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Rheumatoid factor as a cause of positive reactions in tests for Epstein–Barr virus-specific IgM antibodies

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SUMMARY

Sera from twenty-eight patients with rheumatoid arthritis (RA) were titrated in indirect immunofluorescence tests for Epstein–Barr virus (EBV) specific antibodies. All had IgG antibodies to viral capsid antigen (VCA), 64% at titres ≧ 320, and 71% reacted also in tests for VCA-specific IgM antibodies at titres ranging from 20 to 640. The reactions observed in the IgM test were not due to VCA-specific IgM antibodies, however, but rather to rheumatoid factor (RF) usually an IgM antibody to the Fc regions of IgG. The titres recorded in the anti-VCA IgM test correlated significantly with the RF titres and both reactivities were abolished by adsorption onto IgG coated latex particles. In addition, they clearly depended upon the height of the IgG antibody titre to VCA, indicating that the more VCA-specific IgG molecules are present the more likely it is that RF will combine with them in sufficient quantity before or after their attachment to VCA-positive test cells so as to become detectable by the fluorescent antibodies to human IgM. Results comparable in every aspect were obtained with those sera from patients with Hodgkin's disease, nasopharyngeal or cervical carcinomas which reacted in the anti-VCA IgM test. Sera from patients with infectious mononucleosis may also contain RF, but in such cases its removal by adsorption onto IgG-coated latex particles did not generally reduce the VCA-specific IgM antibody titre. Removal of RF from any of the sera studied did not affect the titres of VCA-specific IgG and, where applicable, IgA or heterophil antibody titres. These results re-emphasize the pitfall created by RF noted previously in tests for virus-specific IgM antibodies.

INTRODUCTION

The characteristic pattern of antibody responses in primary Epstein–Barr virus (EBV) infectious permits serological confirmation of a diagnosis of infectious mononucleosis (IM) with a single acute phase serum (Henle, Henle & Horwitz, 1974). At onset of the disease, patients show high titres of IgM and IgG antibodies to EB viral capsid antigen (VCA) and over 85% have antibodies to the D (diffuse) component of the EBV-induced early antigen (EA) complex, whereas antibodies to the EBV-associated nuclear antigen (EBNA) are absent or at most barely detectable. During convalescence, VCA-specific IgM antibodies and anti-D gradually return to non-detectable levels, IgG antibodies to VCA decline to lower titres, and anti-EBNA emerges at gradually increasing titres. Both VCA-specific IgG antibodies and anti-EBNA persist indefinitely at nearly constant titres due to the persistent viral carrier state established in the lymphoreticular system following primary EBV infections.

The apparently stable equilibrium between the persistent virus and host defences can be upset by immunosuppressive diseases or therapy, leading to enhanced IgG antibody titres to VCA with or without

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emergence of antibodies to EA components. Elevated titres of EBV-related antibodies (as compared to controls) are seen among patients with Hodgkin's disease (HD), non-HD lymphomas, various types of leukaemias (Johansson *et al.*, 1970; 1971; Levine *et al.*, 1971), sarcoidosis (Hirshaut *et al.*, 1970; Wahren *et al.*, 1971; Nikoskelainen, Hannuksela & Palva, 1974a), systemic lupus erythematosis (Evans, Rothfield & Niederman, 1971), renal transplants (Spencer & Andersen, 1972; Fiala *et al.*, 1975) or immunodeficiency diseases (Berkel *et al.*, 1979). It was of interest that positive reactions in tests for VCA-specific IgM antibodies were obtained in a small proportion of patients with HD or non-HD lymphomas (unpublished observations) or nasopharyngeal carcinoma (NPC) (Henle *et al.*, 1977). These reactions are not due to IgM antibodies to VCA however, but, as will be shown below, to rheumatoid factor (RF) present in these sera.

In recent studies from patients with rheumatoid arthritis (RA) (Alspaugh *et al.*, in preparation) an over-representation of high titres of VCA-specific IgG antibodies was observed as compared to sera from healthy controls, as well as an increased incidence of antibodies to the EA complex. Most of the RA sera were subsequently found to react also in the VCA-specific IgM test, often at high titres. These results suggested that RF, an antibody usually of the IgM class to the Fc regions of IgG, might be responsible for the immunofluorescence reactions obtained with anti-human IgM conjugates and the results to be reported confirm this suggestion. Such 'secondary' IgM immunofluorescence reactions due to intervention of RF have been noted previously among sera from patients with measles, mumps, rubella and herpes simplex virus infections (Fraser, Shirodaria & Stanford, 1971; Shirodaria, Fraser & Stanford, 1973), as well as in some sera from EB viral carriers (Nikoskelainen, Leikola & Klemola, 1974b; Nikoskelainen & Hänninen, 1975). The present report serves to emphasize once again the pitfall created by RF in tests for virus-specific IgM antibodies.

MATERIALS AND METHODS

Sera. These were selected from collections previously tested for EBV-related antibodies. They were derived from (a) twenty-eight adult patients with RA seen at the Rheumatology Clinic of the Louisiana State University Medical Center. The diagnosis of RA was made when symptoms and laboratory tests met the criteria of classical or definite RA described by the American Rheumatism Association (1973); (b) twenty-seven paediatric patients with juvenile RA or other arthritic conditions treated at The Children's Hospital of Philadelphia, of whom fifteen had antibodies to EBV, the others had not yet been infected; (c) ninety Swedish patients with HD or non-HD lymphomas provided by Drs G. Holm and M. Björkholm, Stockholm. Of these, thirty-two had previously been positive in the anti-VCA IgM test; (d) fifty-one NPC patients from Hong Kong (Dr J. C. Ho), Alaska (Dr A. Lanier) or the Eastern United States. Five of these had reacted previously in the anti-VCA IgM test, the rest were chosen at random; (e) sixteen patients with carcinomas of the head and neck other than NPC from the same sources as (d); (f) sixteen patients with cervical carcinoma, provided by Dr N. Einhorn, Stockholm; (g) seventy patients with acute IM; (h) twenty-four miscellaneous donors with serological evidence of past primary EBV infections; and (i) twenty-seven donors who had not been infected with EBV. All sera had been stored at $-20°C$ for periods up to 3 yr and had been frozen and thawed repeatedly.

EBV-specific serology. The techniques for titration of VCA-specific IgM, IgG and IgA, as well as EA-specific antibodies by indirect immunofluorescence have been described elsewhere (Henle & Henle, 1966; 1976; Henle, Henle & Klein, 1971; Henle *et al.*, 1974; Nikoskelainen *et al.*, 1974b). The sera were diluted, however, in a glycine-buffered saline solution of pH 8·2 (GBSS) instead of the usual veronal-buffered saline, in order to permit the use of the same dilutions for the RF test. This change did not alter the results of the immunofluorescence reactions. For the anti-VCA IgM tests cell smears from the HR-1 line were used as usual, but smears of EB3 cells were also used, both being derived from Burkitt's lymphomas.

Test for RF. IgG-coated latex particles (Rheuma-Wellcotest, Wellcome Reagents Limited) were used for detection of RF. The sera were diluted serially in two-fold steps in GBSS and distributed in the wells of Linbro microtitre U plates. Equal volumes of five-fold diluted antigen (25 μl) were added to each well and the plates were incubated at room temperature for 30 min, centrifuged for 1 min at 2000 rpm, and then mechanically agitated. The results were read 30 min later according to the pattern of sedimented aggregates. Titres of $\geqq 40$ were considered positive.

Absorption of sera. 0·2–1·0 ml of suspended IgG-coated latex particles were sedimented at 7000 rpm in an angle head table centrifuge and resuspended in 1·0 ml of ten- or twenty-fold diluted test serum. After incubation at room temperature for 1 hr, the latex particles were removed by centrifugation and the procedure was repeated once and, if necessary, twice. The final supernates were tested for activity in the RF and anti-VCA IgM, IgG and when applicable IgA tests.

Tests with RF from an anti-VCA negative serum. A serum from a Red Cross blood donor (RC-153) was found which had an RF titre of 320 but no anti-VCA. This serum was used for (a) three-step immunofluorescence tests, charging HR-1 and EB3 cell smears consecutively with anti-VCA IgG positive, but RF and anti-VCA IgM negative sera, serum RC-153, and anti-

human IgM or IgG conjugate. Either the first serum was serially diluted and serum RC-153 was kept constant (1:20) or the first serum was kept constant (1:10) and serum RC-153 was serially diluted. (b) Dissociation of RF from IgG and reassociation in the presence of VCA-specific IgG antibodies. For this purpose, 1·0 ml of serum RC-153, diluted 1:5 in GBSS, was overlayed on 1·0 ml of a 15% sucrose solution and centrifuged at 150,000 *g* for 2 hr. After removal of the top 1·0 ml, the RF-enriched bottom fraction was dialysed at 4°C overnight against GBSS before adjustment of the volume to 2·0 ml. Sufficient 1N HCl was added to reduce the pH to 2·8–3·0 and, after 15 min in an ice bath, aliquots of this preparation were added to equal volumes of RF and VCA-specific IgM negative sera with high titres of anti-VCA IgG antibodies, diluted 1:5 in GBSS. The pH was immediately adjusted to 8·2 by addition of 1N NaOH. These preparations as well as untreated samples of the sera were then titrated for their reactivities in the RF and anti-VCA IgM and IgG tests.

RESULTS

The results obtained with twenty-eight sera from adult RA patients are shown in Fig. 1. In the left panel, the VCA-specific IgG antibody titres of the individual patients are plotted against the titres recorded in the test for the corresponding IgM antibodies. All sera contained VCA-specific IgG antibodies, eighteen

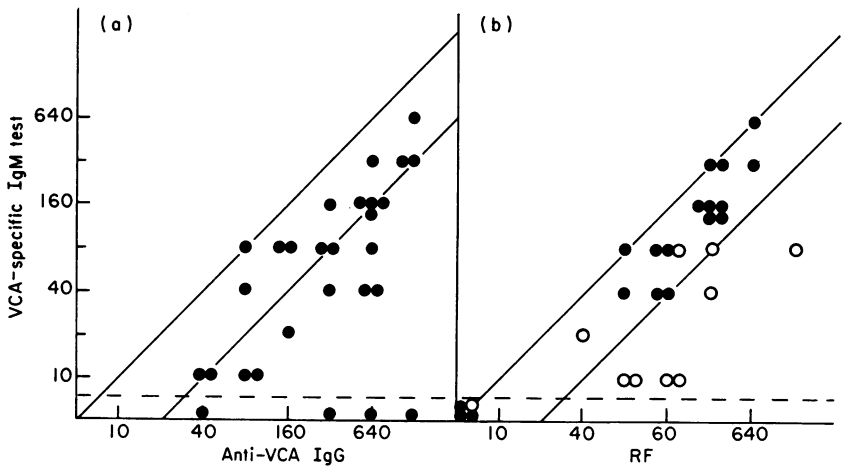

FIG. 1. Relation between the titres obtained in the anti-VCA IgM test and the anti-VCA IgG titres (a) and the RF titre (b). Points between the diagonal lines present IgM test titres equal to or up to four times lower than the anti-VCA IgG or RF titres, respectively. (●) Anti-VCA IgG≧1:320, (○) ≦1:160.

(64%) at high titres (≧320). The geometric mean titre (GMT) of 294 was 3·3 times higher than the GMT of 89 observed in the healthy controls included in this study. In the test for VCA-specific IgM antibodies, four (14%) of the RA sera failed to react, another four gave doubtful reactions at dilution 1:10, but the remainder yielded titres ranging from 20 to 640, with a GMT for the whole set of 49. The sera of control donors gave uniformly negative results (see Table 2). In the right panel of Fig. 1, the RF titres of the RA patients are plotted against the titres obtained in the anti-VCA IgM test, revealing a significant correlation between the two reactivities. Sera which were negative for RF were also negative in the anti-VCA IgM test. With increasingly higher RF titres the reactivity in the anti-VCA IgM test also tended to increase. The RF titres were however, two to four times higher as a rule (GMT 124) than the titres obtained in the anti-VCA IgM test (GMT 49). Greater differences between the two titres were noted with sera which had relatively low VCA-specific IgG antibody titres (≦160), identified in the figure by open circles. The dependance of the titres obtained in the anti-VCA IgM test on the titre of VCA-specific IgG antibodies is further illustrated in Table 1. While there was no striking correlation between the anti-VCA IgG and RF titres, the ratios between the GMTs recorded in the RF and anti-VCA IgM tests rose from 1·7 to 7·0 as the VCA-specific IgG titres declined from ≧640 to ≦80.

The fluorescence elicited in the IgM test with RA sera tended to be more flaky or grossly granular than the finely granular or diffuse staining obtained with sera from acute IM patients. Furthermore, EB3 cell

TABLE 1. Influence of VCA-specific IgG antibody titres of RA sera on the ratio between the titres obtained in the RF and anti-VCA IgM tests

Anti-VCA IgG titre	Number of sera	Range of titres (GMT) RF test (a)	Anti-VCA IgM test (b)	Ratio of GMTs (a)/(b)
640–1280	11	80–640 (279)	40–640 (166)	1·7
160–320	7	40–1280 (216)	20–160 (65)	3·3
40–80	6	80–320 (126)	10–80 (18)	7·0
Total	24	40–1280 (211)	10–640 (71)	3·0

smears could be used instead of HR-1 smears in the case of RA sera, whereas for IM sera EB3 cells were distinctly inferior.

In Table 2 the results obtained with the adult RA patients are compared with those recorded for the other groups studied. RF titres of $\geqq 40$ and titres of $\geqq 20$ in the anti-VCA IgM test were considered significant. Of the twenty-seven sera from paediatric patients with juvenile RA or other arthritic conditions, whether anti-VCA IgG positive (fifteen) or negative (seventeen), none reacted in either test. Among the ninety sera from HD or non-HD lymphoma patients were thirty-two which had been selected because they previously had shown reactivity in the anti-VCA IgM test (GMT 32; range 20–320) and 72% of these revealed RF titres (GMT 255, range 40–320). Of the fifty-eight sera with titres of $\leqq 10$ in the anti-VCA IgM test, 31% showed low or moderate RF titres (GMT 54). As seen with the RA sera, the ratios between the GMTs recorded in the two tests tended to increase with a decrease in the VCA-specific IgG antibodies. Of the seventeen NPC sera which were positive in the anti-VCA IgM test (GMT 57), 77% had RF titres as high as 320 (GMT 152,) whereas only one (3%) of the thirty-four NPC sera which were non-reactive in the anti-VCA IgM test showed an RF titre of 40. None of the sixteen sera from patients with carcinomas of head and neck other than NPC reacted in either of the two tests. Three of the

TABLE 2. Relation between results obtained in the anti-VCA IgM test and RF in various groups of patients

Patients (number)	Anti-VCA IgM test Result	Number	RF test Number positive	(%)	Anti-VCA IgM test Range of titres	(GMT)	RF test Range of titres	(GMT)
RA adults	+	20	20	(100)	20–640	(114)	40–1280	(240)
(28)	−	8	4	(50)	$\leqq 10$		80–160	(113)
RA juvenile	+	0						
(27)	−	27	0	(0)				
HD	+	32	23	(72)	20–320	(38)	40–320	(255)
(90)	−	58	18	(31)	$\leqq 10$		40–320	(54)
NPC	+	17	13	(77)	20–640	(57)	40–320	(152)
(51)	−	34	1	(3)	$\leqq 10$		40	
CA head and neck	+	0						
(16)	−	16	0	(0)				
CA cervix	+	3	2	(67)	20–40	(25)	160–320	(233)
(16)	−	13	4	(31)	$\leqq 10$		40–160	(47)
IM	+	70	12	(17)	40–1280	(242)	40–320	(89)
(70)								
Controls*	+	0						
(51)	−	51	0	(0)				

* Anti-VCA-positive (24) or -negative (27).

sixteen sera from patients with cervical carcinoma had titres of 20–40 in the anti-VCA IgM test and two of these revealed RF titres of 160 and 320. The other thirteen sera in this group were negative in the IgM test, but four (31%) showed the presence of RF at relatively low titres (GMT 47). Of the seventy acute IM sera, which by definition contained high titres of VCA-specific IgM antibodies (GMT 242), twelve (15%) had RF titres $\geqq 40$ (GMT 89). None of the sera from control donors with serological evidence of past EBV infection (twenty-four) or no infection (twenty-seven) were positive in either of the two tests.

A number of RF-positive sera from RA, HD, or NPC patients were adsorbed with IgG-coated latex particles (Table 3). Complete removal of RF also abolished the reactivity of the sera in the anti-VCA IgM test. If RF was incompletely adsorbed also residual activity in the anti-VCA IgM test was noted

TABLE 3. EBV-related antibody titres before and after removal of RF by adsorption onto IgG-coated latex particles

Serum	Diagnosis	RF	Anti-VCA			Heterophil antibody
			IgM	IgG	IgA	
4	RA	160/< 10*	80/< 10	320/320	< 10/—	
12	RA	320/80	160/20	640/640	< 10/—	
15	RA	640/< 10	320/< 20	2560/2560	< 10/—	
16	RA	320/< 10	160/< 20	1280/640	< 10/—	
21	RA	2560/< 10	320/< 20	160/160	< 10/—	
2a	NPC	160/< 10	40/< 10	1280/1280	80/80	
94a	NPC	80/< 10	20/< 10	640/1280	160/160	
107b	NPC	40/< 10	40/< 10	5120/5120	640/640	
169-23	HD	240/40	160/10	1280/1280	40/40	
169-42	HD	320/< 10	160/< 10	2560/2560	640/640	
169-52	HD	160/< 10	80/< 10	640/640	< 10/—	
169-75	HD	40/< 10	20/< 10	640/640	< 10/—	
164-5	HD	80/< 10	160/< 10	640/320	< 10/—	
164-25	HD	320/40	40/10	2560/2560	40/40	
164-45	HD	< 10/< 10	40/< 10	160/160	< 10/—	
164-135	HD	10/< 10	40/< 10	1280/1280	< 10/—	
M-698	IM	160/< 10	640/320	640/640	< 10/—	160/160
M-715	IM	320/< 10	80/20	640/640	< 10/—	640/640
H-1	IM	80/< 10	1280/1280	1280/1280	10/—	320/320
H-7	IM	160/< 10	1280/1280	1280/1280	< 10/—	640/640
H-8	IM	40/< 10	2560/2560	1280/1280	< 10/—	1280/1280
H-10	IM	40/< 10	640/640	320/640	10/—	1280/1280

* Titre before/after adsorption.

(sera RA-12, 169-23 and 164-25). The reactivity in the anti-VCA IgM test of several HD sera with insignificant amounts of RF was nevertheless absorbed onto IgG-coated latex particles (sera 164–45 and 164–135). In contrast, after complete adsorption of RF from acute IM sera, the IgM antibodies to VCA were not, or only slightly, reduced in titre, with the exception of serum M-715. This serum was obtained relatively late after onset of illness as it already had antibodies to EBNA at a titre of 5. The anti-VCA IgM titre was reduced in this case from 80 to 20, whereas the RF titre declined from 320 to <10. VCA-specific IgG antibodies and, where applicable, VCA-specific IgA and heterophil antibodies were not affected by the absorption procedure.

In a search for RF-positive but anti-VCA-negative sera, one (RC-153) was found which had an RF titre of 1:320 and had been collected several years ago from an apparently healthy Red Cross blood donor. When this serum was used as the second charge in three-step immunofluorescence tests (i.e. after exposure of the cell smears to RF-free sera with high titres of VCA-specific IgG but no IgM antibodies),

the subsequent addition of anti-human IgM conjugate stained the VCA-positive cells (Table 4). Positive reactions were obtained when the anti-VCA titre of the first charge was ≧32 and when the RF-positive serum was diluted no more than 1:20 or 1:40. There was, however, considerable background fluorescence which made reading of endpoints hazardous.

Assuming that the RF in serum RC-153 was in part combined with IgG, an attempt was made to effect a dissociation at low pH and permit reassociation in the presence of high concentrations of VCA-specific IgG antibodies (see the Materials and Methods section). As shown in Table 5, all test sera (the same as used in the three-step immunofluorescence test) were negative in the anti-VCA IgM test before treatment, but gave titres of 80 to 160 thereafter. The staining was clean-cut and no unusual background fluorescence was noted. The RF titres of the treated sera corresponded to the titre of serum RC-153 and the VCA-specific IgG antibody titres were of similar order before and after the treatment. Unfortunately serum RC-153 had been exhausted by the various tests so that the question whether its mere addition to the test sera would have yielded similar results remains unanswered.

TABLE 4. Immunofluorescence after successive exposure of cell smears to anti-VCA IgG-positive/IgM and RF-negative serum, anti-VCA IgG-negative/RF-positive serum (RC-153) and anti-human IgM conjugate

First step			Second step			
				Serum RC-153 diluted		
Serum number	Dilution	Anti-VCA IgG titre	GBSS	1:20	1:80	1:320
M-714	1:10	128	0*	+	0	0
	1:40	32	0	±		
	1:160	8	0	0		
M-1000	1:10	128	0	+	0	0
NPC-12	1:10	128	0	+	±	0
BL-8	1:10	256	0	+	±	0
	1:40	64	0	±		
	1:160	16	0	0		
BL-65	1:10	512	0	+	±	0
	1:40	128	0	±		
	1:160	32	0	0		
M-37	1:10	< 2	0	0	0	0

* Immunofluorescence elicited by anti-human IgM conjugate: + = definite; ± = doubtful and 0 = no staining. Both HR-1 and EB-3 cell smears were used.

DISCUSSION

The results reaffirm the insufficiently appreciated fact that RF of the IgM class may interact in immunofluorescence tests for virus-specific IgM antibodies and thus may lead to erroneous interpretations (Fraser *et al.*, 1971; Shirodaria *et al.*, 1973; Nikoskelainen *et al.*, 1974b). While RF was shown to arise at low frequency in patients with primary EBV infections with the techniques used, it is not likely to cause great problems because the VCA-specific IgM antibody titres usually far exceed the RF levels. Accordingly, removal of RF from early acute phase IM sera by adsorption onto IgG-coated latex particles does not, as a rule, reduce the VCA-specific IgM titres. In late acute phase or early convalescent sera, removal of RF may be necessary for dependable detection of residual VCA-specific IgM antibodies.

RF poses a vastly greater problem in a search for VCA-specific IgM antibodies in patients with suspected chronic EBV infections or who experience an activation of latent, persistent infections due to immunosuppressive diseases or therapy which could be accompanied by a re-emergence of IgM antibodies to VCA. As shown here, the majority of sera from HD or NPC patients which had previously yielded moderate or high titres in the anti-VCA IgM test, contained RF at titres of ≧40 and both

TABLE 5. Antibody titres obtained after dissociation of RF from IgG and reassociation of RF with VCA-specific IgG antibodies

	Titres in test for:		
		Anti-VCA	
Reassociation mixture	RF	IgM	IgG
Serum M-174+GBSS	< 10	< 10	1280
+RF*	160	80	640
Serum M-1000+GBSS	< 10	< 10	1280
+RF	160	80	1280
Serum NPC-12+GBSS	< 10	< 10	1280
+RF	160	160	1280
Serum BL-8+GBSS	< 10	< 10	2560
+RF	320	80	1280
Serum BL65+GBSS	< 10	< 10	5120
+RF	320	160	2560
Serum M-37+GBSS	< 10	< 10	< 10
+RF	320	< 10	< 10

* From anti-VCA-negative serum RC-153.

reactivities were removed by IgG-coated latex particles. Low titres obtained in the anti-VCA IgM test were not regularly accompanied by significant levels of RF, yet they too were removed by IgG-coated latex particles. Conversely, some sera with low RF titres failed to yield definite reactions in the anti-VCA IgM test. These results do not preclude an occasional re-emergence of VCA-specific IgM antibodies in immunosuppressed viral carriers. It is likely though that such a re-emergence would be of limited duration and its detection might thus depend on the chance of collecting sera at the right moment. In such an event, the specificity of the reaction must be ascertained by showing that it is not removed by IgG-coated latex particles.

The titres recorded in the anti-VCA IgM test depended on the level of RF in the sera. They were also influenced to a considerable degree by the height of the VCA-specific IgG antibody titre. When both the RF and anti-VCA IgG titres were high, the titres observed in the anti-VCA IgM test were also high so that the ratio between the mean titres obtained in the RF and anti-VCA IgM tests was low. When the RF titres were high but the anti-VCA IgG titres low, this ratio increased substantially. When the RF and anti-VCA IgG titres were both low, no significant reactivity was found in the anti-VCA IgM test, but it yielded low titres when the anti-VCA titres were high. Thus, the more VCA-specific IgG molecules are present the more likely it is for even small amounts of RF to interact in the anti-VCA IgM test. This relationship stands to reason because of the chain reaction involved: anti-VCA IgG antibodies attach to VCA-positive lymphoblasts in the test smears; RF combines with the Fc regions of anti-VCA IgG before or after its attachment to the antigen-containing cells; and the anti-human IgM conjugate then binds to RF.

The availability of a small amount of an RF-positive but anti-VCA-negative serum permitted a limited exploration of the interactions between RF and IgG antibodies to VCA. RF clearly combined with VCA-specific IgG antibodies from RF-free sera after their attachment to VCA-positive cells in the test smears. However, the anti-human IgM conjugate induced fluorescence only when relatively large quantities of VCA-specific IgG antibodies and of RF were employed in the three-step test procedure. The interaction between anti-VCA IgG and RF was therefore possibly less efficient than that observed with the RF and anti-VCA IgG positive sera from RA and other patients. This suggests, in turn, that RF in these sera might partly be combined with IgG, among it anti-VCA. Indeed, attempts to dissociate IgG from the RF of the anti-VCA negative serum at low pH and to permit reassociation in the presence of

large quantities of anti-VCA IgG antibodies from RF-free sera yielded preparations which gave higher titres in the anti-VCA IgM test than those noted with the same sera in the three-step test procedure. Because most of the sera had been stored for many months and had been repeatedly frozen and thawed, they possibly contained aggregated or denatured IgG that facilitated association with RF. Further, adequately controlled studies are planned should another RF-positive serum from an anti-VCA negative donor become available.

RA sera, and probably others, may contain not only RF of the IgM but also other IgG classes. The presence of IgG RF cannot de detected by the immunofluorescence techniques employed nor would it be expected to cause a significant problem. All the RA and other sera used in this study contained VCA-specific IgG antibodies at titres likely to exceed those of any IgG RF that might be present. Indeed, removal of RF by adsorption onto IgG-coated latex particles did not reduce the titres obtained in the anti-VCA IgG test. While the VCA-specific IgG antibodies served to detect IgM RF, the reverse procedure, using VCA-specific IgM or IgA antibodies to detect IgG RF, is not available because these antibodies have never been observed in the absence of anti-VCA IgG. RF of the IgA class will neither be frequent nor abundant in RA sera because they mostly showed no, or at most low, reactivity in the anti-VCA IgA test. In the NPC or HD sera with moderate to high anti-VCA IgA titres, this reactivity was not reduced by the removal of RF.

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