Immunofluorescence investigations of antibodies against treponemal group antigen

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In the immunofluorescence test, apart from the antibodies directed specifically against pathogenic *T. pallidum*, antibodies directed against the protein antigen group common to pathogenic spirochaetes, both saprophytic and cultivated, are also detected (Deacon and Hunter, 1962; Fribourg-Blanc and Neil, 1966, 1967; Poetschke and Killisch, 1959; Covert, Kent, and Stevens, 1961; Király, 1966a, 1966b). The second type of antibody is an immunological response to spirochaetes saprophytizing on the mucous membranes of man.

A particularly common cause of nonspecific positive results to the FTA test is the antigenic effect of *Borrelia refringens* (Manikowska-Lesińska and Lesiński, 1968; Manikowska-Lesińska, 1969). The FTA-ABS test (Hunter, 1962; Hunter, Deacon, and Meyer, 1964) makes it possible to absorb from the serum tested antibodies directed against the group antigen. This absorption causes a slight and variable but evident fall in the fluorescence titre (Manikowska-Lesińska, 1966; Manikowska-Lesińska and Lesiński, 1968; Manikowska-Lesińska, Pucilo, and Linda, 1969).

The aim of these investigations was to discover whether there is a characteristic relationship between the specific and group antibodies revealed by the FTA and the various classes of immunoglobulins.

Material and methods

Of 228 syphilitic sera studied, 66 were from cases of primary syphilis, 109 from secondary, 23 from early latent, and 30 from late syphilis.

The sera were tested by the immunofluorescence method with the three-layer modification as described briefly in the foregoing paper (Manikowska-Lesińska and Jakubowski, 1970) and in detail in our previous report (Manikowska-Lesińska and Jakubowski, 1969).

The absorption of the syphilitic sera by the Reiter treponeme ultrasonicate was performed by means of the technique employed in the Białystok Laboratory for the FTA-ABS test (Manikowska-Lesińska and Lesiński, 1966).

All the sera were examined quantitatively before and after absorption by the ultrasonicate, and the titre of the monovalent fluorescence of the antibodies fixed with the various classes of immunoglobulins was determined (Manikowska-Lesińska, 1964).

Results

These are shown in the Figure (overleaf). Comparison of the fluorescence titres obtained before and after absorption with Reiter's ultrasonicate enabled us to establish the percentage of specific antibodies and group antibodies in the sera investigated. The diagram therefore shows the relationship of both types of antibody with immunoglobulins IgG, IgM, and IgA during the course of untreated syphilis.

Antibodies directed against the group antigen during primary syphilis are mainly fixed with immunoglobulins IgG and IgA. They comprise 38 per cent. of the total of IgG antibodies in sero-negative primary syphilis. The percentage of non-specific antibodies associated with IgG immunoglobulins falls considerably in the later stages of syphilis; in the late latent stage it was only 9 per cent.

Of the antibodies associated with IgM immunoglobulins, those directed specifically against pathogenic *T. pallidum* predominate in early syphilis. The percentage of IgM antibodies directed against group antigen was only 3.6 per cent. in sero-negative primary syphilis. The highest percentage was observed in latent syphilis.

A large percentage of IgA antibodies was directed against group antigen at all the stages of syphilitic infection. The percentage of nonspecific IgA antibodies increased significantly in the course of untreated syphilis, from 42 per cent. in primary sero-negative syphilis to 60 per cent. in late syphilis.

Discussion and conclusions

These results confirm the observation of Király, Backhausz, Jobbágy, Lajos, and Kováts (1967) and of Király and Jobbágy (1969) that both the antibodies specific for pathogenic *T. pallidum* and those directed

FIGURE Percentage specific and group antibodies present in IgG, IgM, and IgA immunoglobulins in the course of untreated syphilis

against group antigen are present in all three immunoglobulins investigated.

Specific antibodies produced in response to pathogenic *T. pallidum* in the early stages of syphilis are found chiefly in the IgM immunoglobulins. They not only form the largest proportion of antibodies detected in the FTA test but are also almost entirely directed against the specific antigen of *T. pallidum*. In the later stages of syphilis their number diminishes and the percentage of antibodies against group antigen increases.

We have previously shown that, in the early stages of syphilis, the IgG antibodies are few, and the present investigations prove that a large proportion of them is directed against group antigen. It may be concluded that *T. pallidum* infection acts first as a stimulus increasing the titre of IgG antibodies against saprophytic spirochaetes, and only later induces the production of specific IgG antibodies. In the later stages of *T. pallidum* infection the production of IgG antibodies against specific *T. pallidum* antigens increases so markedly that the percentage of IgG group antibodies is greatly reduced.

Summary

A new absorption method of immunofluorescence testing was used to study the relationship of antibodies directed against group antigen and specific *T. pallidum* antigen with immunoglobulins IgG, IgA, and IgM in 228 sera from patients at various stages of syphilis.

The specific antibodies produced as a result of *T. pallidum* infection in the early stages of syphilis are found mainly in IgM immunoglobulins. Antibodies associated with IgG and IgA immunoglobulins at this stage are directed chiefly against group antigen.

In the late stages of infection the production of IgG antibodies directed against specific *T. pallidum* antigen increases, and most of the IgM and IgA antibodies are directed against group antigen.

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Recherches sur les anticorps immuno-fluorescents vis-à-vis de l'antigène tréponémique de groupe

SOMMAIRE

On a utilisé une nouvelle méthode d'absorption de l'épreuve d'immuno-fluorescence pour étudier la relation entre les anticorps vis-à-vis de l'antigène de groupe et de l'antigène *T. pallidum* spécifique avec les immuno globulines IgG, IgA et IgM pour 228 sérums de malades à des stades variés de la syphilis.

Les anticorps spécifiques en rapport avec une infection à *T. pallidum* aux stades précoces de la syphilis appartiennent principalement au groupe des immuno globulines IgM. A ce stade, les anticorps des groupes des immuno globulines IgG et IgA sont en rapport principalement avec l'antigène de groupe.

Au stade tardif de l'infection la production d'anticorps IgG contre l'antigène spécifique *T. pallidum* augmente et la plupart des anticorps IgM et IgA sont en rapport avec l'antigène de groupe.