INFECTION AND IMMUNITY, July 1979, p. 408-416
0019-9567/79/07-0408/09 $02.00/0

Vol. 25, No. 1

Measles Virus-Specific Antibodies and Immunoglobulin M Antiglobulin in Sera from Multiple Sclerosis and Rheumatoid Arthritis Patients

P. V. SHIRODARIA,[1]* K. B. FRASER,[1] MARILYN ARMSTRONG,[1] AND S. D. ROBERTS[2]

Department of Microbiology and Immunobiology, The Queen's University of Belfast, Belfast BT12 6BN,[1] and Department of Rheumatology, The Royal Victoria Hospital, Belfast BT12 6BA,[2] Northern Ireland

Received for publication 5 January 1979

When rheumatoid factor in rheumatoid arthritis and multiple sclerosis sera was titrated by the fluorescent antibody method on measles virus-infected cells, there was a marked and variable drop in titer on acetone-fixed cells as compared with unfixed cells. This was accounted for by the failure of measles virus hemolysin-inhibiting (HLI) antibody of the immunoglobulin G class to bind to acetone-fixed infected cells. It was shown by staining unfixed and acetone-fixed measles virus-infected cells that rheumatoid factor in most rheumatoid arthritis sera combined with measles virus-specific hemagglutinin-inhibiting and HLI antibodies, whereas rheumatoid factor in multiple sclerosis sera combined only with HLI antibody. Rheumatoid factor of similar specificity was also observed in normal sera and occasionally in rheumatoid arthritis sera. Both rheumatoid arthritis and multiple sclerosis sera showed almost identical increases in average titer above normal of measles virus-specific fluorescent staining immunoglobulin G and HLI antibodies.

Sera from some multiple sclerosis patients contain a measles virus-specific immunoglobulin M (IgM) called MS-IgM, which binds to the surface of unfixed measles virus-infected cells but not to acetone-fixed preparations (8). Previous observations on acetone-fixed measles virus-infected cells showed that the presence of rheumatoid factor (RF) in sera can result in a secondary IgM staining (21). In view of this and of the failure of Salmi and his colleagues (20) to demonstrate the presence of measles virus-specific IgM in multiple sclerosis patients sera which had been depleted of IgG, we reexamined the properties of MS-IgM and found it to be an antiglobulin, not a measles virus-specific antibody (6, 6a).

Recent observations on the differing sensitivities of measles virus hemolysin and hemagglutinin to acetone fixation (7) and on the differences in titers of antihemolysin and antihemagglutinin in most human sera have allowed us to design experiments to investigate secondary IgM staining of measles virus-infected cells by RF-like substances (antiglobulins).

In this paper we report results of secondary IgM staining by RF of unfixed and acetone-fixed measles virus-infected cells and cells infected with other viruses. We also show that patients with rheumatoid arthritis have the same increase in measles virus-specific antibody as has been reported often in patients with multiple sclerosis (2, 3, 5), thus pointing to something in common in the immunology of the two diseases.

MATERIALS AND METHODS

Patients. Serum samples were obtained from patients clinically diagnosed as having multiple sclerosis according to the criteria described by Allison and Millar (1) and elaborated by Leibowitz and Alter (15) and from selected patients with definite rheumatoid arthritis according to the criteria of the American Rheumatism Association (19). The sera were collected over a period of 2 years and stored at −20°C until tested.

For the preliminary survey, sera from three groups were selected: (i) patients with multiple sclerosis, (ii) patients with rheumatoid arthritis, and (iii) normal controls (healthy blood donors). In each group 15 subjects were included, 7 females and 8 males. The age range was 39 to 74 years. The sera were sex matched and age matched within a 2-year range.

Cells and media. HEp-2 cells were used for measles, herpes simplex, and respiratory syncytial viruses and were grown at 37°C in Eagle medium with 10% fetal bovine serum and 10% tryptose phosphate broth. Vero cells were used for Sendai virus and were grown at 37°C in M199 medium with 6% fetal bovine serum. After infection cells were maintained in Eagle or M199 medium with 1% fetal bovine serum.

Virus preparations. The Edmonston strain of measles virus grown in HEp-2 cells and designated TC243 (9), herpes simplex virus (H.F.E.M. strain) grown in HEp-2 cells, respiratory syncytial virus (Long strain) grown in HEp-2 cells, and Sendai virus grown

in allantoic fluid and kindly supplied by B. W. J. Mahy, Virology Division, Cambridge University, were used in the preparation of infected cells. Cells grown on round glass cover slips were inoculated with each virus preparation so as to give about 30% infected cells after 48 h of incubation.

Serological methods. Before serological assays, all of the sera were absorbed overnight at 4°C with acetone-treated, washed, pelleted HEp-2 cells (30%, vol/vol). Absorbed sera were inactivated at 56°C for 30 min.

Fluorescent antibody test. The indirect test described previously (8) was applied to either unfixed or acetone-fixed infected cells on cover slips for the titration of the virus-specific secondary IgM staining by RF (21) and virus-specific IgG staining. Endpoint values were interpolated when the endpoint of the titration was greater or less than an arbitrary 1+ fluorescence (see Table 1).

HAI test. The standard micro method for the measles hemagglutination inhibition (HAI) test was used. Serial twofold dilutions of serum were prepared in Dulbecco phosphate-buffered saline, pH 7.4, and an equal volume of the virus with 4 hemagglutinating doses was added to each dilution. After incubation at 37°C for 1 h, an equal volume of a 1% suspension of monkey erythrocytes was added. The erythrocytes were allowed to settle at 37°C for 1 h. The highest dilution of serum which gave 50% inhibition of agglutination was taken as the endpoint.

HLI test. For the hemolysin inhibition (HLI) test 0.25-ml amounts of serial twofold dilutions of serum were mixed with equal volumes of the virus preparation containing 8 hemolysing doses. After incubation at 37°C for 60 min, equal volumes of a 2% suspension of monkey erythrocytes were added. After further incubation at 37°C for 18 h, the samples were centrifuged at 200 × *g* for 5 min, and the supernatants were examined for lysis. The highest dilution of serum which gave 50% reduction of hemolysis was taken as the endpoint.

RF. The standard latex slide agglutination test (RA-test; latex-globulin reagent; Hyland Div., Travenol Laboratories, Costa Mesa, Calif.) was used to detect IgM RF in sera.

Absorption with human aggregated gamma globulin and latex-globulin reagent. Heat-aggregated human gamma globulin was prepared from human normal immunoglobulin (Central Public Health Laboratory, London, England) as described previously (21). Aggregated gamma globulin was washed three times and resuspended in phosphate-buffered saline. Latex particles coated with globulin (Hyland latex-globulin reagent) were washed five times and resuspended in phosphate-buffered saline.

The suspension of either aggregated gamma globulin or latex-globulin reagent was pelleted by centrifugation at 2,000 × *g* for 20 min, and a volume of serum was added to the pelleted material to obtain the final concentration of the absorbents indicated below. Absorptions were carried out either at room temperature (20 to 22°C) for 30 min or overnight at 4°C. Sera were clarified by centrifugation at 6,000 × *g* for 30 min before use.

RESULTS

Secondary IgM staining of unfixed and acetone-fixed virus-infected cells by RF. A difference was observed between measles virus and other viruses when IgM RF in a rheumatoid serum was titrated by the fluorescent antibody method on virus-infected HEp-2 cells or Vero cells, unfixed or fixed in acetone at room temperature for 10 min. The ratio of titer on unfixed cells to titer on acetone-fixed cells infected with herpes simplex virus, respiratory syncytial virus, or Sendai virus was not much more than two. In contrast, the ratio with measles virus-infected cells ranged from less than 4 to 12. When six more rheumatoid sera were used, the range of ratios of titers on unfixed cells to titers on acetone-fixed cells was 1.5 to 2.5 for cells infected with herpes simplex virus, 1 to 2.5 for cells infected with respiratory syncytial virus, and 3.5 to 12 for cells infected with measles virus. Experimental data showing the intensities of fluorescence for one rheumatoid serum on herpes simplex, respiratory syncytial, and measles viruses and one multiple sclerosis serum on measles virus (included for comparison) are given in Table 1.

When six sera from multiple sclerosis patients were titrated on measles virus-infected unfixed or acetone-fixed cells, a ratio of >12 to >80 was obtained. Since most of these sera gave no staining on acetone-fixed virus-infected cells at the 1:3 dilution, ratios for RF in these sera are minimal values.

Secondary IgM staining and measles virus-specific IgG. The above results suggest that the loss of titer of secondary IgM staining on acetone-fixed measles virus-infected cells may be due to loss of antigen as a result of acetone fixation. If this were so, then the variation in the ratios of the titer of measles virus-specific IgG on unfixed cells to the titer on acetone-fixed, infected cells would be clearly equal to or proportional to the ratios of secondary IgM staining by RF on unfixed and acetone-fixed infected cells. To test this, eight rheumatoid sera and two normal sera with RFs were examined by the fluorescent antibody method for virus-specific secondary IgM and virus-specific IgG fluorescence titers on unfixed and acetone-fixed infected cells. The fluorescent antibody titers and the expected and observed values of secondary IgM fluorescence staining on acetone-fixed infected cells are shown in Table 2.

It can be seen that the titers of secondary IgM staining by RF on unfixed or acetone-fixed virus-infected cells depends on two factors: (i) quantity

TABLE 1. *Comparison of intensities of fluorescence and antibody titers of secondary IgM on unfixed and acetone-fixed cells infected with different viruses*

Serum	Infecting virus	Fixation	Reaction with the following fluorescent antibody titer: 8	16	32	64	128	256	512	Reciprocal of titer
Rheumatoid	Herpes simplex	Unfixed	++++	++++	+++	++	+	tr[a]	−	128
		Acetone-fixed	++++	+++	++	+	±	−	−	106
	Respiratory syncytial	Unfixed	++++	+++	++	+	±	−	−	106
		Acetone-fixed	+++	++++	++	+	+	−	−	128
	Measles	Unfixed	++++	+++	++	+	+	−	−	128
		Acetone-fixed	+++	++	−	−	−	−	−	24
Multiple sclerosis	Measles	Unfixed	+++	++	++	++	−	−	−	80
		Acetone-fixed	−	−	−	−	−	−	−	

[a] tr, Trace.

TABLE 2. *Fluorescent IgG and IgM titers of rheumatoid arthritis sera on unfixed and acetone-fixed measles virus-infected cells*

Serum	RF titer	Ratio of HLI to HAI	Immunofluorescence titer with: Unfixed cells IgM	Unfixed cells IgG	Acetone-fixed cells IgM	Acetone-fixed cells IgG	Ratio of virus-specific IgG in unfixed vs acetone-fixed cells	Expected titer of secondary IgM staining on acetone-fixed cells[a]
RA 1	6	16	12	768	<3	192	4	3
RA 2	192	8	192	768	48	192	4	48
RA 3	12	32	24	768	<3	48	16	1.5
RA 4	384	2	192	768	48	192	4	48
RA 5	6	12	24	3,072	3	384	8	3
RA 6	3	8	12	3,072	<3	384	8	1.5
RA 7	192	4	96	192	48	96	2	48
RA 8	24	16	96	384	12	48	8	12
Control 1	12	4	24	192	<3	48	4	6
Control 2	12	2	24	384	<3	192	2	12

[a] The expected IgM titer of rheumatoid (RA) sera on fixed tissue was calculated from the titer on unfixed tissue on the assumption that IgM binding is proportional to the amount of bound virus-specific IgG. Compare with IgM immunofluorescence titer with acetone-fixed cells.

of RF and (ii) quantity of virus-specific IgG. In seven rheumatoid sera (Table 2, RA 2 through 8) the decrease in the titer of secondary IgM staining after fixation compared with the titer on unfixed infected cells seemed to bear the same ratio as the decrease in virus-specific IgG titers on unfixed compared with acetone-fixed infected cells. One rheumatoid serum (RA 1) and two normal control sera (Table 2) were exceptions in that the secondary IgM staining was observed only on unfixed cells and not on acetone-fixed cells, although by calculation of expected values of secondary IgM these sera should have shown secondary IgM staining on acetone-fixed cells (Table 2). These sera had low RF titers and hence low titers of secondary IgM staining on unfixed infected cells. It is also noteworthy that in some rheumatoid sera with low-titer RF, secondary IgM staining accompanied high titers of virus-specific IgG antibodies.

Binding capacity of RF with virus-specific HLI and HAI IgG antibodies. In view of the above results, it was necessary to compare the binding capacity of RF to HLI antibody with its binding capacity to HAI antibody. In reconstruction experiments, equal amounts of HLI or HAI antibody were added to unfixed and acetone-fixed measles virus-infected cells, followed by the addition of a fixed dilution of rheumatoid serum containing RF but not measles virus-specific antibodies (Fig. 1a and b). As most human sera contain higher titers of HLI antibodies than HAI antibodies, HAI antibody could only be applied when HLI antibody was present. Application of the serum to acetone-fixed cells ensured that only the HAI antibody was taken up at the cell membrane. HLI antibody was added to unfixed cells at a dilution of serum which was beyond the endpoint of HAI activity. The fluorescence assay was used for HLI and HAI anti-

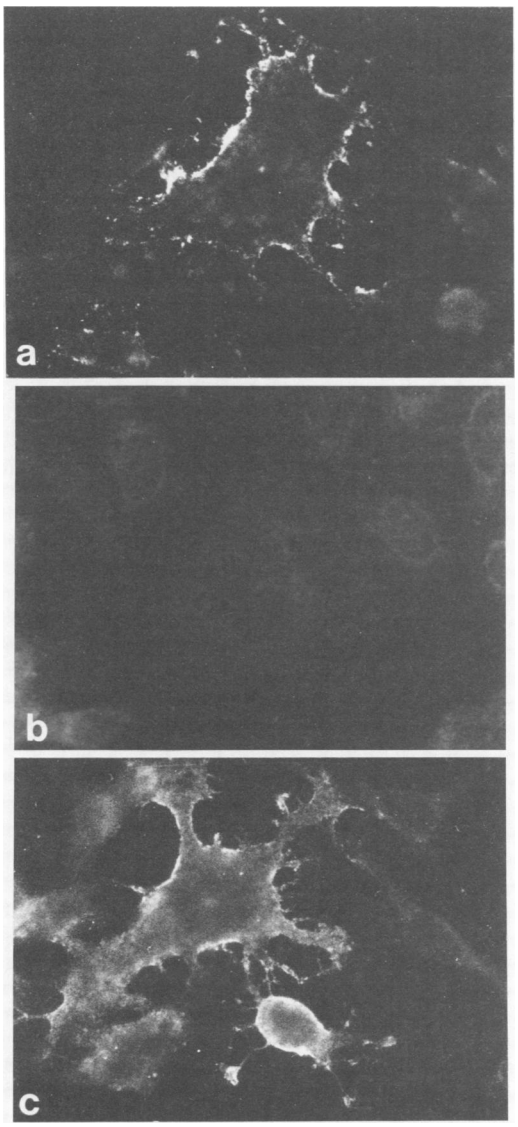

FIG. 1. *Indirect immunofluorescence showing secondary IgM staining of measles virus-infected cells by RF with either HLI or HAI antibody. (a) Membrane staining of unfixed cells after HAI antibody treatment. (b) Absence of staining of acetone-fixed cells after HLI antibody treatment. (c) Membrane staining of acetone-fixed cells after HAI antibody treatment.*

bodies and RF throughout, on the assumption that equal intensities of fluorescence revealed by the same conjugated globulin represented equal amounts of bound antibody, but functional tests were also made to exclude the presence of HAI antibody in all dilutions of serum containing HLI antibody.

Results (Table 3) given by three rheumatoid sera with RF latex agglutination titers of 1,024, 512, and 256 and corresponding immunofluorescence titers (determined by titrating the diluted sera in the presence of excess measles virus-specific IgG antibodies) of 3,200, 800, and 800 agreed in that they showed similar binding power of RF to HLI and HAI antibodies, as judged by the intensities of the fluorescence. It is also clear that RF caused no secondary IgM staining when added to acetone-fixed cells to which HLI antibody had been applied. It is difficult to use sera from multiple sclerosis patients in such reconstruction experiments because in most sera the titer of RF is much lower than the titers of measles virus-specific antibodies and RF can only be tested for in the presence of unequal amounts of HAI or HLI antibody. Nevertheless, as Table 3 shows, multiple sclerosis serum with an RF immunofluorescence titer of 48 combined only to HLI antibody and failed to adhere to a great excess of HAI antibody.

Absorption of RF and secondary IgM staining. Rheumatoid sera used in previous tests had a high RF titer. In most multiple sclerosis sera the titers of RF are low, and RF is seldom present above a titer of 1:48. Therefore, we wanted to determine whether absorption of RF from a serum by increasing concentrations of aggregated gamma globulin or by fixed amounts of latex-globulin reagent yielded residual material which was less specific on acetone-fixed cells than original RF, thus showing differences in its reactions with measles HLI and HAI antibodies. Four rheumatoid sera with various titers of RF were absorbed with increasing concentrations of aggregated gamma globulin. The sera were also absorbed under various conditions with fixed concentrations of aggregated gamma globulin or latex reagent. Absorptions with 2, 5, and 10% (vol/vol) aggregated gamma globulin did not entirely remove RF from these sera, whereas 20% aggregated gamma globulin removed RF activity from three of four sera (Table 4). One serum (Table 4, RA 3) gave residual RF which stained unfixed virus-infected cells but not acetone-fixed infected cells, although there was sufficient virus-specific IgG to have fixed RF on acetone-fixed cells. The latex-globulin reagent was a much more efficient absorbent of RF than was aggregated gamma globulin. The different temperatures and times of absorption did not affect the results (Table 5).

Comparison of measles virus-specific antibody titers in multiple sclerosis, rheumatoid arthritis, and normal control sera. Since rheumatoid arthritis and multiple sclerosis sera may both contain RF or similar forms of IgM antiglobulin, we also examined measles antibodies in patients having these diseases. In-

TABLE 3. *Intensity of IgM-specific fluorescent staining after the application of RF in rheumatoid sera to measles virus-infected cells pretreated with measles virus-specific HLI or HAI IgG antibody*[a]

Treatment of measles virus-infected cells					Equivalent no. of units in multiple sclerosis serum	Fluorescence intensity of specific IgM staining with:			
Fixation	Antibody	No. of units applied[b]				RA 1	RA 2	RA 3	Multiple sclerosis serum
		RA 1	RA 2	RA 3					
	RF	4	2	4	16				
Unfixed	HLI	10	12	8	320	+	+	+	++
Acetone-fixed	HLI	10	12	8	320	0	0	0	0
Acetone-fixed	HAI	12	12	8	100	+	+	+	0

[a] For comparison, specific IgM staining of infected cells by RF in a multiple sclerosis serum (MS-IgM) in the presence of excess HLI or HAI IgG antibody is given.

[b] Number of units applied refers to the fluorescent antibody titer of measles HLI or HAI antibody and RF. The endpoint of a serum was determined by fluorescent antibody titration and a dilution of approximately 10 times the final concentration of measles virus antibody was applied. The endpoint of the immunofluorescence titer of RF in rheumatoid (RA) sera, determined by diluting the sera in the presence of excess measles virus-specific antibodies, was taken as one RF unit. A dilution two to four times more concentrated than the endpoint was applied.

TABLE 4. *Absorption of RF from four rheumatoid sera with 20% (vol/vol) human aggregated gamma globulin and its effect on secondary IgM staining of unfixed and acetone-fixed measles virus-infected cells*

Serum	RF titer	% Human aggregated gamma globulin (vol/vol)	Immunofluorescence staining titer with:			
			Unfixed cells		Acetone-fixed cells	
			IgM	IgG	IgM	IgG
RA 1	6	Unabsorbed	24	768	<3	48
RA 2	24		96	384	12	48
RA 3	48		192	768	48	384
RA 4	192		96	384	24	96
RA 1	<3	20	<3	768	<3	48
RA 2	<3		12	384	<3	48
RA 3	<3		24	768	<3	384
RA 4	12		24	384	3	96

creased measles virus-specific antibody titers have been reported in patients with rheumatoid arthritis (12–14, 23), although no formal comparison with multiple sclerosis patients has been reported. We therefore made a preliminary survey of sera from patients with multiple sclerosis or rheumatoid arthritis and from normal controls by the fluorescent antibody method and by functional tests of inhibition of virus hemagglutination and hemolysin.

The geometric mean titers of the antibodies are shown in Table 6. The comparisons of mean log titers were made by the paired t test because subjects in three groups were age and sex matched. The conventional level of significance ($P < 0.05$) was used throughout. The results of statistical analysis are shown in Table 7. The individual titers of antibodies are shown in Fig. 2A and B.

TABLE 5. *Comparison of the efficiency of human aggregated gamma globulin and latex-globulin reagent on the absorption of RF at room temperature or 4°C from five rheumatoid sera and effect of adsorptions on the secondary IgM staining of unfixed and acetone-fixed measles virus-infected cells*

Serum	Absorbent[a]	Temp	RF titer	Immunofluorescence staining titer with:			
				Unfixed cells		Acetone-fixed cells	
				IgM	IgG	IgM	IgG
RA 1	Unabsorbed		6	24	768	<3	48
	AGG (20%)	Room or 4°C	<3	<3	768	<3	48
	L-GR (1.6%)	Room or 4°C	<3	<3	384	<3	48
RA 2	Unabsorbed		24	96	384	12	48
	AGG (20%)	Room or 4°C	<3	12	384	<3	48
	L-GR (1.6%)	Room or 4°C	<3	12	384	<3	48
RA 3	Unabsorbed		48	192	768	48	384
	AGG (20%)	Room or 4°C	<3	24	768	<3	384
	L-GR (1.6%)	Room or 4°C	<3	12	768	<3	384
RA 4	Unabsorbed		192	96	384	24	96
	AGG (20%)	Room or 4°C	12	24	384	3	96
	L-GR (1.6%)	Room or 4°C	12	48	384	3	96
RA 5	Unabsorbed		384	192	768	48	192
	AGG (20%)	Room or 4°C	24	48	768	12	192
	L-GR (1.6%)	Room or 4°C	24	96	768	12	192

[a] AGG, Aggregated human gamma globulin; L-GR, latex-globulin reagent.

TABLE 6. *Geometric mean titers of measles virus-specific antibodies by immunofluorescence, HAI, and HLI in three groups*

Clinical condition	No. of patients	Titer in:			
		Immunofluorescence (IgG antibody) test		HLI test	HAI test
		Unfixed cells	Acetone-fixed cells		
Multiple sclerosis	15	842 (2.9255 ± 0.4237)[a]	192 (2.2833 ± 0.3598)	865 (2.9372 ± 0.4650)	133 (2.1228 ± 0.4081)
Rheumatoid arthritis	15	804 (2.9054 ± 0.3109)	146 (2.1629 ± 0.3178)	826 (2.9172 ± 0.3554)	110 (2.0425 ± 0.4287)
Normal controls	15	291 (2.4639 ± 0.2967)	73 (1.8618 ± 0.3908)	299 (2.4757 ± 0.3338)	50 (1.7013 ± 0.4755)

[a] Numbers in parentheses are mean logarithmic titers ± standard deviations.

TABLE 7. *Comparison of mean log titers of measles virus-specific antibodies by immunofluorescence, HAI, and HLI in three groups by the paired t test*

Groups compared	*P* value			
	Immunofluorescence (IgG antibody) test		HLI test	HAI test
	Unfixed cells	Acetone-fixed cells		
Multiple sclerosis vs control	0.001–0.01[a]	0.001–0.01[a]	0.01 –0.02[a]	0.02–0.05[a]
Rheumatoid arthritis vs control	0.001–0.01[a]	0.05 –0.02[a]	0.001–0.01[a]	0.05–0.10
Multiple sclerosis vs rheumatoid arthritis	>0.9	0.3–0.4	>0.9	0.6–0.7

[a] Significant at $P < 0.05$.

A comparison of the mean log titers of antibodies in three groups showed that patients with multiple sclerosis and patients with rheumatoid arthritis had significantly higher titers of measles virus-specific IgG by fluorescence and HLI antibody tests than did normal controls. The patients with multiple sclerosis also had significantly higher titers of HAI antibodies than normal controls (Tables 6 and 7). Although geometric mean titers of HAI antibodies were higher in patients with rheumatoid arthritis than in normal controls (Table 6), the mean log titers just failed to show a significant difference (Table 7). This may be due to the small number of patients in the group. It can also be seen that titers of HLI antibodies were higher than titers of HAI antibodies and were similar to higher titers of IgG antibody in immunofluorescence tests on unfixed virus-infected cells. There were no significant differences in the titers of measles antibodies between multiple sclerosis and rheumatoid arthritis (Tables 6 and 7).

DISCUSSION

It has been reported previously that MS-IgM in sera of patients with multiple sclerosis is an antiglobulin, not a measles virus-specific antibody (6; Fraser et al., in press). The unfixed measles virus-infected cells revealed a secondary IgM staining which acetone-fixed infected cells did not show. Our present findings suggest that there are two reasons for this discrepancy.

The first is that IgG staining of measles virus on cell surfaces is more sensitive to acetone fixation than is IgG staining of other surface-maturing viruses. The second is that the RF-like substance IgM has greater affinity for measles HLI antibody than for HAI antibody. The combined results of the two factors can result in differences of more than 80-fold in the titers of IgM shown on unfixed compared with fixed infected cells.

Measles virus antigen at the cell surface is a complex of two antigenic determinants which are independently accessible to their specific antibody. Loss of combining power after acetone fixation, as shown by immunofluorescence, is not due to an equal reduction in affinity of both antigens for antibody, but to the destruction of one antigen, hemolysin, and the retention of the other, hemagglutinin (7). Since the two antibody levels differ widely in individual sera, the loss of titer on acetone-fixed infected cells compared with the titer on unfixed infected cells differs in each serum and is greatest when the difference between HLI and HAI antibodies is largest (Table 2). These facts explain the greater decrease in titer given by measles virus antibody compared with the titer shown by other virus antibodies which, presumably, do not react with an acetone-sensitive antigen (Table 1). They also explain the wide range in the ratio of titer of

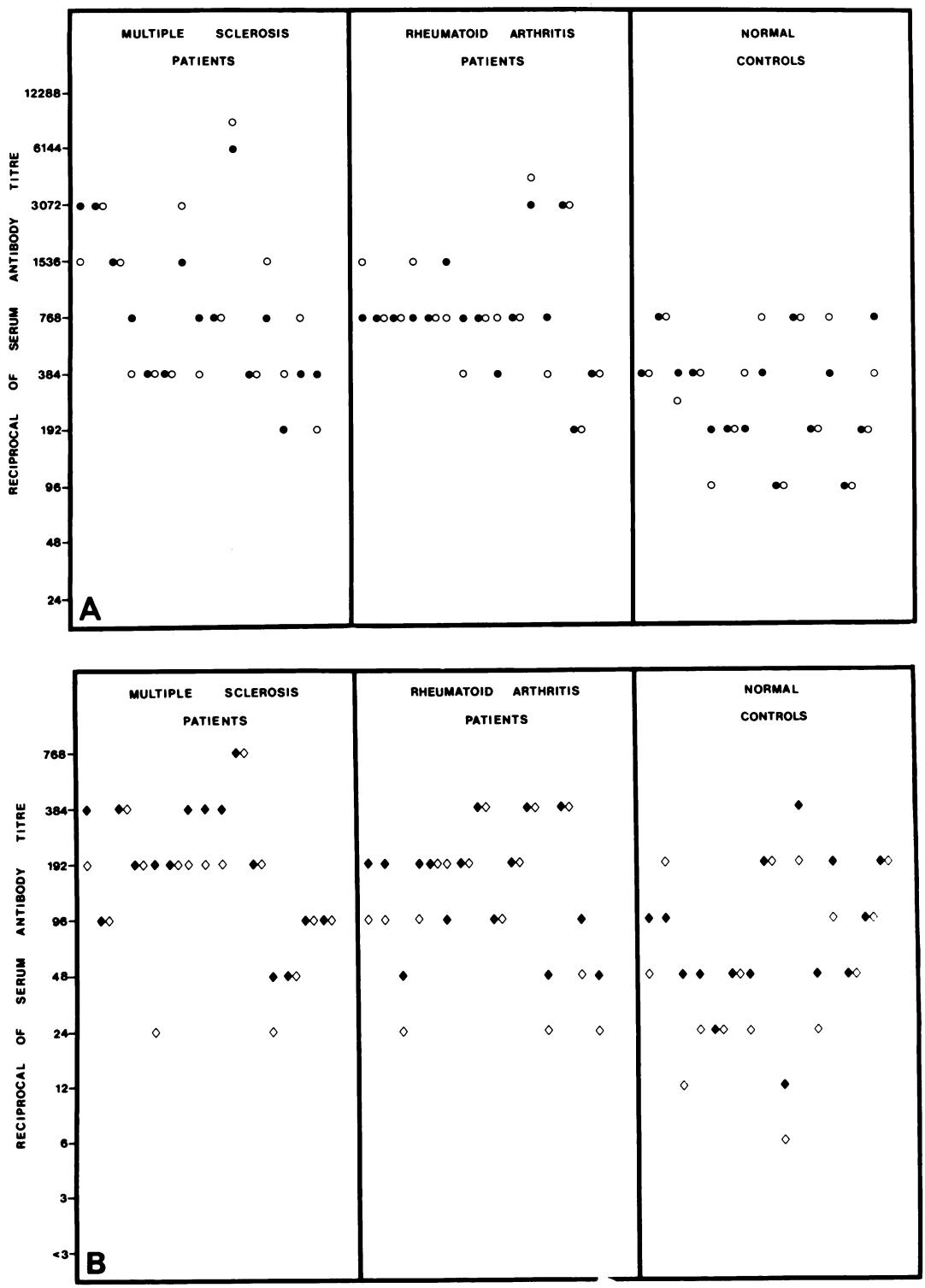

FIG. 2. *Titers of measles virus-specific antibodies in three study groups. (A) Symbols: ●, IgG antibody on unfixed infected cells (immunofluorescence); ○, antihemolysin antibody (HLI). (B) Symbols: ◆, IgG antibody on acetone-fixed infected cells (immunofluorescence); ◇, antihemagglutinin antibody (HAI).*

measles antibody on unfixed cells to titer on fixed cells seen with different human sera (see above). Differences in titer are even greater with sera from multiple sclerosis patients, for some contain much more antihemolysin than normal (5).

Two serum factors interact in titrating secondary IgM staining. They are the amount of IgG antibody available to combine with virus antigen and the amount of RF available to bind to specifically adherent IgG. For this reason RF was first measured by a standard agglutination, and Table 2 shows that the fluorescent antibody method is normally more sensitive than the latex agglutination method. It also shows that one rheumatoid serum and two control sera showed secondary IgM staining only on unfixed infected cells, although the staining should have been present on acetone-fixed cells if the ratios presented in Table 2 are applied, thus resembling secondary IgM staining obtained with RF in multiple sclerosis sera.

Our results show that three high-titer RF-containing rheumatoid sera all gave similar brightness of fluorescence, that is, bound equally well when added to infected cells coated with equivalent amounts of HLI or HAI antibody. RF in multiple sclerosis sera produced fluorescence only in the presence of HLI antibody, not with HAI antibody. Also, when RF as tested by latex agglutination had been removed, fluorescent antibody testing revealed secondary IgM staining in one rheumatoid serum (Table 4) on unfixed virus-infected cells but not on acetone-fixed cells, although there was sufficient virus-specific IgG to have shown staining on acetone-fixed cells. Thus, two types of secondary IgM staining by RF can be observed. One (shown in the majority of rheumatoid sera) is due to standard RF which binds to HLI and HAI antibodies on infected cells, and the other is observed with low-titer RF in sera from multiple sclerosis, in control sera, and occasionally in rheumatoid sera which binds only to HLI antibody. The reason for these differences is not known, but it might be due to differences in the subclass specificities of the HLI and HAI antibodies. For instance, it has been reported that complement may be activated by measles antibodies by two different pathways which are related to HLI and HAI antibodies (4). It is known that cultured lymphocytes from rheumatoid arthritis patients secrete RF (22). It will be important to know whether cultured lymphocytes from multiple sclerosis patients secrete RF and also whether in vitro-secreted RF from patients with rheumatoid arthritis and patients with multiple sclerosis show the differences in their specificities for measles virus-specific HLI and HAI antibodies.

Different temperatures have no effect on the absorption of RF from rheumatoid sera. Neither absorbing material reduced the IgG staining titers, and coated latex particles were, volume for volume, by far the most effective absorbent (Table 5). Since the removal of RF did not affect virus-specific IgG titer, we concluded that above average titer of antibody in multiple sclerosis was not a consequence of a secondary in vitro action of RF.

We wondered whether the measles virus antibody titers in multiple sclerosis and rheumatoid arthritis were in any way related to our observation that sera from patients with both diseases contain IgM antiglobulins, sometimes of low titer. The most striking feature of the survey of groups of patients and of normal persons was the fact that the average increase in titer of antibody to measles virus hemolysin above normal is equally great in multiple sclerosis and rheumatoid arthritis sera (Table 6 and Fig. 1a). This is also apparent in the mean titer of fluorescent antibodies on unfixed tissue, which is to be expected from the fact that most of this is HLI antibody. The similar increase in the two sera was unexpected despite the existence of reports that increases in the amounts of circulating antibody against measles virus and some other viruses have been reported in groups of patients with a variety of rheumatic diseases (10–14, 16–18, 23, 24). If our present result holds widely, it will greatly alter the idea that there is a special relationship between measles infection and multiple sclerosis, unless the same is true for rheumatoid arthritis. It even raises the possibility that a common cause for the altered immune responses in both diseases could exist.

ACKNOWLEDGMENTS

This work was supported by a grant from the Arthritis and Rheumatism Council and the Medical Research Council.

We thank Margaret Haire for providing multiple sclerosis and control sera, J. D. Merrett, Department of Medical Statistics, for his valuable help with statistical analysis, R. Woods and Doris Campbell for preparing photographic prints, and Elizabeth Fleming for excellent technical help.

LITERATURE CITED

1. **Allison, R. S., and J. H. D. Millar.** 1954. Prevalence and familial incidence of disseminated sclerosis in Northern Ireland. Ulster Med. J. **23**(Suppl. 2):1–92.
2. **Brody, J. A., J. L. Sever, A. Edgar, and J. McNew.** 1972. Measles antibody titers of multiple sclerosis patients and their siblings. Neurology **22**:492–499.
3. **Cathala, E., and P. Brown.** 1972. The possible viral aetiology of disseminated sclerosis. J. Clin. Pathol. **25**(Suppl. 6):141–151.
4. **Ehrnst, A. C.** 1977. Complement activation by measles virus cytotoxic antibodies: alternative pathway C acti-

vation by hemagglutination-inhibition antibodies but classical activation by hemolysin antibodies. J. Immunol. **118**:533–539.

5. **Fraser, K. B.** 1975. Population serology, p. 53–79. *In* A. N. Davison, J. H. Humphrey, A. L. Liversedge, W. I. McDonald, and J. S. Porterfield (ed.), Multiple sclerosis research (Proceedings of a joint conference held by the Medical Research Council and the Multiple Sclerosis Society of Great Britain and Northern Ireland, 17–18 October 1974). Her Majesty's Stationary Office, London.
6. **Fraser, K. B.** 1978. False-positive measles-specific IgM in multiple sclerosis. Lancet **i**:91–92.

6a. **Fraser, K. B., M. Armstrong, P. V. Shirodaria, and M. Gharpure.** 1979. Affinity for measles virus antihaemolysin of a residual immunoglobulin M in sera of some patients with multiple sclerosis. Clin. Exp. Immunol. **36**:304–310.

7. **Fraser, K. B., M. Gharpure, P. V. Shirodaria, M. A. Armstrong, A. Moore, and E. Dermott.** 1978. Distribution and relationship of antigens of measles virus membrane complex in the infected cell and the nature of haemolysin antigen, p. 771–780. In B. W. J. Mahy, and R. D. Barry (ed.), Negative strand viruses and the host cell. Academic Press, London.
8. **Fraser, K. B., P. V. Shirodaria, and M. Haire.** 1974. Enzyme treatment of unfixed cell cultures as a means of determining different specificities of IgM in multiple sclerosis and measles. Med. Microbiol. Immunol. **160**: 221–230.
9. **Gould, E. A.** 1974. Variants of measles virus. Med. Microbiol. Immunol. **160**:211–219.
10. **Hollinger, F. B., J. T. Sharp, M. D. Lindsky, and W. E. Rawls.** 1971. Antibodies to viral antigens in systemic lupus erythematosus. Arthritis Rheum. **14**:1–11.
11. **Hurd, E. R., W. Dowdle, H. Casey, and M. Ziff.** 1972. Virus antibody levels in systemic lupus erythematosus. Arthritis Rheum. **15**:267–274.
12. **Kalliomaki, J. L., and P. Halonen.** 1972. Antibody levels to parainfluenza, herpes simplex, varicella-zoster, cytomegalo virus and measles virus in patients with connective tissue diseases. Ann. Rheum. Dis. **31**:192–195.
13. **Kalliomaki, J. L., P. Halonen, and A. Salmi.** 1975. Virus antibodies in serum and synovial fluid of patients with rheumatoid arthritis and other connective tissue diseases. Ann. Rheum. Dis. **34**:43–47.
14. **Laitinen, O., T. Vesikari, and A. Vaheri.** 1972. Virus antibody levels in rheumatoid arthritis and systemic lupus erythematosus. Acta Med. Scand. **192**:37–40.
15. **Leibowitz, U., and M. Alter.** 1973. Multiple sclerosis: clues to its cause. North Holland Publishing Co., Amsterdam.
16. **Lucas, C. J., R. Brouwer, T. E. W. Feltkamp, J. H. Ten Veen, and J. J. Van Loghem.** 1972. Measles antibodies in sera from patients with autoimmune diseases. Lancet **i**:115–116.
17. **Philips, P. E., and C. L. Christian.** 1970. Myxovirus antibody increases in human connective tissue diseases. Science **168**:982–984.
18. **Philips, P. E., and C. L. Christian.** 1973. Virus antibodies in systemic lupus erythematosus and other connective tissue diseases. Ann. Rheum. Dis. **32**:450–456.
19. **Ropes, M. W., G. A. Bennett, S. Cobb, R. Jacox, and R. A. Jessar.** 1959. 1958 revision of diagnostic criteria for rheumatoid arthritis. Arthritis Rheum. **2**:16–20.
20. **Salmi, A., B. R. Ziola, T. Vuorimaa, T. Arnadottir, and M. Reunanen.** 1978. Measles IgM antibodies and IgM anti-IgG activity in subacute sclerosing panencephalitis (SSPE) and multiple sclerosis (MS). Ir. J. Med. Sci. **147**:121.
21. **Shirodaria, P. V., K. B. Fraser, and C. F. Stanford.** 1973. Secondary fluorescent staining of virus antigens by rheumatoid factor and fluorescein-conjugated anti-IgM. Ann. Rheum. Dis. **32**:53–57.
22. **Slaughter, L., D. A. Carson, F. C. Jensen, T. L. Holbrook, and J. H. Vaughan.** 1978. In vitro effects of Epstein-Barr virus on peripheral blood mononuclear cells from patients with rheumatoid arthritis and normal subjects. J. Exp. Med. **148**:1429–1434.
23. **Stanford, F.** 1972. A comparison of complement-fixing antibody titres between patients with rheumatoid arthritis and matched controls. Ann. Rheum. Dis. **31**:330–333.
24. **Triger, D. R., T. R. Gamlen, E. Paraskevas, R. S. Lloyd, and R. Wright.** 1976. Measles antibodies and autoantibodies in autoimmune disorders. Clin. Exp. Immunol. **24**:407–414.