SEROLOGICAL STUDIES ON GENERAL PARALYSIS OF THE INSANE

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SYNOPSIS

Thirty-six patients suffering from general paralysis of the insane were examined for syphilitic and autoimmune antibodies. All the patients were reactive for the fluorescent treponemal antibody absorption and the *Treponema pallidum* haemagglutination tests; 63·9% were reactive for the Venereal Disease Research Laboratory slide test; and all were negative for the various autoimmune antibodies.

INTRODUCTION

It has been established that treponemes contain phospholipids which are related to those in mammalian tissues (Vaczi *et al*, 1966). Cardiolipin-like substances extracted from beef cardiac muscle utilised in serological tests for syphilis e.g. the Venereal Disease Research Laboratory (V.D.R.L.) test, are found in all tissue cells in the inner membranes of mitochondria (Fleischer *et al*, 1967). Syphilitic patients with general paralysis of the insane (G.P.I.) possess a cross-reacting antibody to a highly encephalitogenic preparation extracted from brain tissue (Field *et al*, 1963). The question arises as to whether this antibody in G.P.I. is a reaction to the treponeme, or an auto-antibody to tissue component(s). In an attempt to clarify the tissue, we screened thirty-six proven and well-documented G.P.I. patients for autoimmune antibodies to cell nuclei, mitochondria and microsomes, as well as to smooth muscle cells and brain tissue, by the indirect fluorescent antibody technique (Weller and Coons, 1954). In addition we examined these patients' sera for syphilitic antibodies by the V.D.R.L. slide test (Harris and Bossak, 1951), the fluorescent treponemal antibody absorption (FTA-ABS) test (Hunter *et al*, 1964), and the *Treponema pallidum* haemagglutination (TPHA) test (Tomizawa and Kasamatsu, 1966).

MATERIAL AND METHODS

1. G.P.I. Patients' Sera

Sera were obtained from thirty-six G.P.I. cases (all males) aged 40 to 73 years the majority of whom have been in the Singapore Woodbridge mental hospital for at least 10 years. These patients have shown sero-positivity for syphilis prior to this investigation, and their blood groups are known.

2. Indirect Fluorescent Antibody Tests

Anti-human globulin (rabbit) labelled with fluorescein isothiocyanate supplied by Burroughs and Wellcome, U.K., was used in the double-layer method for detection of auto-antibodies to cell nuclei, mitochondria, microsomes, smooth muscle cells and brain tissue. Tissue antigens used were rat liver for antinuclear factor, rat kidney for anti-mitochondrial antibody, human thyroid for anti-microsomal antibody, rat stomach wall for anti-smooth-muscle antibody, and human brain (obtained 6 hours after death) for brain tissue antibody.

3. Micro-TPHA Test

The quantitative micro-TPHA test kit (KS 6000) supplied by the Fuji Zohki Pharmaceutical Co., Tokyo, Japan, was used according to the instructions provided. All thirty-six G.P.I. sera were titrated from $\frac{1}{80}$ dilution upwards in the absorbing diluent which contained sheep and ox red cell membrane, Reiter protein, and rabbit testis extract in optimal concentrations (Tomizawa *et al*, 1969). The test was performed in a micro-diluting equipment obtained from Cooke Laboratory Products, U.S.A. The highest dilution which gave the minimum degree of haemagglutination of 1+ ("smooth mat of cells surrounded by smaller red circle") was recorded as the titre of haemagglutination antibody.

4. FTA-ABS Test

The FTA-ABS test was performed on the thirty-six G.P.I. sera with use of the "sorbent" described by Stout *et al*, 1967 instead of the "sonicate" of Reiter. The FTA-ABS test antigen was supplied by Baltimore Biological Laboratory (B.B.L.), U.S.A. Specific fluorescence was recorded from R1 to R4 as standardised by the Centre for Disease Control (C.D.C.), U.S.A.

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5. V.D.R.L. Test

The V.D.R.L. slide test (U.S.P.H.S. Manual of Tests for Syphilis, 1969) was performed on the thirty-six G.P.I. sera; the V.D.R.L. antigen was supplied by B.B.L., U.S.A.

RESULTS

1. Autoimmune Antibodies

All thirty-six sera were negative for auto-antibodies to nuclei, mitochondria, microsomes, smooth-muscle cells, and brain tissue.

2. Occurrence of TPHA Antibody in G.P.I. Sera

86·2% of G.P.I. patients possess TPHA antibody titres of between 80 to 1280, and only 13·9% of these patients have titres above 1280 (Fig. 1). This indicates that the TPHA antibody does persist to the G.P.I. stage, though not in very high titres.

3. FTA-ABS Assay

Of the thirty-six sera tested for FTA-ABS, all were at least R2; there were eight R2's, fourteen R3's, and fourteen R4's (See Table I). This finding

TABLE I

SOME DATA OF THE 36 CASES OF G.P.I. (ALL MALES)

Patient No.	Age	Race	Blood Gp.	VDRL	FTA-ABS	TPHA Titre
Gp 1	50	Chinese	B	R2	R3	640
Gp 2	46	Malay	O	R2	R3	640
Gp 3	48	Chinese	AB	R4	R4	640
Gp 4	41	Chinese	O	WR	R3	640
Gp 5	40	Chinese	O	R8	R4	640
Gp 6	44	Chinese	A	WR	R3	160
Gp 7	47	Chinese	B	NR	R4	2560
Gp 8	48	Chinese	A	R1	R2	320
Gp 9	48	Chinese	B	WR	R2	80
Gp 10	44	Chinese	O	R1	R3	640
Gp 11	51	Chinese	B	R4	R3	640
Gp 12	53	Chinese	O	R1	R3	320
Gp 13	56	Chinese	O	R4	R4	640
Gp 14	57	Chinese	B	R1	R4	320
Gp 15	58	Chinese	B	R2	R3	320
Gp 16	59	Chinese	A	R1	R3	2560
Gp 17	59	Chinese	O	WR	R2	320
Gp 18	59	Chinese	B	NR	R2	160
Gp 19	60	Chinese	A	R2	R3	320
Gp 20	61	Chinese	A	R2	R3	640
Gp 21	61	Chinese	A	WR	R2	160
Gp 22	62	Chinese	O	R2	R2	80
Gp 23	62	Chinese	A	RN	R3	320
Gp 24	63	Chinese	O	RN	R4	80
Gp 25	65	Chinese	A	R1	R3	2560
Gp 26	67	Chinese	B	WR	R4	320
Gp 27	59	Chinese	B	R2	R4	640
Gp 28	65	Chinese	A	WR	R4	640
Gp 29	68	Malay	B	R1	R4	160
Gp 30	68	Chinese	O	R2	R2	80
Gp 31	68	Chinese	B	R1	R4	320
Gp 32	71	Malay	A	R2	R4	1280
Gp 33	72	Malay	O	R4	R3	1280
Gp 34	72	Chinese	B	WR	R2	80
Gp 35	72	Chinese	O	WR	R4	160
Gp 36	73	Indian	O	R1	R4	320

R = reactive; WR = weakly reactive; NR = non-reactive; RN = "rough" negative.

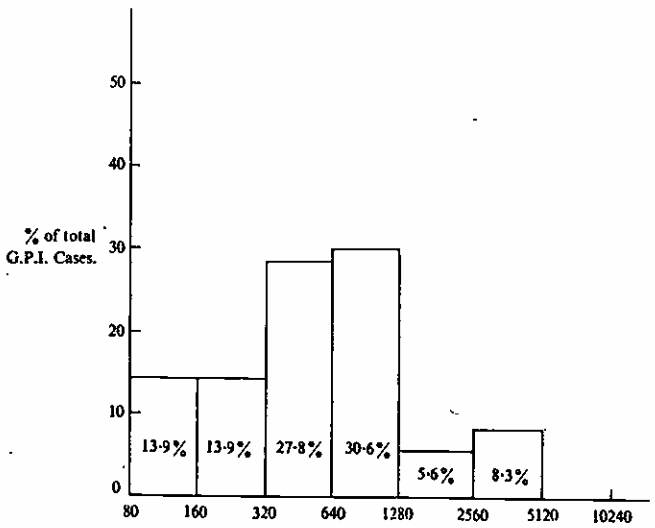

Fig. 1. TPHA antibody titres of G.P.I. sera

confirms that the fluorescent treponemal antibody persists for a long time, even to the G.P.I. stage.

4. V.D.R.L. Assay

Only 63·9% of the thirty-six G.P.I. patients were V.D.R.L. reactive. Of these, nine were R1's, nine R2's, four R4's, and one R8 (See Table I). This shows that the cardiolipin antibody tends to fall off after a period of time. On the other hand, only two of the thirty-six patients had non-reactive sera which did not give rough flocculation.

DISCUSSION

In order to ascertain whether the serologic manifestations of G.P.I. are autoimmune in nature, we examined thirty-six G.P.I. sera for auto-antibodies to nuclei, mitochondria, microsomes, smooth-muscle cells and brain tissue. If it is the result of an autoimmune process, one would expect to detect some auto-antibodies or other. However, all auto-antibody tests proved negative; even that to brain tissue, although Field *et al* (1963) detected a cross-reacting antibody to the brain extract they prepared. Also, Wright *et al* (1970) showed that after penicillin treatment the antibodies against mitochondrial cardiolipin disappear in all the cases of syphilis they tested, with the exception of one gumma case. Since our G.P.I. cases have been penicillin-treated, their negative result for anti-mitochondrial antibody supports the findings of these workers. There seems to be no correlation between blood grouping and predisposition to G.P.I. Anyway, the number of patients investigated on in this study is too small for us to decide on the importance of blood groups to G.P.I. Schmidt *et al* (1970) showed that in 32 patients suffering from G.P.I., serological tests for syphilis were reactive in only half, and the *Treponema pallidum* immobilisation (T.P.I.) test was reactive in the serum in nearly 90%. Our findings show that in 36 patients suffering from G.P.I., 63·9% were reactive for the V.D.R.L., and 100% reactive for both the FTA-ABS and TPHA tests.

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REFERENCES

1. Field, E. J., Caspary, E. A. and Ball, E. J.: "Some biological properties of a highly active encephalitogenic factor isolated from human brain." Lancet, ii, 11, 1963.
2. Fleischer, S., Rouser, G., Fleischer, B., Casu, A. and Kritchevsky, G.: "Lipid composition of mitochondria from bovine heart, liver, and kidney." J. Lipid Res., 8, 170, 1967.
3. Harris, A. and Bossak, H. N.: "A rapid method for performing quantitative tests for syphilis in large-scale testing programs." J. Vener. Dis. Inform., 32, 203, 1951.
4. Hunter, E. F., Deacon, W. E. and Meyer, P. E.: "An improved FTA test for syphilis, the absorption procedure (FTA-ABS)." Publ. Hlth. Rep. (Wash.), 79, 410, 1964.
5. Schmidt, H., Dein, E. and Rasmussen, E. B.: "Immunological and immunochemical investigations of patients suffering from general paralysis." Brit. J. Vener. Dis., 46, 135, 1970.
6. Stout, G. W., Kellogg, D. S., Jr., Falcone, V. H., McGrew, B. E. and Lewis, J. S.: "Preparation and standardization of the sorbent used in the fluorescent treponemal antibody absorption (FTA-ABS) test." Hlth. Lab. Sci., 4, 5, 1967.
7. Tomizawa, T. and Kasamatsu, S.: "Haemagglutination tests for diagnosis of syphilis." A preliminary report. Jap. J. Med. Sci., Biol., 19, 305, 1966.
8. Tomizawa, T., Kasamatsu, S. and Yamaha, S. I.: "Usefulness of the haemagglutination test using *Treponema pallidum* antigen (TPHA) for the serodiagnosis of syphilis." Jap. J. Med. Sci., Biol., 22, 341, 1969.
9. U.S. Public Health Service: Manual of Tests for Syphilis—1969, PHS Publication No. 411, Washington, D.C., U.S. Govt. Printing Office.
10. Vaczi, L., Kirley, K. and Rethy, A.: "A WHO venereal diseases and treponematoses research." 66, 93, 1966.
11. Weller, T. H. and Coons, A. H.: "Fluorescent antibody studies with agents of *Varicella* and *Herpes Zoster* propagated *in vitro*." Proc. Soc. Exp. Biol., N.Y., 86, 789, 1954.