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STUDIES ON GRANULOMA INGUINALE

V. ISOLATION OF A BACTERIUM RESEMBLING *DONOVANIA GRANULOMATIS* FROM THE FAECES OF A PATIENT WITH GRANULOMA INGUINALE*†

BY

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Anderson (1943), and later Anderson, DeMonbreun, and Goodpasture (1945) were able to isolate a bacterium from the lesions of three cases of granuloma inguinale. These authors were able to show that this bacterium, provisionally named *Donovania granulomatis*, morphologically resembles the Donovan body and that, when prepared as antigen, it reacts with sera from cases of granuloma inguinale. The bacterium has unusual nutritional requirements, requiring egg-yolk material for growth and does not grow on any of the enriched media used to grow fastidious bacteria. These unusual requirements are strong evidence that this bacterium is not similar to any of the various bacteria previously isolated from granuloma inguinale.

In a previous publication (Goldberg, 1959), we indicated that there is increasing evidence for assuming that *Donovania granulomatis*, the supposed aetiological agent of granuloma inguinale, might be a faecal organism.

The purpose of this report is to present data which indicate that a bacterium has been isolated from the faeces of a patient with proven granuloma inguinale, which is morphologically and antigenically very similar or identical to the strains of *D. granulomatis* which have been isolated from clinical cases of granuloma inguinale.

In subsequent papers, additional evidence will be presented tending to support the faecal habitat of the aetiological agent and the epidemiology of the disease will be discussed in more detail.

Isolation of the Bacterium

The patient from whom the bacterium was isolated is a 55-year-old female Jamaican referred to Dr. D. B. Stewart, Professor of Obstetrics and Gynecology, University College of the West Indies, who diagnosed granuloma inguinale of the cervix. The biopsy report stated:

"Granulation tissue with small collections of polymorphonuclear leucocytes and histiocytes, compatible with granuloma inguinale."

A smear prepared from the lesion showed Donovan bodies, and in addition, the complement-fixation test for granuloma inguinale was positive to a titre of 1:20.

A faecal specimen was obtained from the patient and frozen at −20°C. The material was transported to our laboratory in the frozen state and kept frozen until just before use.

The specimen was thawed and emulsified in sterile saline. Serial dilutions in sterile saline were made and several 1-ml. aliquots of each of the dilutions were inoculated into tubes of lactalbumin hydrolysate (brilliant green) sodium azide medium. Previous work in our laboratory had indicated that sodium azide in the concentration of 0·4 mg./ml. and brilliant green 0·2 mg./ml. were not inhibitory to the three strains of *Donovania* under cultivation. Lactalbumin hydrolysate medium containing the brilliant green and sodium azide in the above concentrations was prepared.*

The inoculated tubes were incubated at 37°C. for 4 days. Gram- and Wright-stained preparations were made from each tube. Most of the tubes contained mixed bacteria, but what appeared to be a pure culture of a Gram-negative bacterium with the typical bipolar appearance of *Donovania* when stained with Wright's stain was observed in several of the tubes. Transfers were made from these tubes into lactalbumin hydrolysate medium without inhibiting agents. Serial cultivation of the bacterium was accomplished without difficulty. Aliquots of the culture were streaked upon blood agar plates and no growth was observed either under aerobic or anaerobic conditions. When the bacterium was inoculated into the yolk sac of 5-day-old embryonic eggs, the organism grew without causing the death of the embryo. Smears of the yolk material from these eggs, stained with Wright's stain, showed bacteria indistinguishable from *Donovania*.

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* For method of preparation see Goldberg (1959).

Identification of the Organism

The identification of a member of the genus *Donovania* presents great difficulty, for few strains have been isolated and characterized. Fortunately, we have under cultivation in our laboratory three strains of *Donovania*, including the prototype, the Anderson strain. It was thus possible to compare our isolate with these strains, not only with reference to their morphological and cultural characteristics, but also to their antigenic similarity.

The following characteristics have been found to be consistent with all strains of *Donovania* that we have examined:

I. MORPHOLOGICAL

(1) Frankly Gram-negative, non-spore-forming rod, showing no evidence of bipolar staining with the Gram technique, but typical bipolar staining with Wright's stain.

(2) Pleomorphism, not necessarily associated with one transfer, but which might be present from transfer to transfer. A characteristic feature, and one which is difficult to describe, is the variation of morphology from transfer to transfer. On repeated transfers one may detect subtle differences; one transfer may contain cells which are somewhat longer than usual, may have less bipolar staining, etc., while in the next transfer the bipolar staining may be very pronounced and the typical safety-pin form commonly seen.

II. CULTURAL

(1) Dependence upon egg-yolk material for growth. It has been found that lactalbumin hydrolysate may replace the egg-yolk factor/s.

(2) Growth only in liquid media. Inability to grow either aerobically or anaerobically on solid media.

(3) Growth in single cells or very short chains of two to three organisms. Absence of clusters of bacteria. Occasional appearance of a matrix surrounding cells.

Using the above criteria, the isolated bacterium, named Jamaica 1–59 strain, was found to be identical culturally and morphologically with all of the standard strains of *D. granulomatis*.

III. SEROLOGICAL

The antigenic analysis of the Jamaica 1–59 strain was done using two groups of sera. The first group comprised 33 sera from patients with granuloma inguinale, which had been found to be reactive with antigens prepared from strains of *D. granulomatis* isolated from clinical cases of granuloma inguinale. Fifteen sera were from patients residing in the United States and eighteen from Jamaicans. Each serum was tested against bacterial antigens prepared from the Anderson strain of *D. granulomatis*, the Franklin strain*, and the Jamaica 1–59 strain. These antigens were essentially suspensions of washed bacterial cells of the respective strains in buffered saline. In addition, a filtrate antigen of the Anderson strain was also used. This latter antigen is a protein-free antigen prepared from the bacterium when it still retained its original ability to produce large amounts of carbohydrate material in the culture medium. It most probably represents capsular material. None of the antigens showed any anticomplementary activity. The usual complement-fixation test with a total volume of 1 ml., each reagent at 0·2-ml. volume, was used. Primary incubation was 75 minutes at 37°C. The highest dilution of serum showing three to four plus fixation of complement was the end-point.

The results obtained are outlined in Table I (opposite).

Of the fifteen sera from granuloma inguinale cases occurring in the United States, seven reacted in a positive manner. Of the eighteen sera from Jamaican patients, seven were positive. It will be noted that many of the sera were not positive to *any* of the *bacterial* antigens tested. Of the fifteen sera which were positive to the Anderson and Franklin *bacterial* antigens, eleven were positive to the Jamaica 1–59 *bacterial* antigen. In addition, three sera were positive to the Jamaica 1–59 bacterial antigen but negative with the others. The antigenic components of *Donovania* vary and each strain is a complex of antigens, many of which are shared by other members of the species (Goldberg, 1956). The results obtained accord with these previous findings.

Further to compare the antigenic components of the Jamaica 1–59 strain with the other strains, antisera against the Jamaica 1–59 and Anderson strains were prepared. Originally rabbit antisera were prepared, but it was subsequently found that guinea-pigs produced a much more potent antiserum. The guinea-pigs were inoculated with multiple intradermal inoculations of equal volumes of bacteria and complete Freund's adjuvant. Four closely-spaced intradermal inoculations per pig were given with a total volume of approximately 0·2 ml. 2 weeks after the inoculation, the guinea-pigs were bled by cardiac puncture and the serum tested with the homologous

* The Franklin strain of *D. granulomatis* was isolated from a pseudobubo in a patient with granuloma inguinale in 1949.

TABLE I

SEROLOGICAL REACTIONS OF VARIOUS ANTIGENS WITH SERA FROM CASES OF GRANULOMA INGUINALE

Case No.	Source of Serum	Antigens Used			
		Bacterial			Filtrate (Anderson)
		Anderson	Franklin	Jamaica 1–59	
1	U.S.A.	0	0	0	80
2		0	0	0	20
3		0	0	0	10
4		0	0	0	640
5		0	0	0	80
6		40	0	0	80
7		80	0	0	80
8		0	20	5	0
9		10	0	10	0
10		160	160	320	0
11		10	10	0	0
12		0	0	10	0
13		320	80	10	0
14		160	160	40	0
15		10	5	5	0
16	Jamaica	0	0	0	10
17		0	0	0	10
18		0	0	0	160
19		0	0	0	320
20		0	0	0	20
21		0	0	0	40
22		0	0	0	160
23		0	0	0	80
24		0	0	0	20
25		0	0	0	80
26		80	40	80	640*
27		640*	10	0	80
28		160	0	40	640*
29		0	0	20	0
30		0	0	20	0
31		10	40	10	0
32		40	20	40	0
33		0	20	80	0

* = 4 plus at highest dilution tested.

and heterologous antigens. In addition, both of the antisera were absorbed with the homologous and heterologous antigens and re-tested with each of the antigens. The absorptions were carried out by mixing equal volumes of bacterial cells and antiserum. The mixture was kept at 37°C. for 60 minutes and refrigerated overnight. In the morning, the mixture was centrifuged at 14,000 G and the supernatant serum tested in the usual manner. The complement-fixation test as outlined above was used.

The results obtained are outlined in Table II. The antigen prepared from the Jamaica 1–59 strain reacted with the homologous serum to a titre of 1:640 and also with the Anderson antiserum to a titre of 1:80. The Anderson antigen reacted with the Jamaica 1–59 antiserum to a titre of 1:2560 (two dilutions higher than the homologous antigen) and also to the Anderson antiserum to a titre of 1:160. This is evidence that the two strains do have antigens in common. One absorption of each antiserum by either of the antigens lowered the titres for either antigen when the absorbed antiserum was re-tested; this is a further indication that the antigenic make-up of the two organisms is very similar.

TABLE II

RESULTS OF COMPARISON OF JAMAICA 1–59 AND ANDERSON STRAINS

Antiserum Strain		Jamaica 1–59			Anderson		
Treatment . .		None	Absorbed with		None	Absorbed with	
			Jamaica 1–59	Anderson		Jamaica 1–59	Anderson
Antigen Tested	Jamaica 1–59	640*	40	160	80	10	10
	Anderson	2,560	80	320	160	40	20

* Reciprocal of highest titre showing 3 or 4 plus inhibition of haemolysis.

Discussion

The identification of members of the genus *Donovania* presents great difficulty. The strains which have been isolated previously have all been isolated from clinical lesions of the disease, either from the cutaneous lesion or from material aspirated from the psuedobuboes. With such organisms it is usually sufficient to demonstrate a frankly Gram-negative reaction, typical morphology when stained with Wright's stain, inability to grow on all of the common laboratory media, and the necessity of egg-yolk material for growth. When such an organism was isolated from the faeces, it was felt necessary further to confirm the identity by serological methods. The results indicate that the isolated bacterium was indeed a *Donovania*. The morphology, growth characteristics and cultural requirements were identical with those of the other strains isolated from clinical lesions. In addition, the antigenic analysis indicated that the isolated strain contained antigens in common with all three of the so-called "standard" strains. We feel that these findings confirm the faecal habitat of the *Donovania* and may shed light on the epidemiology of the disease. A more detailed discussion of this will be presented in a subsequent communication.

The significance of the fact that the patient from whose faeces the strain was isolated also had granuloma inguinale is not known. Further work is in progress to determine whether additional strains of *Donovania* can be isolated from patients with granuloma inguinale and possibly from the faeces of normal individuals.

Summary

A bacterium has been isolated from the faeces of a patient with granuloma inguinale. This bacterium has been found to be morphologically similar to *Donovania granulomatis*, to have cultural characteristics identical with *D. granulomatis*, and to react with sera from patients with granuloma inguinale to the

same degree as antigens prepared from known strains of *D. granulomatis*. In addition, this bacterium was shown (by the use of complement-fixation tests utilizing the absorbed antisera) to contain antigens similar to those possessed by the prototype strain of *D. granulomatis*.

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Etude du granulome inguinal
V. Culture de bactérie ressemblant au *Donovania granulomatis* des fèces d'un cas de granulome inguinal

RÉSUMÉ

Une bactérie cultivée des fèces d'un malade atteint de granulome inguinal ressemblait au *Donovania granulomatis* des points de vue sérologique et morphologique.

On trouva d'ailleurs, par la fixation du complément avec l'antisérum absorbé, que cette bactérie contenait des antigènes semblables à ceux de la souche archétype du *Donovania granulomatis*.