Identification of a Low Molecular Weight IgM Antibody with Treponema Pallidum Specificity in Sera of Patients with Chronic Syphilis

F. Müller and S. Oelerich
Division of Serology, Department of Medical Microbiology, Institute of Hygiene, Hamburg

Nachweis eines treponemaserzifischen IgM-Antikörpers mit niedrigem Molekulargewicht bei Patienten mit chronischer Syphilis


Schlüsselwörter: Syphilis – Immunologie der Syphilis – 8S (IgM)-Antikörper – LMW-Antikörper

Summary. A low-molecular (8S) treponema-specific IgM antibody was isolated by means of Sephadex G 200 G gel filtration and/or sucrose gradient ultracentrifugation in 78 of 4,120 sera of patients who had been identified to have had syphilis. The IgM specificity can be shown by indirect immunofluorescence using a μ-chain specific antiserum. The low molecular IgM (LMW-IgM) antibodies are not identical with the 19S-IgM as demonstrated in studies with gel filtration and sucrose gradient methods. They are not identical neither with 7S IgG or IgA. Neither the presence of antinuclear nor rheumatic factor could be shown in the LMW-IgM fraction. In most of the patients with LMW-IgM antibody, there existed a treponema infection of late latency.

Key words: Syphilis – Treponema infection – Immunology of syphilis – 8S (IgM) antibody – LMW antibody in syphilis

More than ten years ago, Killander [10] and Rothfield et al. [18] first reported on the occurrence of immunoglobulin M with low molecular weight (LMW-IgM) in sera of human beings. Because of its μ-chain specificity and its low molecular weight, this protein was called 7S-IgM and later 8S-IgM. LMW-IgM was found in sera of patients with Type I dysgammaglobulinemia, autoimmune diseases and infectious diseases [2, 5, 6, 9, 10, 11, 13, 18-23]. Some biochemical differences between LMW-IgM and monomers of the 19S-IgM macromolecule were shown by Swedlund et al. [23] as well as Hansson and Laurell [7].

Up to now, very little is known about the antibody properties of this immunoglobulin. In 8S-IgM preparations Killander [10] showed activities against blood group substances. Rothfield et al. [18] noticed a reaction of LMW-IgM with cell nuclei (antinuclear antibody activity). Swedlund et al. [23] described 8S-IgM antibody activities against tetanus toxoid. Finally, Hunter et al. [8] found an 8S-IgM antibody in sera of infants reacting with cow’s milk casein.

Employing the indirect immunofluorescence technique our investigations on treponema-specific 19S-IgM antibodies in sera of syphilitic patients had an accessory result. In several patients at a chronic stage of infection we found a treponema-specific antibody with μ-chain specificity which could be distinguished from 19S-IgM by the relative late elution on Sephadex G 200 G gel filtration and sucrose density gradient.

The following paper gives a report about the identification of the treponema-specific LMW-IgM antibody. Additional experiments were carried out to characterize this immunoglobulin.
Materials and Methods

Sera. Sera from 4,120 patients with treated or untreated syphilis at several stages of the disease were studied by IgM-FTA-ABS and IgM-FTA-19S tests. All of them were found to be reactive in TPHA, FTA-ABS and TPI tests. The sera were heat inactivated (30 min at 56°C) and stored at -40°C till used.

Gel Filtration. The micro-method of Sephadex G 200 gel filtration described by Müller and Loa [15] was applied. 0.7 ml serum was passed through an 1.5 x 30 cm column by phosphate buffered saline (pH 7.3; 15 mS conductivity at 22°C). Fractions of 1.3 ml were collected.

Sucrose Density Gradient Ultracentrifugation. The sucrose gradient with concentrations from 12.5% to 37.5% was overlayed with fractions after gel filtration. The fluorescence reaction of LMW-IgM was not diminished by absorption of the whole serum or the fractions (after gel filtration) with ultra-sonicate of Treponema phagedenis indicating the Nichols specificity of the antibody. In some but not in all sera with treponema-specific LMW-IgM we found the rheumatoid factor with titre till 1 in 320. But there was no direct correlation between LMW-IgM and RA activity of these sera was demonstrated in the first elution peak. After gel filtration the RA activity of these sera was demonstrated in the first elution peak of 19S IgG only (second elution peak).

Results

In 123 sera of 78 syphilitic patients we found a low molecular weight antibody with μ-chain specificity of IgM but with a lower sedimentation rate than 19S. The incidence was calculated to be 1.9% of the investigated 4,120 patients. Figure 1 shows the elution pattern of this immunoglobulin in the ascending part of the second peak of Sephadex G 200 gel filtration. Though the separation of treponema-specific LMW-IgM and IgG antibodies is not complete, these two immunoglobulins are still differentiated because of the chain specificity of the immunofluorescence technique. It must be mentioned that IgA antibodies are eluted in the same position as LMW-IgM.

In 28 experiments we tried to differentiate the LMW-IgM from 19S-IgM by sucrose density gradient. Figure 2 shows the results of two different sera. Case A serum (from untreated primary syphilis) indicates a normal treponema-specific IgM antibody in the 19S region (first elution peak). In case B serum (from untreated late latent syphilis) the treponema-specific immunoglobulin with IgM determinants is located in the region of the 7S-IgG only (second elution peak).

The fluorescence reaction of LMW-IgM was not diminished by absorption of the whole serum or the fractions (after gel filtration) with ultra-sonicate of Treponema phagedenis indicating the Nichols specificity of the antibody.

No ANA activity could be found in sera with treponema-specific LMW-IgM using chicken red blood cells as the antigen.

In some but not in all sera with treponema-specific LMW-IgM we found the rheumatoid factor with titre till 1 in 320. But there was no direct correlation between LMW-IgM and RA. After gel filtration the RA activity of these sera was demonstrated in the first elution maximum of 19S globulins.

By absorption experiments it could be demonstrated that LMW-IgM cannot be identical with IgG or IgA. Only anti-IgM absorbed the LMW-IgM completely (Table 1).