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Immunology

Measles virus-specific IgD antibodies in patients with subacute sclerosing panencephalitis*

(immunoglobulin/slow virus disease)

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ABSTRACT **Indirect immunofluorescent analysis revealed that sera from five patients with subacute sclerosing panencephalitis possessed IgD antibodies directed against measles virus components in persistently infected HeLa cells. IgD levels in these sera were within the normal range. Control studies indicated that the reaction was specific for measles virus. The detection of IgD measles antibodies in these patients suggested that IgD may be involved in the pathogenesis of this viral disease.**

Since the initial isolation and structural characterization of IgD from the serum of a patient with multiple myeloma (1), the biological function(s) of IgD has remained a question. Recent studies have revealed that a high percentage of human lymphocytes possess membrane-bound IgD (2). This report led to the suggestion that IgD may function as a lymphocyte receptor for B-cell differentiation (3). The receptor role for IgD, however, does not account for IgD found in the serum (4) nor its apparent antibody activity (5). Investigations with serum IgD have been hampered because of normally low levels of IgD in the serum and the degradation of IgD molecules by plasmin activity (6). Nonetheless, IgD has been detected in some patients' sera directed against chronically present antigens; these include penicillin (7), bovine gamma globulin (8), diphtheria toxoid (8), insulin (9, 10), and nuclear components (5).

In the course of our investigations on the biological function(s) of IgD, antibody activity within the IgD class was observed in children with subacute sclerosing panencephalitis (SSPE), a slow degenerative disease of the central nervous system (11). SSPE is characterized by extremely high titers of antibodies directed against measles-like viruses (12) in the serum and spinal fluid of these patients (13). Antigen–antibody complexes have been related to the pathogenesis of the disease (14, 15). Although antibodies of the IgG class predominate in the patient's serum, the presence of IgM measles-specific antibodies has been reported (16). In this report evidence is presented that IgD antibodies directed against measles virus components are present in sera of children with SSPE. This represents evidence of IgD antibody activity against a viral agent.

MATERIALS AND METHODS

Sera. Five SSPE sera with complement fixing antibody titers to measles virus that ranged between 1:256 and 1:4096 were obtained from D. A. Fuccillo (NINDS). Human IgD myeloma sera were provided by R. Kyle (University of Rochester), J. D. Capra (University of Texas), and T. Waldmann (NIH). Sera from eight multiple sclerosis patients were supplied by B. Pirofsky of the Division of Immunology, University of Oregon Health Sciences Center. Seropositive control sera were obtained from normal blood donors at the Blood Bank Service of the American Red Cross, and convalescent patients clinically diagnosed as having had measles (complement fixing titer 1:32).

Immunofluorescent Procedure. The presence of measles virus specific IgG and IgD antibodies was determined by indirect immunofluorescence. Coverslip cultures were fixed with 95% methanol at room temperature for 5 min, rinsed with phosphate-buffered saline (0.14 M NaCl, 0.01 M PO_4, pH 7.2), and covered with the appropriate sera diluted 1:10 in phosphate-buffered saline. After 30 min at 37°, the cells were rinsed with phosphate-buffered saline and stained with either a 1:10 dilution of fluorescein isothiocyanate (FITC) conjugated anti-human IgG (anti-γ) or a 1:4 dilution of FITC conjugated anti-human IgD (anti-δ) (Meloy Laboratory, Springfield, Va.) for 30 min at 37°. The coverslips were counterstained with Evans blue (0.06% in phosphate-buffered saline) for 5 min at room temperature, rinsed thoroughly, mounted, and observed with a Zeiss microscope with integral illuminator 6 V 15 W.

Viruses and Cells. The Edmonston strain of measles virus was obtained from the American Type Culture Collection. Mumps virus was obtained from the Center for Disease Control. Newcastle disease virus, Herts strain, was obtained from J. S. Youngner (University of Pittsburgh). HeLa, BHK-21, and WI-38 cells were grown on round coverslips in Linbro trays in Eagle's minimum essential medium supplemented with 10% heat-inactivated fetal calf serum with 100 units/ml of penicillin and 100 μg/ml of streptomycin (17).

Virus-Infected Cells. A persistent infection of HeLa cells with the Edmonston strain of measles virus ($HeLa_{PI}$ cells) was established and has been characterized (18, R. C. Armen and J. V. Hallum, manuscript in preparation). These cells were used as the primary tissue substrate throughout these studies. $HeLa_{PI}$ cells release 10^3 to 10^4 plaque forming units/ml of culture fluid and are resistant to superinfection with wild-type measles virus. Electron micrographs reveal the presence of nucleocapsid structures in both the cytoplasm and nucleus of these infected cells. Ninety-five percent of the $HeLa_{PI}$ cells demonstrate measles virus components by hemadsorption with monkey erythrocytes or immunofluorescence with measles virus convalescent antiserum. The techniques for the preparation of cells infected with Newcastle disease virus and mumps virus have been described elsewhere (17, 19).

Specificity of FITC Conjugated Anti-δ. By Ouchterlony analysis, a single precipitin line was detected between the FITC conjugated anti-δ antiserum and a pool of normal

Abbreviations: SSPE, subacute sclerosing panencephalitis; FITC, fluorescein isothiocyanate; $HeLa_{PI}$ cells, HeLa cells persistently infected with measles virus.

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Table 1. Measles specific IgG and IgD antibodies in patient sera*

Group	No. tested	No. positive		Mean IgD serum level (mg/100 ml)
		IgG antibody	IgD antibody	
SSPE	5	5	5	3.0
Multiple sclerosis	8	8	0	2.6
IgD myeloma	10	ND†	0	337.0
Sero-positive controls	22	22	0	3.1

* Sera were diluted 1:10 prior to testing.
† ND = not determined.

human sera known to contain IgD. Precipitin lines were not observed when the conjugate was absorbed with purified IgD-myeloma proteins. Fluorescence was not detected when the adsorbed conjugate was reacted with SSPE sera. FITC conjugated anti-δ from other sources (Behring Diagnostics, Somerville, N.J. and Cappel Laboratories, Inc., Downington, Pa.) gave similar results in indirect immunofluorescent tests. Additional absorption of these antisera (Behring and Cappel) with pooled normal human IgG did not diminish the fluorescent anti-δ reactivity, even though we could demonstrate free kappa, lambda, and gamma determinants in the absorption mixture by gel diffusion.

Immunoglobulin Quantitation. Serum IgD levels were measured by radial immunodiffusion in agarose gel with anti-δ antiserum as previously described (20, 21).

RESULTS

Specificity of IgD Antibodies to Measles Virus. IgD antibodies in SSPE sera to measles virus components were determined to be specific by a series of control experiments. Positive fluorescence for IgD was not detected when SSPE serum was reacted with uninfected HeLa cells, uninfected WI-38 cells, uninfected BHK-21 cells, BHK-21 cells infected with mumps virus, and HeLa cells infected with Newcastle disease virus. Fluorescence was not removed after adsorption of SSPE serum with uninfected HeLa cells, uninfected WI-38 cells, uninfected BHK-21 cells, or BHK-21 cells infected with mumps virus. The fluorescence was removed by adsorption with $HeLa_{PI}$ cells or after extensive absorption with a concentrated measles virus preparation grown in primary African Green Monkey kidney cells. Additionally, positive fluorescence was observed when SSPE serum was reacted with WI-38 cells infected with measles virus. These results indicated that IgD antibodies in SSPE serum are directed against measles virus antigens rather than cellular components, viral-induced structural changes, or other paramyxoviruses, such as Newcastle disease virus and mumps virus.

IgG and IgD Measles Antibodies. Sera from the five SSPE patients contained IgG and IgD measles specific antibodies (Table 1). IgD anti-measles virus antibodies were detected in three of the SSPE sera at a 1 to 40 dilution. Measles antibodies of the IgD class were not detected in sera from any other group tested, i.e., measles convalescent, multiple sclerosis, IgD-myeloma, and normal human serum. The multiple sclerosis sera were included because of the slightly higher measles virus antibody titers previously reported (22). The fact that positive fluorescence was not observed with the IgD myeloma plasma suggested that IgD molecules are not cytophilic for $HeLa_{PI}$ cells. In contrast, measles antibodies of the IgG class were found in all sera tested. Significant differences in serum IgD levels were not observed in SSPE patients when compared to normal human serum.

Microscopic Examination. In positive preparations for IgG antibodies, greater than 95% of the $HeLa_{PI}$ cells revealed a granular fluorescence in both the nucleus and cytoplasm. $HeLa_{PI}$ cells stained with FITC anti-δ demonstrated the same percentage of infected cells; however, the fluorescence was primarily cytoplasmic, with some nuclear fluorescence.

DISCUSSION

IgD antibodies to measles virus in sera of SSPE patients provide evidence of antiviral activity by IgD molecules. Several investigators have found IgD antibodies against nuclear components in sera of some patients with autoimmune disease, i.e., systemic lupus erythematosus, discoid lupus erythematosus, systemic scleroderma, rheumatoid arthritis, and Raynaud's disease (5). Heiner and Rose demonstrated anti-bovine serum albumin and anti-bovine gammaglobulin antibodies from the IgD class in three patients with sensitivity to cow's milk and IgD antibodies to diphtheria toxoid following booster injections with the toxoid in another individual (8). IgD antibodies to insulin have been reported in 14% of the diabetics tested after long-term treatment with nonhuman insulin (10), and to penicillin in three hypersensitive individuals (7). From these reports it would appear that chronic antigenemia is a prerequisite for the development of IgD antibodies. The continual presence of viral components in patients with SSPE both within the cell as well as on the cell membrane would provide the long-term stimulation necessary for IgD production (11).

The significance of IgD antibody activity is currently unknown. IgD measles virus antibodies could be detected at a 1:40 dilution by indirect immunofluorescence despite the fact that serum IgD levels were within the normal range. This would suggest that a significant proportion of the total IgD is directed against measles virus. Furthermore, in the above studies, IgD antibodies were detected in a small percentage from each group studied while in this study IgD measles virus antibodies were in all five SSPE patients examined. These facts suggest a functional role for IgD in these patients. The interaction of IgD with viral-coded membrane proteins may block the binding of complement fixing antibodies and subsequent lysis. On the other hand, IgD antibodies may form circulating complexes with the virus and result in a specific suppression of thymus-derived lymphocyte functions. Circulating measles virus-antibody complexes in SSPE patients which result in a suppression of thymus-derived lymphocyte function have been reported (14, 15). Whatever its role, it would appear that IgD antibodies can develop in response to a wide variety of antigenic stimuli.

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1. Rowe, D. S. & Fahey, J. L. (1965) *J. Exp. Med.* **121**, 171–184.
2. Van Boxel, J. A., Paul, W. E., Terry, W. D. & Green, I. (1972) *J. Immunol.* **109**, 648–665.
3. Vitetta, E. S. & Uhr, J. W. (1975) *Science* **189**, 964–969.
4. Leslie, G. A., Lopez, R. H. & Holmes, J. N. (1975) *Int. Arch. Allergy Appl. Immunol.* **49**, 350–357.
5. Schmidt, K. & Mueller-Eckhardt, S. (1973) *Z. Immunitaetsforsch. Exp. Ther.* **145**, 385–391.
6. Spiegelberg, H. L. (1972) in *Contemporary Topics in Immunochemistry*, ed. Enman (Plenum Pub., New York), Vol. 1, pp. 165–180.
7. Gleich, G. J., Biegen, R. C. & Stankievic, R. (1969) *Science* **165**, 606.
8. Heiner, D. C. & Rose, B. (1970) *J. Immunol.* **104**, 691–697.
9. Devey, M., Sanderson, C. J., Carter, D. & Coombs, R. R. A. (1970) *Lancet* ii, 1280–1282.
10. Lertora, J. J. L., Gomez, F. J. & Leslie, G. A. (1975) *Int. Arch. Allergy Appl. Immunol.* **49**, 596–606.
11. ter Meulin, V., Katz, M. & Muller, D. (1972) in *Current Topics in Microbiology and Immunology*, ed. Arber, W. (Springer-Verlag, New York), Vol. 57, pp. 1–38.
12. Payne, F. & Baublis, J. (1973) *J. Infect. Dis.* **127**, 505–511.
13. Connolly, J. H., Allen, I. V., Hurwitz, L. J. & Millar, J. H. D. (1967) *Lancet* **i**, 542–544.
14. Sell, K. W., Thurman, G. B., Ahmed, A. & Strong, D. M. (1973) *N. Engl. J. Med.* **288**, 215–216.
15. Ahmed, A., Strong, D. M., Sell, K. W., Thurman, G. B., Knudsen, R. C., Wistar, R. & Grace, W. R. (1974) *J. Exp. Med.* **139**, 902–924.
16. Connolly, J. H., Haire, M. & Hadden, D. S. M. (1971) *Br. Med. J.* **1**, 23–25.
17. Cummiskey, J. F., Hallum, J. V., Skinner, M. S. & Leslie, G. A. (1973) *Infect. Immun.* **8**, 657–664.
18. Armen, R. C. & Hallum, J. V. (1974) *Abstracts of the Annual Meeting of the American Society for Microbiology*, p. 250.
19. Truant, A. L. & Hallum, J. V. (1974) *Abstracts of the Annual Meeting of the American Society for Microbiology*, p. 261.
20. Luster, M. I., Leslie, G. A. & Bardana, E. J. (1976) *Int. Arch. Allergy Appl. Immunol.* **50**, 212–219.
21. Leslie, G. A. & Swate, T. E. (1972) *J. Immunol.* **109**, 47–50.
22. Henson, T. E., Brody, J. A., Sever, J. L., Cyken, M. L. & Cannon, J. (1970) *J. Am. Med. Assoc.* **211**, 1985–1988.