IMMUNOLOGICAL ASPERMATOGENESIS IN MAN

I. BLASTOID TRANSFORMATION OF LYMPHOCYTES IN RESPONSE TO SEMINAL ANTIGEN IN CASES OF NON-OBSTRUCTIVE AZOOSPERMIA

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Summary. Lymphocytes from twenty-one (16.6%) of 126 men with non-obstructive azoospermia or severe oligospermia showed blastoid transformation in culture when exposed to normal semen homogenate, indicating their ability to produce antibodies against seminal antigen(s).

In the diagnosis of aspermatogenesis of immunological origin, the blastoid transformation test is believed to have advantages over other tests which depend on the direct estimation of circulating antibodies against spermatozoa.

There is evidence that testicular injury may be involved in the production of immunological aspermatogenesis in man.

INTRODUCTION

Clinical experience shows that knowledge of the aetiology and pathogenesis of male infertility is far from complete. Although many infertile husbands can readily be assigned to a definite category, others have nothing in their history or examination that assists the examiner in his survey and this particularly applies to certain cases of severe oligospermia and azoospermia. It has been shown that the infertility of one group of these affected males may be due to auto-immune factors (Wilson, 1954, 1956; Rümke & Hellings, 1959; Nakabayashi, Tyler & Tyler, 1961; WHO report, 1966).

Lymphocytes cultured *in vitro* show blastoid transformation and mitosis when exposed to an antigen to which they have been previously sensitized (Pearmain, Lycette & Fitzgerald, 1963; Hirschhorn, Bach, Kolondy, Firschein & Hashem, 1963; Hashem & Barr, 1963; Elves, Roath & Israels, 1963; Ling & Husband, 1964; Sarkany, 1967). This phenomenon was used to investigate the probable presence of an immunological disorder suppressing spermatogenesis in cases of non-obstructive azoospermia and oligospermia in man.

MATERIAL

Investigations were carried out on 126 males attending a Fertility Clinic who were found to have complete azoospermia or severe oligospermia. The history

of each included the age, nationality, years of marriage, previous history of infective or traumatic genital lesions, family history regarding infertility, and sexual history. The ages of the men ranged from 20 to 61 years, and the number of years they had been married from 1 to 30. Those who showed clinical evidence of gross hormonal imbalance, or of obstruction, or of a sex chromosomal abnormality, as Klinefelter's syndrome and its variants, were excluded. At the time of examination, 117 men had azoospermia and nine had severe oligospermia. Seven men had a history of testicular biopsy, five had a history of testicular injury and ten gave a history of orchitis 1 to 6 years before the present investigation.

The testes of seventy-four men were of normal size and consistency, while fifty-two had testes which were smaller in size and softer in consistency than the average (Table 1).

TABLE 1
HISTORY OF TESTICULAR INJURY AND SIZE AND CONSISTENCY
OF TESTES IN 126 MEN WITH AZOOSPERMIA OR SEVERE
OLIGOSPERMIA

<i>History of testicular injury</i>			<i>Size and consistency of testes</i>	
<i>Infection</i>	<i>Trauma</i>	<i>Biopsy</i>	<i>Small and soft</i>	<i>Normal</i>
10	5	7	52	74

Thirty-one adult males ranging in age between 25 and 50 years were used as controls. All were attending the Fertility Clinic, for premarital examination. Their seminal analyses gave counts mostly within the range of 60 to 90 $\times 10^6$ spermatozoa/ml, with 60%, or more active spermatozoa of normal morphology.

METHOD

Three leucocyte cultures were prepared from each individual. An 8-ml sample of peripheral blood was drawn into a heparinized syringe, transferred to a centrifuge tube and centrifuged at 2000g for 5 min. The plasma and buffy layer were transferred into a second centrifuge tube, mixed, and left to settle for 10 to 15 min, after which 0.6-ml aliquots from the supernatant, each containing approximately 7×10^6 leucocytes, were transferred to three 1-oz culture bottles designated A, B and C. To bottle B, 0.6 ml of a 2% seminal extract in Hanks's B.S.S. was added. The seminal extract was prepared by mixing normal semen specimens from ten to twelve adults. The 2% suspension of the mixture in Hanks's B.S.S. was frozen and thawed five times. Bottles A and C received an equal volume of Hanks' B.S.S. The three bottles were incubated at 37° C for 20 min, and 6 ml Eagle's B Medium containing 16% foetal calf serum were added to each. Bottle A also received 0.2 ml phytohaemagglutinin (PHA)M and all three bottles were incubated at 37° C. A supplement of 3 ml fresh medium was added to each 96 hr later, and the incubation continued for a further 48 hr. The leucocyte cultures were incubated

with Colcemid for 2 hr, and then subjected to hypotonic treatment, fixation in 50% acetic acid, staining with aceto-orcein and squashing.

Two thousand cells were randomly counted in each culture and the percentages of large blast cells and of metaphase figures were estimated. A reaction was considered to be positive when:

1. Bottle A (positive control culture containing PHA) showed a significant percentage of blast cells and metaphases.
2. Bottle B (test culture containing the seminal antigen) showed blast cells and metaphases.
3. Bottle C (blank control culture) showed no blast cells or metaphases, or a percentage significantly lower than that of B.

RESULTS

A positive reaction, as specified by the three criteria for a successful positive control culture A, blastoid transformation and mitoses in test culture B and a

TABLE 2
PERCENTAGE OF MITOTIC FIGURES AND BLASTOID CELLS IN THE CULTURES
OF TWENTY-ONE INFERTILE MEN WITH POSITIVE LYMPHOCYTE BLASTOID
TRANSFORMATION TESTS

Case No.	Culture A		Culture B		Culture C	
	Mitoses	Blastoid cells	Mitoses	Blastoid cells	Mitoses	Blastoid cells
1	1.5	39.6	0.2	7.2	0.0	1.0
8	4.5	85.0	0.7	8.2	0.2	3.8
21	1.4	60.1	0.8	15.7	0.2	4.1
25	3.5	59.8	0.7	8.0	0.4	3.2
27	4.2	62.0	0.9	8.1	0.4	4.0
28	8.5	80.2	0.3	7.0	0.0	2.1
48	4.0	65.1	0.5	8.3	0.1	4.0
50	7.0	70.1	0.8	7.4	0.0	3.7
51	6.8	72.0	0.3	5.9	0.0	1.2
54	1.2	60.4	0.3	3.4	0.0	0.4
85	5.0	82.4	1.4	12.1	0.4	5.6
92	4.2	80.2	0.8	8.3	0.1	2.1
97	7.5	68.3	0.9	10.1	0.3	2.7
113	4.3	54.1	0.6	8.9	0.0	1.1
120	7.2	52.6	0.8	18.9	0.2	4.2
121	4.8	56.2	1.5	18.0	0.3	4.0
123	7.7	65.7	0.9	10.1	0.1	2.6
131	2.5	24.6	0.6	17.6	0.0	2.6
134	2.4	40.2	1.8	20.2	0.3	5.1
145	1.8	48.1	0.5	9.0	0.0	2.3
160	1.6	30.1	0.7	10.2	0.0	3.8

negative blank control C, was noted for twenty-one individuals (16.6%). The cultures from the remaining 105 men, all considered negative, showed either no transformation in the test culture B or an almost equal degree of transformation in both the test culture B and the blank control culture C. In the twenty-one positive cases, the percentage of blastoid cells in test culture B ranged from 3.4 to 20.2, and the percentage of mitotic figures ranged from 0.2 to 1.8. In the blank control cultures, the percentages were 0.4 to 5.6 and 0 to 0.4

respectively, and in the PHA cultures they were 24.6 to 85.0 and 1.2 to 8.5 respectively (Table 2). The mean age of the twenty-one positive cases was 37 years, and that of the 105 negative ones was 35 years. History of injury to the testes in the form of orchitis, biopsy, or direct trauma was present in eight of the twenty-one positive cases and in fourteen of the 105 negative ones. The testes were smaller in size and softer in consistency than normal in fourteen of the twenty-one positive cases and thirty-eight of the 105 negative ones (Table 3).

TABLE 3
HISTORY OF TESTICULAR INJURY AND SIZE AND CONSISTENCY
OF TESTES IN TWENTY-ONE INFERTILE MEN WITH POSITIVE
LYMPHOCYTE BLASTOID TRANSFORMATION TESTS

Case No.	History of testicular injury			Size and consistency of testes	
	Infection	Trauma	Biopsy	Small and soft	Normal
1	—	—	+	+	—
8	—	—	+	—	+
21	—	—	+	+	—
25	—	—	—	+	—
27	—	—	—	+	—
28	—	—	—	+	—
48	—	—	—	+	—
50	—	+	—	+	—
51	—	—	—	+	—
54	—	—	—	+	—
85	—	—	—	—	+
92	—	—	+	+	—
97	—	—	—	—	+
113	+	—	—	+	—
120	+	—	—	+	—
121	—	—	—	—	+
123	—	—	—	—	+
131	+	—	—	—	+
134	—	—	—	+	—
145	—	—	—	—	+
160	—	—	—	+	—
Total 21	3	1	4	14	7

None of the thirty-one controls showed a positive blastoid transformation reaction.

DISCUSSION

The semen contains various antigens, both in spermatozoa (Otani, Ino & Kagami, 1965; Weil, 1965) and in seminal plasma (Stevens, Post & Balows, 1965) and under certain conditions, some of the antigens evoke antibody formation. Several instances of male and female infertility, both in humans (Nakabayashi *et al.*, 1961; Franklin & Dukes, 1964) and in experimental animals (Ashitaka, Isojima & Ukita, 1964) have been ascribed to the formation of antibodies against spermatozoa. These cause infertility through sperm agglutination, sperm immobilization, reduced ability of the spermatozoa to

invade ovulatory cervical mucus, or by suppression of spermatogenesis (Wentworth & Mellen, 1964; Bishop & Carlson, 1965).

A positive blastoid transformation test indicates sensitization of lymphocytes to some antigens present in the semen. The seminal extract used here obviously contained several sperm antigens and antigens of other cellular or non-cellular elements, including the ABO group agglutinogens which might have passed to the semen of some secretor donors. One or more of these could hypothetically be the antigen(s) involved in the positive test. With the extract dilutions used, most of the minor antigens were rendered ineffective; if any of them had been involved, some positive results would have appeared among the controls. The fact that twenty-one of the 126 infertile males, and none of thirty-one controls, gave a positive reaction, strongly suggests that the antigens involved in the test are specifically related to the suppression of spermatogenesis present in this group of patients.

The lability of the antibody titres, as well as the appearance of positive results in some fertile males, strongly limit the reliability of some of the tests used to detect circulating antibodies against spermatozoa such as the haem-agglutination, spermagglutination and sperm immobilization techniques (Bratanov, Dikov & Popova, 1964; Stevens *et al.*, 1965; WHO Report, 1966). Some of these difficulties may be overcome by testing for the ability of the patient's lymphocytes to produce the antibodies, rather than by testing for the presence of the circulating antibodies themselves. The blastoid transformation test gives this information and the fact that none of the controls in the present series gave a positive reaction is strongly in favour of the reliability of the test. It should be mentioned here, however, that the percentage of positive reactors obtained in this series only refers to the blastoid transformation test. Testing for the immunological response by determination of the circulating antibodies might give a different percentage of reactors, depending on the method used.

Of the 126 men examined in the present series, 105 had a negative blastoid transformation test and fourteen (13%) of these had a previous history of testicular injury. A similar history was recorded for eight (38%) of the twenty-one men with positive tests. The difference between both groups is significant ($P < 0.02$) and suggests that testicular injury in the form of biopsy, orchitis or direct trauma may play an aetiological rôle in the production of auto-antibodies, resulting in immunological aspermatogenesis or oligospermatogenesis. Earlier reports dealing with the aetiological rôle of testicular injury in the production of antibodies against spermatozoa dealt mostly with cases of 'obstructive' azoospermia (Rümke & Hellinga, 1959; Phadke & Padukone, 1964; Rümke, 1965), while the present series was concerned only with the 'non-obstructive' type.

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