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New Developments in the Immunological Understanding and of Serodiagnosis in Syphilis

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There are many serologic tests for syphilis. By means of the usual serologic tests, it is not possible to differentiate between patients who need therapy and those who are cured. In this paper I want to discuss the scientific developments and demonstrate the results of immunologic research in syphilis, which makes it possible to differentiate between treated and untreated cases.

Key Words: Serologic test, syphilis.

The bacterial origin of syphilis has been known since 1905 when Treponema pallidum was described as the causative agent of this infectious disease (Schaudinn *et al.*, 1905). Since 1943 it has been known that Treponema pallidum is sensitive to penicillin (Mahoney *et al.*, 1943) and that syphilis can be cured by the administration of adequate doses of long-acting penicillin preparations (Idsøe *et al.*, 1972). To date, no resistance of Treponema pallidum to penicillin has been observed (Department *et al.*, 1968). Nevertheless, a rising number of infections has been noted in various countries all over the world. This seems to be a contradiction which is easy to explain: love between human beings – whatever is understood by that – and sexuality are closely linked. Because there will always be love, there will always be syphilis as one of the sexually transmitted diseases. Therefore, it will remain one of the responsibilities of medical research to investigate this treponemal infection from different points of view.

Syphilis in humans develops in stages. Stages with clinically characteristic symptoms alternate with latent stages in which virulent treponemes can destroy tissues in the infected body without clinical signs. Many years after an untreated infection late manifestations can appear in the blood vessels and the central nervous system which are then irreparable.

It was the merit of Wassermann and co-workers (Wassermann *et al.*, 1906) to describe a test for the serological diagnosis of syphilis even in the latent stages. Today we know that Wassermann was mistaken in assuming treponemal specificity of the Wassermann reaction. Nevertheless, the test, demonstrating antilipoidal antibodies, has proved its diagnostic value for more than 50 years.

In 1949 the first test for the detection of Treponema pallidum-specific antibodies was described by Nelson and Mayer (Nelson *et al.*, 1949). Until a few years ago the Treponema pallidum immobilization (TPI) test was used for serological diagnosis of syphilis in patients with clinically uncharacteristic symptoms in whom a treponemal infection had to be diagnosed or excluded. After the introduction of the Fluorescent treponemal antibody absorption (FTA-ABS) test and the Treponema pallidum haemagglutination assay (TPHA) we no longer need the TPI. Earlier than the TPI and with at least the same specificity, these two reactions show whether an infection by Treponema pallidum has taken place or not (Treponemal *et al.*, 1982).

Until 1974 syphilis serology was – and in several countries of the world still is – an auxiliary science, which provides serological test results for the clinician, especially for the dermatologist. The clinician made an interpretation of the laboratory findings and drew the conclusions for the necessity of treatment. Since 1974 we in Hamburg have been engaged in explaining serological findings in syphilitic patients by the immune reaction provoked by Treponema pallidum in the infected organism. During this time we have learned to draw conclusions about the stage of infection from certain antibody constellations. We have furthermore learned to distinguish between adequately treated and spontaneously cured syphilitic patients on the one hand and patients who need treatment on the other hand (Herbst *et al.*, 1979; Leyh *et al.*, 1978; Müller, 1978, 1981, 1979; O'Neill *et al.*, 1972; Shannon *et al.*, 1977)

In my lecture I should like to report on the scientific developments in this field and to demonstrate that results of immunological research in syphilis can be adapted to clinical diagnostics.

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Recent results of research in syphilis immunology

The point of departure in our investigations was

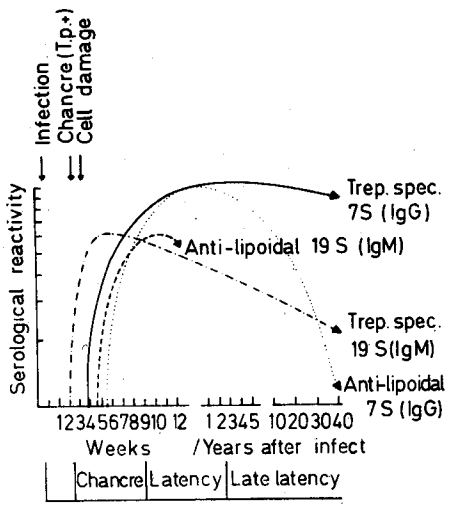

Fig. 1.

the possibility of separating serum fractions by gel filtration, that is to isolate immunoglobulins of the different classes and to investigate these fractions for their content of treponemal antibodies (Leyh *et al.*, 1978; Müller, 1974, 1978, 1981, 1982). One of the important results is shown in the first slide (Fig. 1):

About 10-14 days after infection the organism starts to synthesize treponemal antibodies of the IgM class. Then, after a gap of 4-8 days, treponemal IgG antibodies are produced and can be demonstrated in the patients' sera. As soon as the infectious agent has reached the local lymph nodes, lipoid-containing mitochondria are released from cells as the result of inflammation. The mitochondrial lipoids are not identified by the infected organism as its own but as antigen. This antigen induces the synthesis of antilipoidal antibodies, first of the IgM and shortly after of the IgG class.

Using the gel filtration technique we have investigated sera from patients in clinically defined stages of untreated and treated syphilis (Herbst *et al.*, 1979; Müller, 1981, 1978). In the fractions after gel filtration we have estimated treponemal antibodies

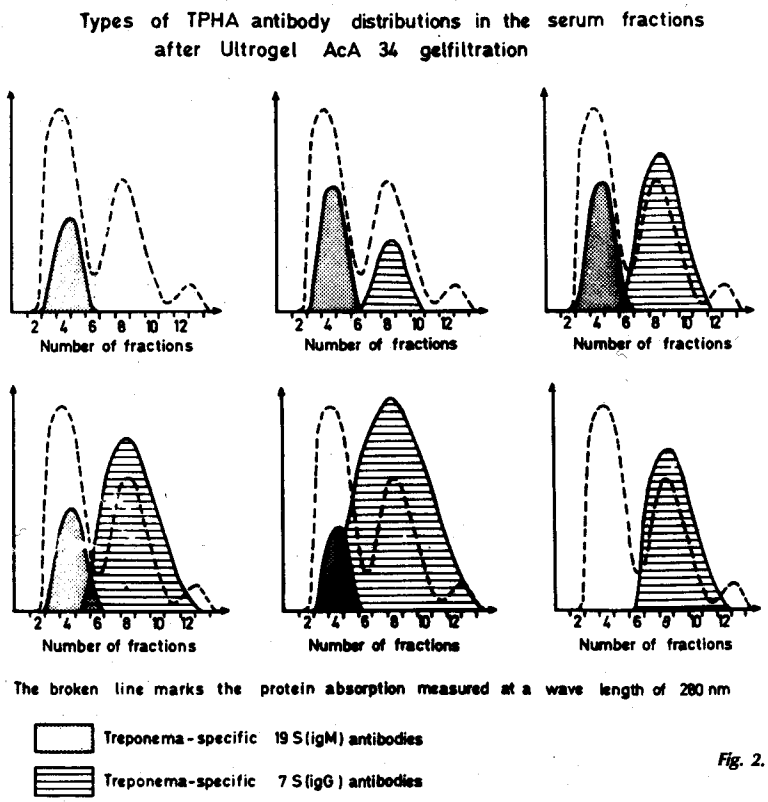

Fig. 2.

by indirect immunofluorescence using FITC-labeled μ-chain and γ-chain specific antisera. As shown schematically in the second slide (Fig. 2) we have found typical IgM and IgG antibody constellations in the various stages of untreated and treated syphilis (Müller, 1982): in primary syphilis almost exclusively treponemal IgM antibodies are to be found. During the course of infection the titer of treponemal IgG antibodies increases considerably. Not shown in this slide is the observation that only about 40% of patients with late latent infection have treponemal IgM antibodies with mostly low titers. In 60% of the patients no treponemal IgM but only IgG antibodies were found (Müller, 1983). It should be noted that treponemal IgM antibodies could not be demonstrated in any patients with a sufficiently treated infection (graph f).

Atwood and Miller (Atwood *et al.*, 1969, 1970) had already suspected that the infected human organism synthesizes treponemal IgM antibodies only as long as virulent treponemes are present. This hypothesis, which was based on the observations of Bienenstock and Bloch (Bienenstock *et al.*, 1966) and their own findings, seems to be confirmed by our investigations. Today we are able to supply a proof of the theory (Moskophidis *et al.*, 1984). I should like to explain the results of experiments, which were performed on a molecular basis, in the following two slides.

After disintegration of [^{35}S]-methionine labeled pathogenic Nichols treponemes with SDS Triton X-100, SDS gel electrophoresis was performed to separate treponemal protein antigens on a molecular basis. Using the double-antibody precipitation technique either IgM or IgG antibodies from sera of syphilitic patients were brought to react with the electrophoretically separated antigens. Finally, the antibody-antigen reactions were made visible by autoradiography of the gel. In slide three (Fig. 3) only IgG antibodies from patients' sera were precipitated. You can see in the first two lines that sera from patients with primary syphilis show nearly no IgG reaction. This is in accord with our immunological observations. But sera from patients with untreated secondary syphilis (lines c and d) or treated syphilis (lines e and f) react with a large number of radiolabeled treponemal protein antigens. This result was to be expected because we know that IgG antibodies are synthesized by long-life memory cells showing an antibody synthesizing activity even after the infection is cured.

In the next slide (Fig. 4) the reaction of IgM antibodies from sera of the same patients with the same portein antigens of Treponema pallidum can be seen. In sera from patients with untreated primary syphilis

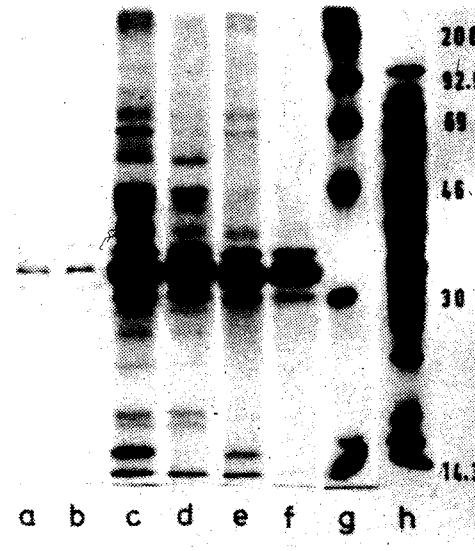

Fig. 3.

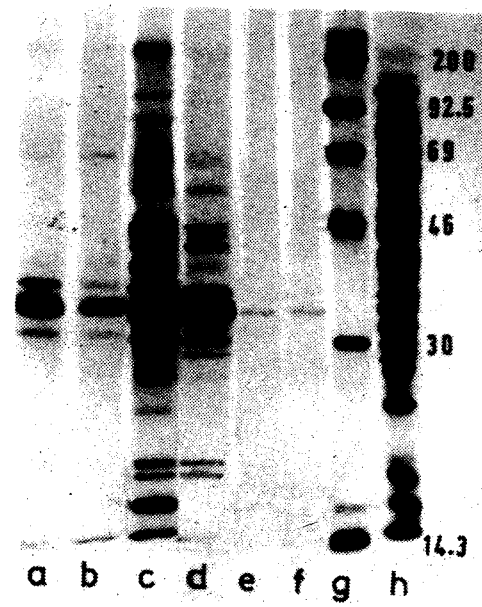

Fig. 4.

(lines a and b) and untreated secondary syphilis (lines c and d) you can see, parallel with the progress of the infection, an increasing number of reacting antigens indicating the presence of IgM antibodies to different partial protein antigens of Treponema pallidum. It is noteworthy that in sera from adequately treated patients (lines e and f) as well as from some patients with an unknown and therefore untreated

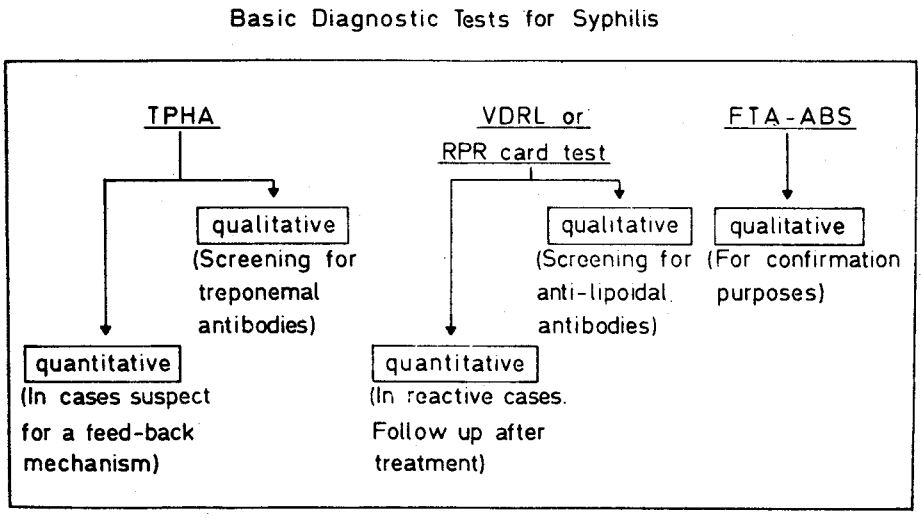

Fig. 5.

treponemal infection (not shown in this slide) no IgM reaction with the treponemal antigens could be observed. On the basis of these findings we believe we are justified in concluding that in most syphilitic patients without specific IgM reaction, virulent treponemes are no longer present in the body, which means that the patients are cured.

Serodiagnostical consequences

From the results of our immunological and molecular biological investigations some conclusions may be drawn as regards serodiagnosis in human syphilis. First I should like to explain our procedure in performing serological diagnosis in syphilis. From the next slide (Fig. 5) you can see that for syphilis screening the TPHA is performed. If this assay shows a nonreactive result a treponemal infection acquired within the last three weeks before investigation can be ruled out. If the TPHA is reactive the result should be confirmed by the FTA-ABS test. At the same time an antilipoidal test should be performed quantitatively. From the Bruusgaard study (Bruusgaard, 1929) and own investigations on healthy blood donors (Müller *et al.*, 1979) it is well known that more than 50% of all treponemal infections heal spontaneously. The next step, which is absolutely imperative, should be therefore to decide whether the seroreactive patient needs treatment or not.

This differentiation can be made by the 19S-IgM-FTA-ABS test or one of its recently described modifications. We prefer our original method using Ultrogel AcA 34 as separating gel and performance of μ-chain specific indirect immunofluorescence in the serum fractions of the 19S elution peak (Müller, 1982, 1979). For some months this test technique has been used for the purpose mentioned in the Department of Dermatology, Yonsei University College of Medicine, with indeed very good results.

In untreated primary, secondary and early latent syphilis treponemal IgM antibodies can always be shown with high serum titres. In patients with untreated tertiary, late latent syphilis or neurosyphilis, IgM antibody titers are normally low, indicating need of treatment. In 2-3% of these patients the 19S-IgM-FTA-ABS test might be false nonreactive. The cause of biologically false non-reactivity is a very high titer of IgG antibodies of the same specificity (Müller, 1983a). IgG antibodies block the release of IgM antibodies from competent B lymphocytes. The so-called feedback reaction occurs when the TPHA-IgG titer is 1/20,000 or higher. These patients must be treated even when the 19S-IgM-FTA-ABS is nonreactive.

In newborn babies and infants of syphilitic mothers reactivity of the 19S-IgM-FTA-ABS test indicates need of treatment even in cases without clinical symptoms of neonatal syphilis because of perinatal infection (Müller, 1983c, 1982, 1983). IgM antibodies are normally not transmitted to the fetus diaplacentally.

After successful treatment treponemal IgM antibodies disappear from patients' sera within 3-12 months depending on the stage of infection. This is demonstrated in the last slide (Fig. 6) showing group of patients after treatment of primary and secondary

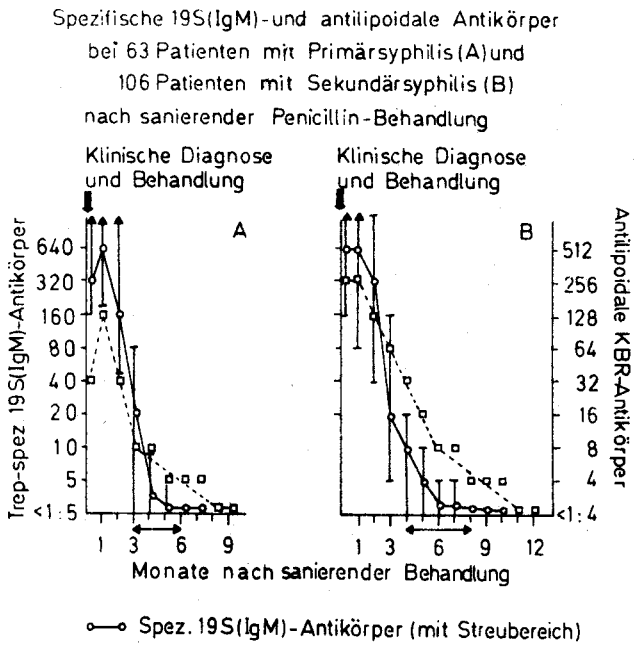

Fig. 6.

syphilis.

Conclusions

I sincerely hope I have succeeded in demonstrating that basic medical research is not only an end in itself but that its results can help clinicians to cure people suffering from a treponemal infection.

All over the world the diagnosis and treatment of syphilis mostly is one of the responsibilities of dermatologists. I hope I have been able to provide some new ideas in this lecture, given in honor of Professor Kung-Sun Oh, who established dermatology in this country.

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