

Mycobacteriophage in Crohn's disease

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SUMMARY Numerous similarities between Crohn's disease and sarcoidosis are being reported. Because of previous findings of culturable mycobacteriophage in the serum of many patients with sarcoidosis, mycobacteriophages were sought in serum of patients with Crohn's disease. No difference was found in the frequency of positive cultures between patients with Crohn's disease and normal control subjects.

Crohn's disease is similar to sarcoidosis in the presence of the granulomas, anergy (Jones, Housley, Ashurst, and Hawkins, 1969; Walker and Greaves, 1969; Parent, Barrett, and Wilson, 1970; Brown, Taub, Present, and Janowitz, 1970), reactivity to Kveim antigen (Mitchell, Cannon, Dyer, Hinson, and Willoughby, 1970), and in the possible presence of a transmissible agent in diseased tissue (Mitchell and Rees, 1970). These likenesses suggest that the two diseases may have related causes. A high incidence of mycobacteriophages cultured from sera taken from patients with sarcoidosis has previously been reported (Mankiewicz and Liivak, 1967). The purpose of this study was to determine if a similarly high incidence of mycobacteriophage is present in sera from patients with Crohn's disease when compared with a group of age-matched control subjects.

Materials and Methods

SUBJECTS

Nineteen patients with typical Crohn's disease and 18 control subjects were studied. Healthy control subjects, matched for age and sex, were obtained in all but one instance. Most of the control subjects were hospital employees.

METHODS

Blood samples were obtained by sterile technique at the University of Minnesota. The serum was separated, coded, and sent to Dr. Edith Mankiewicz at the Royal Edward Chest Hospital, Montreal, Canada, who kindly performed cultures for myco-

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bacteriophage. Her description of the culture technique follows:—

The sera were received by mail. They were filtered on Millipore membrane filters (0.22 μ m) and 0.5 ml amounts were added to each of three screwcap tubes containing 4.5 ml of Redmond and Ward's (1966) RVB medium from which the albumin V fraction had been omitted. One tube was inoculated with 0.2 ml of a Tween-albumin culture of *M. tuberculosis* var. hominis, strain H37Rv; another with the same amount of a culture of *M. tuberculosis* var. bovis, strain BCG, and the third tube was inoculated with *M. smegmatis*, strain ATCC 607. The cultures used for the preparation of the inocula were in their log phase of growth and had been examined for purity and the absence of lytic particles. After these cultures, incubated at 37°C, had reached the end of their log phase of growth, they were filtered on membrane filters and droplets of the filtrates were deposited on lawns of the three indicator strains. When clear plaques appeared on the bacterial lawns, they were isolated in RVB medium enriched with the indicator strain. These cultures were filtered and the lytic activity of the mycobacteriophage was determined on lawns of pathogenic and of saprophytic mycobacteria. When the filtrates of the first cultures containing the patient's serum did not show lytic activity, they were added to newly prepared cultures of indicator strains. 'Blind' passages were repeated three times and the filtrates assessed for lytic activity before a 'negative' result was recorded.

The method used for isolation of mycobacteriophage from serum has been previously described (Mankiewicz and Liivak, 1967). All cultures were

completed and the results tabulated before the code was broken. The Chi square test was used to compare results from the two groups.

Results

The results of cultures of serum for mycobacteriophage in patients with Crohn's disease and in age-matched normal subjects are shown in Table I. No difference was found in the frequency of positive cultures between the two groups ($P > 0.90$).

Subject	No. Tested	Positive
Crohn's disease	19	3
Normal control	18	4

Table Comparison of positive cultures for mycobacteriophage between patients with Crohn's disease and age-matched normal control subjects

Discussion

The likeness of histological findings in Crohn's disease and sarcoidosis was first noted years ago. Now, in addition, it appears that a degree of anergy is characteristic of both diseases (Jones *et al.*, 1969; Walker and Greaves, 1969; Parent *et al.*, 1970; Brown *et al.*, 1970). Although studies of reactivity to Kveim antigen in Crohn's disease have yielded conflicting results, recent data have indicated that over 50% of patients with Crohn's disease will have a positive Kveim test (Mitchell *et al.*, 1970). Since a positive Kveim test may merely represent a reaction associated with persistent adenopathy (Israel and Goldstein, 1971), this similarity may not be a reflection of related aetiologies. Finally, circumstantial evidence for incriminating transmissible agents in the pathogenesis of both diseases has been reported (Mitchell and Rees, 1970).

A recent report has shown that mycobacteriophage can frequently be cultured from serum of patients with sarcoidosis, an uncommon finding in serum from normals and patients with tuberculosis (Mankiewicz and Livaak, 1967). Tuberculous patients have high titres of serum phage neutralizing antibodies while patients with sarcoidosis usually have low or absent titres (Mankiewicz and Van Walbeek, 1962). Neither the significance nor the pathogenetic importance of these observations has yet been established. However, these findings have

led to a postulate that sarcoidosis might represent an infection with lysogenic mutants of tubercle bacilli.

In view of the apparent relationship between sarcoidosis and Crohn's disease, and because of the recent suggestion that Crohn's disease might be caused by an infection with atypical mycobacteria (Morganroth and Watson, 1970), it seemed pertinent to study patients with Crohn's disease for mycobacteriophage. Serum was chosen because of availability, ease of shipping, and the positive cultures found in patients with sarcoidosis. Our findings indicate that, unlike sarcoidosis, an increased incidence of positive cultures of serum for mycobacteriophage is not characteristic of patients with Crohn's disease.

These data suggest two possibilities to us. First, if mycobacteria infected with phage are important in the pathogenesis of sarcoidosis and Crohn's disease, the differences in the cultural results may provide a fundamental clue to basic differences in the diseases. The alternative, and perhaps more likely, suggestion would be that mycobacteriophage are not important in the pathogenesis of Crohn's disease and that the similarities to sarcoidosis are based on other undefined factors.

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