool in an indirect test.

A convalescent serum pool may have to be obtained from 5 different individuals because of the need for adherence to two additional criteria: recovery (ideally) or prolonged remission from disease, and willingness to provide a unit of whole blood.

In the absence of a good definition of "recovery", it will be desirable to choose patients who appear to have been in long-term remission of their disease (e.g., KS), or who have been successfully treated for a serious opportunistic infection to the extent that they are able to return to work and pre-illness lifestyle.

Although under ideal circumstances tumor biopsy specimens could be obtained from the 5 KS patients, and lymph node specimens from them and the 5 lymphadenopathy patients, it is unlikely that such specimens will be available from most otherwise cooperative patients. The aim of this investigation will be to intensively study specimens from 5 autopsies, and additionally 5 lymph nodes from patients with the lymphadenopathy syndrome, and tumor biopsies from 5 patients with KS. Consequently, additional sets of specimens will need to be obtained from documented cases undergoing biopsy and autopsy, according to an established protocol (Appendix B). Autologous control tissue will be obtained at autopsy, and when possible, at biopsy of living patients with fully informed consent.

As outlined in the table, all such biopsies will undergo thorough pathologic examination by Host Factors Division, CID. Impression smears will be made and tested by indirect FA for EBV antigens, T-antigen, and unspecified antigen to be detected by the convalescent pool. Tissue homogenates in PBS with antibiotics will also be inoculated onto cell culture, and lymph node specimens will be subjected to the same procedures outlined above for peripheral WBC. All specimens will undergo EM examination, and will be inoculated into mice to look for immunosuppression (Host Factors Division, CID). Attempt will also be made to start a tumor line. Tumors will be probed with CMV, HSV, selected adenoviruses, and if available, EBV probes.

All viral isolations will be investigated according to established procedures for identification. In addition, insofar as is technically feasible, isolations of a common agent from two or more patients will be analyzed by molecular virologic techniques (e.g., restriction endonuclease analysis in the case of DNA viruses) to establish or rule out strain identity.

