Preliminary Communications

Platelet Aggregation Test with Measles Antigen in Multiple Sclerosis

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Summary: With the measles platelet aggregation test, a new technique recently developed for measuring virus antibody, 153 serum specimens from patients with multiple sclerosis and 164 controls were tested. With one of the three measles antigens used in the test a significantly higher positive rate ($P < 0.001$) was obtained in the specimens from the patients with multiple sclerosis (40%) than in those from the controls (11%). The other two measles antigens also yielded slightly but not significantly higher positive rates in the patients with multiple sclerosis.

INTRODUCTION

Several reports have indicated an increased rate of high antibody levels for measles virus in multiple sclerosis (Adams and Imagava, 1962; Reed *et al.*, 1964; Clarke *et al.*, 1965; Pette and Kuwert, 1965; Adams, 1967; Caspary *et al.*, 1969; Panelius, 1969) or for a number of viruses, including measles (Sever *et al.*, 1968; Ross *et al.*, 1969). Others have found no differences in measles titre between patients with multiple sclerosis and controls (Just *et al.*, 1967) or the increased rate of higher titres has not been significant (Ross *et al.*, 1965). Brody *et al.* (1969) reported higher measles antibody levels in patients with multiple sclerosis and their siblings than in unrelated controls.

The connexion between multiple sclerosis and some infections, particularly measles, has been brought to the fore by the important reports suggesting that in subacute sclerosing panencephalitis measles virus persists in an incomplete form in the brain cells of the patients, producing a latent infection (Chen *et al.*, 1969; Horta-Barbosa *et al.*, 1969; Katz *et al.*, 1969; Payne *et al.*, 1969).

A new technique, the platelet aggregation test, for the demonstration and titration of virus antigens and antibodies has been described (Penttinen and Myllylä, 1968; Penttinen *et al.*, 1969, 1970). The test is based on aggregation of human platelets by a complex between virus antibody and a small-sized virus antigen. The present report describes the results of a platelet aggregation test carried out with three different measles antigens and serum specimens of patients with multiple sclerosis and controls.

PATIENTS AND METHODS

The 317 serum specimens used in the present study were collected earlier from two groups of patients with multiple sclerosis and matched controls with the same birth date and living in the same area (Panelius, 1969). The diagnostic criteria of the multiple sclerosis group 1 were those adopted by Schumacher *et al.* (1965) and the multiple sclerosis group 2 was similar to the "possible" group of Allison and Millar (1954). About two-thirds of the patients in group 1 were in an active phase of the disease, indicated by new symptoms or progression of earlier symptoms within a year. One-third of the patients were in quiescent phase.

ANTIGENS

The same measles virus strain (Halonen *et al.*, 1962) was used in the preparation of each antigen batch. Measles antigen No. 1 was prepared in LLC-MK2 cells maintained in basal medium (Eagle) supplemented with 5% horse serum. The medium was changed two days and five days after virus inoculation and the whole culture was harvested three days after the last medium change, when almost all the cells were fused into very large syncytia. The harvest was frozen and thawed three times and used in the test without further treatment.

Measles antigen No. 2 was prepared in the same cells and in the same way as No. 1, except that the medium was changed only once, and the culture was harvested six days after virus inoculation, by which time the cells were completely degenerated. This culture showed no syncytium formation. After freezing and thawing three times the harvest was centrifuged for 90 minutes at 33,000 r.p.m. in a Spinco rotor No. 40 and the supernatant was antigen No. 2. Antigen No. 3 was prepared in VERO cell roller cultures maintained in B.M.E. medium supplemented with 0.2% bovine serum albumin. After a high multiplicity of infection with measles virus the cells degenerated in three days. The whole culture was frozen and thawed three times, treated with Tween 80 and ether, and clarified by low-speed centrifugation.

Varicella-zoster antigen was prepared with a GMK-AH-1-adapted strain of varicella-zoster virus (kindly provided by Dr. A. Svedmyr, Stockholm), and further adapted to BSC-1 cells. Infected cells at the third passage in BSC-1, together with the medium (minimum essential medium + 0.2% bovine albumin), were harvested when the cytopathic effect was almost complete (on the 12th to 14th day after infection). The harvest was frozen and thawed twice, concentrated seven times with forced dialysis against a vacuum, and centrifuged for 90 minutes at 35,000 r.p.m. in a Spinco rotor 40. The supernatant was used as platelet aggregation antigen.

P.A. TECHNIQUE

The antibody assay based on platelet aggregation has been described in detail elsewhere (Penttinen and Myllylä, 1968; Penttinen *et al.*, 1969, 1970). A fresh batch of washed human platelets prepared by differential centrifugation (Aster and Jandl, 1964) was suspended (200,000 platelets/ml.) each day in phosphate-buffered saline at pH 7.8. A diluent without glucose was used with measles antigen No. 1 and varicella-zoster antigen, whereas glucose in a final concentration of 0.15 mg./ml. was used with measles antigens Nos. 2 and 3. The titrations were performed in microtitre U-plates. Equal volumes (0.05 ml.) of antigen dilutions and serial dilutions of serum were mixed, incubated for one hour at room temperature, and the platelet suspension (0.05 ml.) was added. The plates were incubated overnight at +5° to +8°C. and the sedimentation patterns were read with dark background illumination. The titres are expressed as reciprocal values.

RESULTS

The original plan of the study was to test the whole material of 153 serum specimens of patients with multiple sclerosis and 164 controls with measles antigen No. 1. When 47 multiple sclerosis specimens and 62 controls had been tested,

Rate of Positive Reactions in Platelet Aggregation Test with Three Measles Antigens in Serum Specimens of Two Groups of Patients with Multiple Sclerosis (M.S.) and Controls

Group	Positive*		
	Antigen 1	Antigen 2	Antigen 3
M.S. patients, group 1†	15/38 (38%)	19/85 (22%)	36/81 (44%)
Controls	6/55 (11%)	12/74 (16%)	24/69 (35%)
M.S. patients, group 2†	3/6	4/18 (22%)	8/18 (44%)
Controls	1/6	1/19 (5%)	4/19 (21%)
Total M.S.	18/45 (40%)	23/103 (22%)	44/99 (44%)
Total controls	7/61 (11%)	13/93 (14%)	28/88 (32%)

*Positive ≥ 160 (specimens with titres ≥ 160 but serum controls ≥ 20 are not included in the figures). †See Patients and Methods.

however, the antigen was used up and the rest of the material was tested with antigens Nos. 2 and 3. When specimens positive in serum controls were discarded the final numbers of specimens were 106, 196 and 187 with the respective measles antigens Nos. 1, 2, and 3.

A statistically significantly higher positive (≥ 160) rate in patients with multiple sclerosis (40%) than in the controls (11%) was obtained with measles antigen No. 1, as indicated in the Table. In the rest of the serum material tested with antigens Nos. 2 and 3 the positive rate was also slightly but not significantly higher in patients with multiple sclerosis.

When a titre of 80 was used as a "cut-off point" the positive rate was 47% in patients with multiple sclerosis and 16% in the controls with antigen No. 1, 29% and 24% respectively, with antigen No. 2, and 47% and 34%, respectively, with antigen No. 3. Titres of >160 were observed with varicella-zoster antigen in three out of 37 M.S. patients and in none of 55 controls tested. Two of the varicella-zoster-positive specimens were also positive in the measles platelet aggregation test.

DISCUSSION

If any connexion exists between an earlier measles infection and multiple sclerosis, as seems possible in some cases, probably the virus persists in the organism in some incomplete form, as in subacute sclerosing panencephalitis. Accordingly, it would be expected that the antibody titre would rise selectively for only some and not all of the measles-induced antigens. Thus, tests yielding new information on the various antibodies reacting with one or a few of the measles antigens in multiple sclerosis are of interest. The discrepancies in the literature concerning high measles antibody titres in patients with multiple sclerosis may be partly due to the different types of measles antigens used. With rubella virus the active antigen in the platelet aggregation test is easily separable from infective virus and from the haemagglutinin but seems to be similar to, though not identical with, the small-sized complement-fixation antigen (Myllylä *et al.*, 1969).

The greater sensitivity of measles antigen No. 1 than of either No. 2 or No. 3 in detecting a higher measles platelet-aggregation-positive rate in patients with multiple sclerosis may indicate that the technique of antigen preparation is of importance. Unfortunately, too many variables were combined with the change of the antigens to conclude from these results the nature of the optimal antigen. The differences observed, however, may be of value in studying the antigenic fractions of measles virus or the non-viral cell products induced by measles virus required for detecting higher positive rates in patients with multiple sclerosis.

The significance of a control antigen prepared from non-infected cells has been shown by Caspary *et al.* (1969). Selection of cells for antigen production is also of importance, not only for producing high-titred virus antigens but for virus-specific cell products other than virus particles. These differences in the cell responses are shown by VERO cells used in the present study in the preparation of measles antigen No. 3. VERO cells are defective in their ability to produce interferon (Desmyter *et al.*, 1968).

The negative results with the measles platelet aggregation

test in some of the patients with multiple sclerosis could be explained by a probable multiple aetiology in multiple sclerosis disease, which is assumed to be more of a syndrome (Kurland, 1968) than a disease entity with one aetiology. The finding of a few positive reactions in the varicella-zoster platelet aggregation test in patients with multiple sclerosis is in accordance with earlier reports indicating increased varicella-zoster complement-fixation titres in some patients with multiple sclerosis (Ross *et al.*, 1965, 1969).

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REFERENCES

- Adams, J. M., (1967). *Neurology (Minneapolis)*, **17**, 707.
- Adams, J. M., and Imagawa, D. T. (1962). *Proceedings of the Society for Experimental Biology and Medicine*, **111**, 562.
- Allison, R. S., and Millar, J. H. D. (1954). *Ulster Medical Journal*, **23**, Suppl. No. 2.
- Aster, R. H., and Jandl, J. H. (1964). *Journal of Clinical Investigation*, **43**, 843.
- Brody, J. A., Sever, J. L., and Henson, T. E. (1969). Presented at the workshop on the epidemiology of multiple sclerosis, September 27-28, New York.
- Caspary, E. A., Chambers, M. E., and Field, E. J. (1969). *Neurology (Minneapolis)*, **19**, 1038.
- Chen, T. T., Watanabe, I., Zeman, W., and Mealey, J. (1969). *Science*, **163**, 1193.
- Clarke, J. K., Dane, D. S., and Dick, G. W. A. (1965). *Brain*, **88**, 953.
- Desmyter, J., Melnick, J. L., and Rawls, W. E. (1968). *Journal of Virology*, **2**, 955.
- Halonon, P., Pettay, O., and Salmi, I. (1962). *Acta Paediatrica*, **51**, 27.
- Horta-Barbosa, L., Fuccillo, D. A., and Sever, J. L. (1969). *Nature*, **221**, 974.
- Just, M., Rieder, H. P., and Ritzel, G. (1967). *Klinische Wochenschrift*, **45**, 705.
- Katz, M., Oyanagi, S., and Koprowski, H. (1969). *Nature*, **222**, 888.
- Kurland, L. T., (1968). *The Epidemiology of Multiple Sclerosis*, edited by M. Alter and J. F. Kurtzke, p. 3. Springfield, Illinois, Thomas.
- Myllylä, G., Vaheri, T., Vesikari, T., and Penttinen, K. (1969). *Clinical and Experimental Immunology*, **4**, 323.
- Panelius, M. (1969). *Acta Neurologica Scandinavica*, **45**, Suppl. No. 39.
- Payne, F. E., Baublis, J. V., and Itabashi, H. H. (1969). *New England Journal of Medicine*, **281**, 585.
- Penttinen, K., and Myllylä, G. (1968). *Annales Medicinae Experimentalis Fenniae*, **46**, 188.
- Penttinen, K., Myllylä, G., Mäkelä, O., and Vaheri, A. (1969). *Acta Pathologica et Microbiologica Scandinavica*, **77**, 309.
- Penttinen, K., Saikku, P., Myllylä, G., Brummer-Korvenkontio, M., and Oker-Blom, N. (1970). *Journal of Hygiene*. In press.
- Pette, E., and Kuwert, E. (1965). *Archiv für die Gesamte Virusforschung*, **16**, 141.
- Reed, D., Sever, J., Kurtzke, J., and Kurland, L. (1964). *Archives of Neurology*, **10**, 402.
- Ross, C. A. C., Lenman, J. A. R., and Rutter, C. (1965). *British Medical Journal*, **1**, 226.
- Ross, C. A. C., Lenman, J. A. R., and Melville, I. D. (1969). *British Medical Journal*, **3**, 512.
- Schumacher, G. A., *et al.* (1965). *Annals of the New York Academy of Sciences*, **122**, 552.
- Sever, J. L., Reed, D., Kurtzke, J. F., Huebner, R. J., and Kurland, L. T. (1968). *The Epidemiology of Multiple Sclerosis*, edited by M. Alter and J. F. Kurtzke, p. 107. Springfield, Illinois, Thomas.