Acta Medica Okayama

Volume 24, *Issue* 1 1970 *Article* 7

FEBRUARY 1970

Transformation of antibody from IgM to IgG in experimental syphylitic rabbits. I. Syphylitic serum reaction

Masana Ogata* Kazuo Kumashiro†

*Okayama University,
†Okayama University,

Transformation of antibody from IgM to IgG in experimental syphylitic rabbits. I. Syphylitic serum reaction*

Masana Ogata and Kazuo Kumashiro

Abstract

Localization of IgM and IgG sypylitic antibodies in the sera of patients and the experimental syphylitic rabbits was examined by the gel filtration on Sephadex G.200 column. I) In the case of late syphylitic patients; OGATA test-reactive antibodies were contained in IgM and IgG fractions. On the other hand, RPCF test-reactive antibody was contained only in IgG fraction. This discrepancy may be due to the difference in antigens; Cardiolipin.resicin and T. P. Reiter protein. 2) In the case of the experimental syphylitic rabbits; The results were as follows. a) Variation in the level of the titer. The peaks of the titer were seen 3-4 weeks after inoculation of T. P. Nichols by OGATA test, VDRL test and RPCF test, thereafter titers decreased. On the other hand, the titer kept on rising up to 2 months and maintained high level during the periods of 3, 4 and 5 months by TPHA test. b)Transformation of antibody from IgM to IgG. Transformation of antibody from IgM to IgG was seen 3-4 weeks after inoculation by all four tests; OGATA test, VDRL test, RPCF test and TPHA test, and such a transformation was completed 3 months after inoculation.

*PMID: 4246832 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **24**, 93—99 (1968)

TRANSFORMATION OF ANTIBODY FROM IGM TO IGG IN EXPERIMENTAL SYPHYLITIC RABBITS
I. SYPHYLITIC SERUM REACTION

Masana OGATA and Kazuo KUMASHIRO

Department of Public Health, Okayama University Medical School, Okayama, Japan (Director: Prof. M. Ogata)

Received for publication, October 1, 1969

Ever since Wassermann reported the complement fixation test as a serologic test for syphylis, many investigators have conducted extensive studies on more sensitive and highly specific serum reaction. Especially noteworthy among them is the work of PANGBORN (1), in which she successfully isolated cardiolipin from beef heart, which contributed much to this field of research. However, as the substance, phosphatidic acid, is a non-specific antigen, it has been demonstrated to elicit various biological false reactions (BFP), which led to search for still more specific reatcion. Among these works are included Treponema pallidum immobilization test (TPI) of NELSON (2), Reiter protein complement fixation test (RPCF) of CANNEFAX (3), Fluorescent Treponemal antibody (FTA) by DEACON (4). And more recently TOMIZAWA reported about Treponema pallidum hemagglutination test (TPHA) (5). However, there are still some discrepancies among the results of these tests in the stage of syphylis (6—13). Therefore, it may be said that the tests for syphylis to determine the regimen for the treatment as well as to formulate a precise diagnosis in syphylis have not been yet perfected. It is gerenally known that in viral infection 19S-γ-globulin (IgM) is formed, which is later transformed to 7S-γ-globulin (IgG) within a month after infection (6—13). By applying the above fact it would seem to be possible to tell the lapse of the time after spirochete infection and to explain discrepancies in the results of various tests, if there should be difference in the sensitivity of tests and in the distribution of antibody between IgM and IgG antibodies due to the stage of syphylis. Since the introduction of the gel filtration method for protein by PORATH (1959) (14), the analysis and the purification of serum protein have made a great advance. Therefore, it would be worthwhile to analyze the sera of syphylitic patients by applying the above method. In view of this, the authors attempted to examine the localization of IgM and IgG antibodies in syphylitic patients, and also examined the transformation of IgM to IgG

on the experimental syphylitic rabbits by OGATA, VDRL, RPCF and TPHA tests. The present communication describes briefly the transformation of IgM to IgG in the experimental syphylitic rabbits by using the gel filtration technique on Sephadex G-200 column.

MATERIALS AND METHODS

Materials:

The sera of some syphylitic patients were obtained from the cubital vein, who proved to be positive to OGATA test or VDRL test.

The sera of the experimental syphylitic rabbits: T. P. Nichols was inoculated into the testicles of 9 adults rabbits. The blood was collected one every week for the first month, thereafter collected once every month. The blood of each group was pooled at each step, one group was used in the preliminary test and the other in the experimental proper.

Each serum (1, 2) was inactivated at 56°C for 30 minutes before the gel filtration.

Gel filtration on Sephadex G-200 column (45 cm, by 3 cm): The elution was conducted with 2 ml of each of serum and Mg-saline by the downward technic, the effluent was collected into the fraction collector (TŌYO type-SF-200A) per 4 ml/one tube at the speed 20 ml/hour. The protein content of the each fraction was measured at the optical density of 280 mμ by Hitachi Perkin-Elmer spectrophotometer (type-139). The first peak containing IgM and the second peak containing IgG served as materials.

Mg-saline: 0.167 g of $MgCl_2 \cdot 6H_2O$ and 8.5 g of NaCl were adjusted to the final volume of 1 liter by distilled water.

Quantitative analysis of antibody was done by 4 tests: OGATA, VDRL, RPCF and TPHA tests.

OGATA test (Cardiolipin-reagin complement fixation test); Tests were performed on serial two-fold dilutions in Mg-saline. Each diluted serum was divided into three test runs. Ethyl alcohol 1 : 40 (control), antigen 1 : 320, and antigen 1 : 480 were added into No. 1, No. 2 and No. 3 test runs respectively and tested according to accompanying directions. The antigen for OGATA test was purchased at Sumitomo Pharmaceutical Co. Ltd.

VDRL test (Cardiolipin-reagin precipicin test); Tests were conducted on serial two-fold dilutions, and done according to the accompanying directions. The reagent for VDRL test was purchased at Sumitomo Pharmaceutical Co. Ltd.

RPCF test (Reiter protein complement fixation test); Serum dilutions were done identically as in OGATA test. Mg-saline (Control), antigen 1 : 20, and antigen 1 : 40 were added into No. 1, No. 2, No. 3 test runs respectively, and done according to the accompanying directions. The reagent for RPCF test was purchased at Nihon-Toketu-Kanso Co. Ltd.

TPHA test (Treponema pallidum hemagglutination test); For the prelimi-

nary treatment, the absorbing procedure was taken. In this 0.1 ml (0.5 ml)*1) of each serum was added into 0.9 ml (0.5 ml)*1) of absorbing agents*2), mixed well, left standing at 2—10°C for 30 minutes, centrifuged at 2,500 r.p.m for 5 minutes. The supernatant in each case was submitted for the tests. The tests were performed on serial four-fold dilutions in the accompanying medium*3), and done according to the accompanying directions. The reagents for test was purchased at Fuji-zoki pharmaceutical Co. Ltd.

RESULTS

1) *Localization of antibody in the sera of syphylitic patients*;

Case 1; The serum of 56-year-old woman suffering stomatitis 3 years previously was diagnosed as a late syphylis.

The gel filtrates of this patient's serum were submitted to OGATA test and RPCF test, and the result obtained was as follows: OGATA test-reactive antibody was located in IgM and IgG fractions, and RPCF test-reactive antibody in IgG fraction alone.

Case 2; The serum of a 5-year-old girl, whose parents had suffered from syphylis, was diagnozed as a congenital syphylis.

The gel filtrates of this patient's serum were submitted to OGATA test and RPCF test, and the result showed that OGATA test-reactive and RPCF test-reactive antibodies were located only in IgG fraction.

2) *Titer and localization in the sera of the experimental syphylitic rabbits*;

a) *Variation of the titer in the original sera after inoculation.*

As shown in Table 1 and Fig. 1, the peaks of the titer in the rabbit were seen 3—4 weeks after inoculation by OGATA test, VDRL test, and RPCF test, which decreased thereafter. In contrast, the titer of TPHA test kept rising up to 2 months, and it maintained high titer for the period of 5 months. Variation of the titer by TPHA test seemed to be similar to that of FTA test (12).

b) *Transformation of antibody from IgM to IgG in the gel filtrates on Sephadex G-200.*

As shown in Table 1 and Fig. 2, transformation of antibody from IgM to IgG was similar among all four tests; OGATA test, VDRL test, RPCF test and TPHA test. And IgM antibody appeared 1—2 weeks after

---

*1) ( ) was in the case of fractionates

*2) Absorbing agent: This agent contains mainly Reiter protein for the purpose of removing non-specific substances.

*3) Medium: Phosphate-buffered saline, mixtures of saline, 0.15 M Na_2HPO_4 and 0.15 M KH_2PO_4 at a ratio of 10:7:3 (pH=7.2) was added with arabic gum at 0.25%, normal rabbit serum at 1.0% and tween 80 at 0.01%.

Table 1. Variation of the titer in the original sera, and that of IgM and IgG antibodies in the peak of the gel filtrates of the experimental syphylitic rabbits after inoculation of T. P. Nichols by TPHA, RPCF, OGATA and VDRL tests

Test method / Weeks after inoculation	TPHA test			RPCF test			Ogata test			VDRL test		
	Original sera	IgM	IgG	Original sera	IgM	IgG	Original sera	IgM	IgG	Original sera	IgM	IgG
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	8	0	40	8	0	0	0	0	0	0	0
2	80	512	8	160	16	4	80	8	8	32	2	0
3	320	512	128	320	32	16	320	8	16	128	2	2
4	1280	128	128	640	16	16	320	8	32	128	2	4
8	5120	8	512	320	8	16	160	8	16	64	1	2
12	10240	0	512	160	0	4	40	0	2	8	0	1
16	10240	0	512	80	0	4	40	0	2	8	0	1
20	10240	0	512	80	0	4	20	0	2	8	0	1

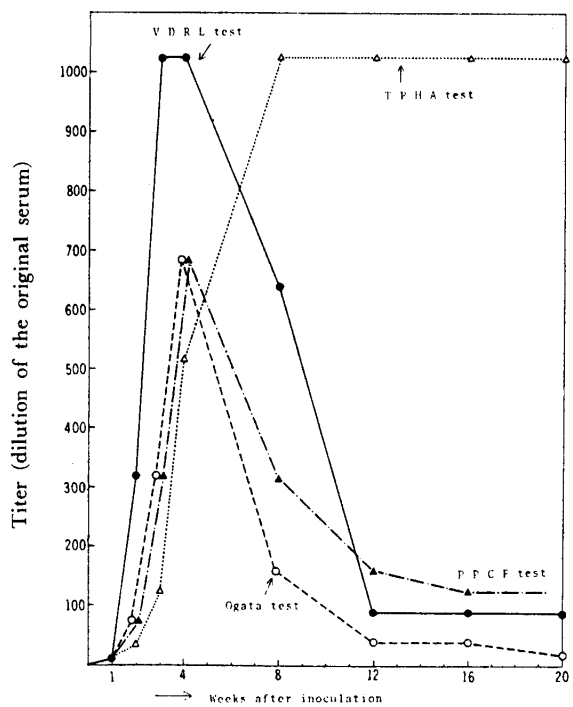

Fig. 1 Variation of the titer in the original sera of the experimental syphylitic rabbits after inoculation of T. P. Nichols by TPHA, RPCF, OGATA and VDRL tests
(TPHA test……titer × 1/10,
VDRL test……titer × 20,)

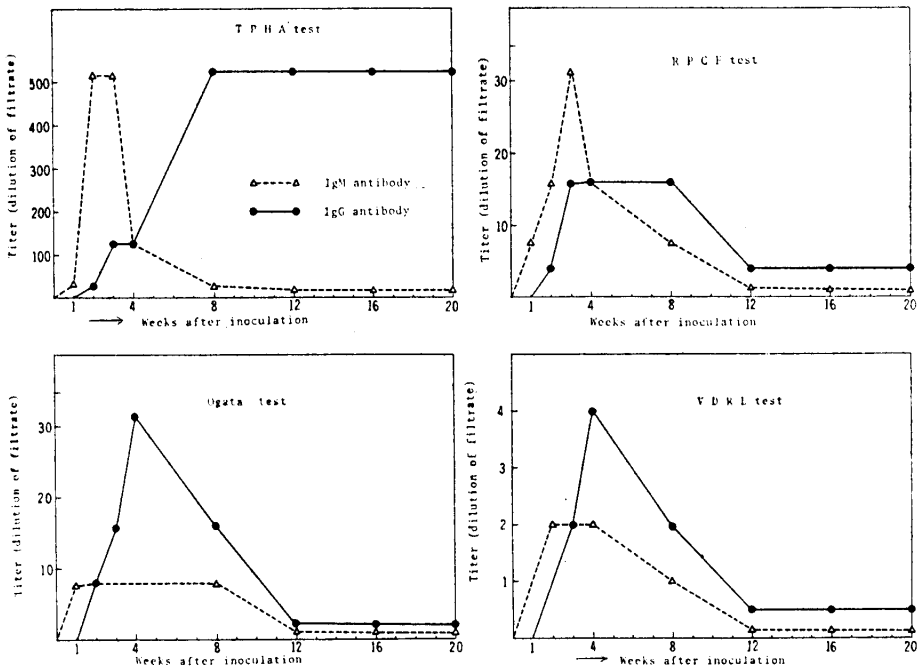

Fig. 2 Variation of the titer of IgM and IgG antibody in the gel filtrates of the experimental syphylitic rabbits after inoculation of T. P. Nichols by TPHA, RPCF, OGATA and VDRL tests

inoculation, kept on rising as the lapse of time, and reached the peak 3—4 weeks after inoculation, thereafter it decreased, and disappeared completely 3 months after inoculation.

On the other hand, IgG antibody appeared about one week after the period of IgM antibody appearance, and it kept on rising, became higher than that of IgM antibody 4 weeks after inoculation, and completely transferred from IgM to IgG antibody 2 months after inoculation.

DISCUSSION

It is reported by MATSUHASHI (10, 11) that there exist both IgM and IgG antibodies in the sera of syphylitic patients, which have been verified by Cardiolipin-reagen test, RPCF test, FTA test, and TPHA test. But there is no report for clarifying the transformation of antibody from IgM to IgG in syphylitic sera after the spirochete infection.

It was recognized that OGATA test-reactive IgM antibody remained even in the serum of late syphylitic patients suffering stomatitis 3 years previously in this experiment. MIZUOKA also reported the presence of FTA

test-reactive IgM antibody in late syphylitic patients. These findings differ from that of the experimental syphylitic rabbits, in which the transformation of antibody from IgM to IgG occurred 2 months after inoculation, and also differs from the immunological results of viral infection of human being, in which transformation took place within 3 weeks to 2 months after infection (15, 16). From these results it is considered that process of human syphylitic infection progresses rather showly as compared with the experimental syphylitic infection in rabbits and also human viral infection.

Difference in the localization of antibody among test methods was recognized in Case 1, indicating that OGATA test-reactive antibody was located in IgM and IgG fractions. On the other hand, RPCF test-reactive antibody was located only in IgG fraction. These discrepancies may be due to the difference in antigens.

CONCLUSION

Localization of IgM and IgG sypylitic antibodies in the sera of patients and the experimental syphylitic rabbits was examined by the gel filtration on Sephadex G-200 column.

1) In the case of late syphylitic patients;

OGATA test-reactive antibodies were contained in IgM and IgG fractions. On the other hand, RPCF test-reactive antibody was contained only in IgG fraction. This discrepancy may be due to the difference in antigens; Cardiolipin-resicin and T. P. Reiter protein.

2) In the case of the experimental syphylitic rabbits; The results were as follows.

a) Variation in the level of the titer.

The peaks of the titer were seen 3—4 weeks after inoculation of T. P. Nichols by OGATA test, VDRL test and RPCF test, thereafter titers decreased. On the other hand, the titer kept on rising up to 2 months and maintained high level during the periods of 3, 4 and 5 months by TPHA test.

b) Transformation of antibody from IgM to IgG.

Transformation of antibody from IgM to IgG was seen 3—4 weeks after inoculation by all four tests; OGATA test, VDRL test, RPCF test and TPHA test, and such a transformation was completed 3 months after inoculation.

ACKNOWLEDGEMENTS

The authors express sincere thanks to Dr. NAOJI KITAMURA, KISHIO ASANUMA, and

MAKIKO YAMADA of Institute for Public Health, Okayama Prefecture, and also Dr. NOBORU YAMADA of FUJI-zoki Parmaceutical Co. Ltd, for many helpful suggestions and discussions relating to this work.

REFERENCE

1. PAGBORN, M. C.: *Proc. Soc. Exp. Biol. Med.* **48**, 484, 1941
2. NELSON, R. A. & MAYER, M. M.: *J. Exp. Med.* **89**, 369, 1949
3. CANNEFAX, G. R. & GARSON, W.: *Pub. Health Rep.* **72**, 335, 1957
4. DEACON, W. E., *et al.*: *Proc. Soc. Exper. Biol. & Med.* **96**, 477, 1957
5. TOMIZAWA, T.: *Jap. J. Med. Sci. & Biol.* **19**, 305, 1966
6. OGATA, M.: *Phisico-Chemical Biology* **12**, (3), 181, 1967 (in Japanese)
7. SEKIGUCHI, M.: *Igaku-no-Ayumi* **66**, (3), 135, 1968 (in Japanese)
8. KOZAKAI, N.: Clinical Serology, p. 12, Kokuseido, Tokyo, 1966 (in Japanese)
9. YAMAMURA, U.: Immuno-Chemistry, p-469, Asakura, Tokyo, 1963 (in Japanese)
10. MATSUHASI, T.: *Nihon Rinsho* **21**, (12), 2506, 1963 (in Japanese)
11. MATSUHASI, T.: *Rinsho Kagaku* **1**, (6), 628, 1965 (in Japanese)
12. MIZUOKA, K.: *Derma et Urologica* **19**, (12), 1225, 1965 (in Japanese)
13. MIURA, K.: *Med. and Biol.* **74**, (3), 134, 1967 (in Japanese)
14. HIRAYAMA, S. & TOMINAGA, K.: *Nihon Rinsho* **21**, 2637, 1963 (in Japanese)
15. SVEHAG, S. E. & MANDEL, B.: *J. Exp. Med.* **119**, 1, 1964
16. FAZEKAS de St. GROTH, S. & WEBSTER, R. G.: Cellular Biology of Myxovirus infections, p. 246, J. & A. Churchill Ltd., London