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Comparative Evaluation of Immunoglobulin M Neutralizing Antibody Response in Acute-Phase Sera and Virus Isolation for the Routine Diagnosis of Enterovirus Infection

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A total of 314 patients exhibiting symptoms consistent with a viral disease provided, during the early stage of hospitalization, at least one specimen from a peripheral site (throat or stools or both) and a serum specimen in order to evaluate the neutralizing immunoglobulin M (IgM) antibody response in acute-phase serum in comparison with virus isolation for the rapid diagnosis of enterovirus (EV) infection. IgM antibodies were fractionated by ion-exchange chromatography and tested by seroneutralization against the various types of EV that have been recently circulating. A total of 189 patients (60%) were negative, and 21 (7%) were positive by both methods; in 51 patients (16%), a virus was isolated without IgM antibody response; 53 patients (17%) showed the opposite pattern. In all age groups except for children under 6 months, the frequency of positive results was higher with IgM serology than with virus isolation (27 and 22%, respectively). Apart from meningitis, for which isolation was more efficient, the other clinical conditions were associated with similar percentages of positivity by both methods. Regarding the 21 cases with positive results by the two techniques, the same serotype was detected in 9 cases and different serotypes were detected in 12, suggesting cross-reactivities. Thus, IgM neutralizing antibody response on acute-phase serum appears to be of limited value in the rapid diagnosis of acute EV infection but may prove useful for the investigation of the wide range of chronic diseases associated with EV.

The conventional diagnosis of enterovirus (EV) infection includes the isolation of the virus and a significant rise of antibody titer on two successive sera collected immediately after the onset of symptoms (acute-phase serum) and a few weeks later (convalescent-phase serum) (17). Since this diagnosis is long and cumbersome, alternative methods based on immunoglobulin M (IgM) serology have been developed with a wide range of techniques. Surprisingly, there are very few studies on the effective value of this serology for the routine diagnosis of EV infection compared with conventional methods (1). We previously described a simple and rapid method of serum fractionation suitable for the determination of neutralizing IgM antibody on a large number of sera (18). In this study, 314 hospitalized patients with symptoms consistent with an EV infection were investigated to evaluate the diagnostic value of IgM serology on acute-phase sera compared with that of virus isolation. Results showed that these two different diagnostic approaches are not equivalent. The type specificity of the IgM serology is also discussed.

MATERIALS AND METHODS

Patients and samples. A total of 314 patients (195 males, 119 females; age range, 0 to 79 years; mean age, 12.5 years) hospitalized in Saint-Etienne during the course of 1986 were included in the study; they exhibited symptoms consistent with an EV infection on admittance. During the first 5 days of hospitalization, each patient provided at least one sample from a peripheral site (stools or throat swab or both) for virus isolation and an acute-phase serum sample for EV serology. A second serum sample, taken at least 8 days after the first one, was available from 153 out of the 314 investigated patients.

Enterovirus isolation and typing. Isolation attempts were made by inoculating two tubes of human diploid lung fibroblasts (P2002 line; Flow Laboratories, Asnières, France) and two tubes of KB cells. Diploid fibroblasts were used for their susceptibility to echoviruses and some coxsackie A viruses since KB cells were chosen for their ability to support growth of coxsackie B viruses and also of a few echoviruses. Culture tubes were kept for at least 10 days before being considered negative; when an enterovirus-like cytopathic effect was noticed, subcultures were made and the viruses were typed with intersecting pools of hyperimmune sera (Statens Seruminstitut, Copenhagen, Denmark) according to the Lim–Benyesh-Melnick scheme (12); in some cases, the identification was achieved by neutralization with a specific monovalent antiserum.

Separation of IgM antibodies. Antibodies of the IgM class were separated by ion-exchange chromatography by a previously described technique (18). Briefly, 0.2 ml of undiluted serum was mixed with DEAE-cellulose exchanger (DE-52; Whatman, Maidstone, England) suspended in Tris buffer of low molarity (20 mM Tris, 40 mM NaCl, pH 7.2), the IgG antibodies were washed out by centrifugation, and the IgM antibodies were eluted in a high-molarity buffer (20 mM Tris, 340 mM NaCl, pH 7.2). The IgM fractions were checked by radial immunodiffusion (Immunodiagnostika, Vienna, Austria) for the efficiency rate and possible contamination by IgG. The fractionation was considered satisfactory when IgM recovery was over 60% of total IgM and IgG content was below 0.16 mg/ml.

Neutralizing antibody assay. Microneutralization tests were performed by using 100 50% tissue culture infective doses of the virus per 0.1 ml as previously described (18). P2002 and KB cell lines were alternatively used according to

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TABLE 1. IgM neutralizing antibody assay on acute-phase serum compared with virus isolation for the routine diagnosis of EV infections in 314 patients

IgM serology result	No. (%) of virus isolation results	
	−	+
−	189 (60)	51 (16)
+	53 (17)	21 (7)

type susceptibility. The whole serum and the IgM fraction were assayed simultaneously against six types of EV. The serotypes included in the panel were determined according to isolations; when at least two isolates of the same serotype arose, this serotype was included in the panel in place of another serotype no longer circulating. Viruses used in the neutralizing antibody test were clinical isolates and not prototype strains. The investigated types were coxsackievirus CA9, CB2, and CB4 and echovirus E4, E7, E11, E21, and E24. According to previous statements (11, 18), a titer of 32 or more was required for IgM positivity.

RESULTS

Table 1 shows the number of EV infections diagnosed by virus isolation and IgM neutralizing serology on acute-phase serum. Although concordant results were obtained in 210 patients (67%), 60% negative and 7% positive in the two tests), discrepancies were noted in a great number of patients; 53 (17%) exhibited positive IgM antibody without virus isolation, and 51 (16%) showed the opposite pattern.

Sex and age influence. No difference was seen in the distribution of EV infections detected by virus isolation and IgM serology according to the sex of patients (data not shown). With regard to the ages of the patients, the IgM serology was not worthwhile in children under 6 months since it was positive in only 2% of the tested cases versus 31% for virus isolation. For all the other ages, the IgM antibody assay allowed us to detect a slightly higher number (27%) of EV infections than did virus isolation (22%).

Seasonal distribution. The assessments of the seasonal distribution of EV infections by the two tests were quite different (Fig. 1); virus isolation showed a classic bimodal pattern, with peak incidences occurring during the summer and the fall; in contrast, acute IgM serology indicated a relatively more constant rate of EV infections during the course of the year despite a slight decrease in winter. However, a certain degree of correlation was observed between the two tests since the two peaks of infection detected by virus isolation (August and October) were followed 1 month later by the two peaks of infection detected by IgM serology (Fig. 1). The absence of infection detected by IgM serology in August was most likely due to the small number of serum specimens (n = 12) examined during this month.

Clinical background. Meningitis and meningism represented the majority of the 125 clinically documented cases of EV infection. For this condition, virus isolation was more sensitive than IgM serology (78 versus 50%, respectively; $P < 0.01$ by chi-square test). For all the other clinical conditions (respiratory infections, hyperthermias, gastroenteritis, etc.), the sensitivity of the IgM serology was equivalent to or higher than virus isolation (Table 2). The IgM serology was positive in culture-negative cases of purpura (five cases), diabetes mellitus (three cases), neurological disorders (three cases), and arthritis (two cases), suggesting

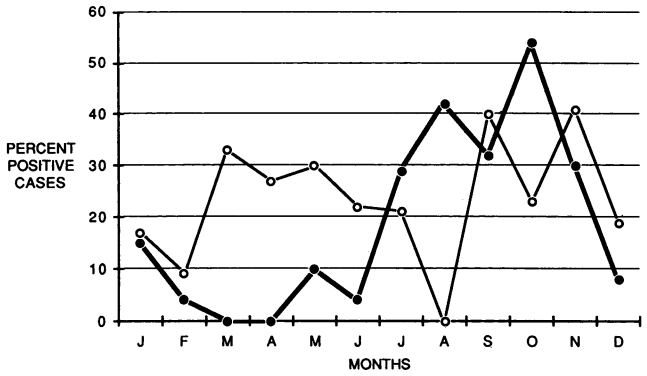

FIG. 1. Diagnosis of EV infections by virus isolation (●) and IgM antibody assay (○) during the course of the year. (Months: January, February, March, April, May, June, July, August, September, October, November, and December.)

either a poor specificity of the IgM test or an etiological relationship between these manifestations and EV infection.

Results according to serotype specificity. As illustrated in Fig. 2, there was a striking discrepancy between the types obtained by virus isolation (mainly E4 and E11) and the type specificity of IgM antibodies detected by the IgM neutralizing assay on acute-phase serum (mainly CA9 and E7). A total of 21 patients (7%) were simultaneously positive by the two tests; from these 21 cases, only 9 showed IgM antibodies directed against a serotype corresponding to the isolated virus (Table 3). Furthermore, IgM antibodies were detected against two or more different serotypes of EV in 19 of 93 cases with positive IgM serology (20%) (Table 3).

Results in patients with paired serum specimens. A second serum specimen, available from 153 out of the 314 patients, acted as a control for the EV-neutralizing serology during the convalescent phase. A total of 19 of these specimens exhibited a significant rise (fourfold or more) of total antibody titer for at least one serotype. Of these 19 rises in antibody titer, 16 (84%) were correlated with virus isolation and 7 (37%) were correlated with IgM antibody on acute-phase serum. Only four patients were simultaneously positive in all three tests (Table 4). It is worthwhile to note that a large proportion of patients with positive isolation (31 out of 47 [66%]) failed to exhibit a rise in antibody titer, suggesting, at least in some cases, a transient viral carriage, although a late-convalescent-phase serum specimen should have been taken to ascertain this finding. The low correlation between positive IgM serology and rise in antibody titer in

TABLE 2. Clinical manifestations associated with EV infections diagnosed by virus isolation and IgM neutralizing antibody assay of acute-phase serum

Clinical condition	No. (%) of positive cases by:		
	Virus isolation	IgM serology	Both tests
Meningitis and meningism (n = 60)	47 (78)	30 (50)	17 (28)
Respiratory infections (n = 13)	7 (54)	6 (46)	
Hyperthermias (n = 11)	6 (55)	6 (55)	1 (9)
Gastrointestinal infections (n = 9)	3 (33)	6 (67)	
Miscellaneous[a] (n = 21)	5 (24)	17 (81)	1 (5)
Not precisely identified (n = 11)	4 (36)	9 (82)	2 (18)

[a] Purpuric eruption (seven cases), diabetes mellitus (three cases), neurological disorders (three cases), arthritis (two cases), and others (six cases).

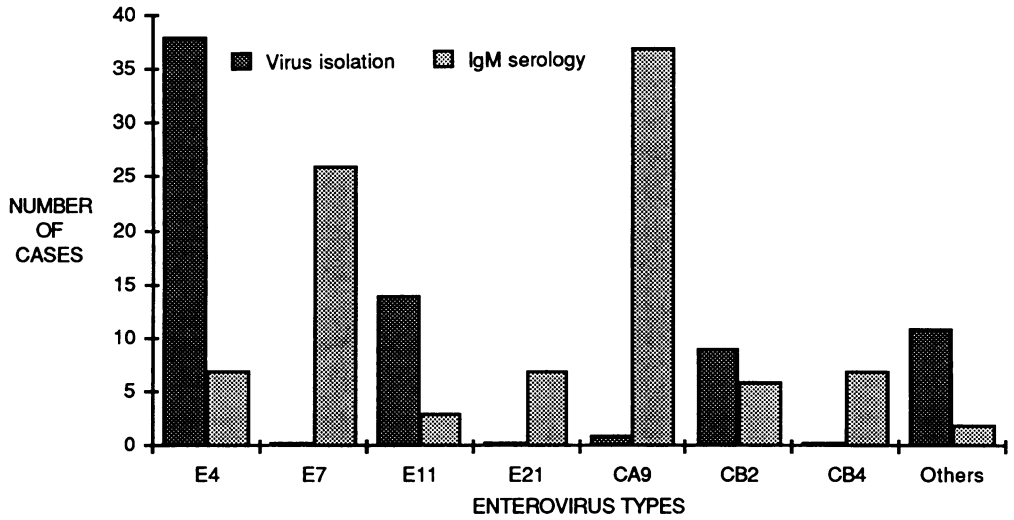

FIG. 2. EV type specificity studied by virus isolation and IgM neutralizing antibody assay. It should be noted that some patients were simultaneously positive for several EV types by IgM serology.

paired serum specimens is less surprising, since the subjects with high levels of antibody in the acute-phase serum are unlikely to exhibit a further rise in antibody titer.

DISCUSSION

As a whole, the results suggest a poor correlation between the two methods, since discrepancies were observed in about one-third of the cases (Table 1). Many factors may be postulated to explain these differences.

In the situation involving a positive culture without early IgM antibody, it can be stated that either a transient viral carriage, as suggested by culture-positive cases without a rise in antibody titer in paired serum specimens (Table 4), or a delayed response of IgM antibody production at an early stage of the infection was responsible. The delayed antibody response was particularly obvious in the first months of life, as illustrated by the lack of results of IgM serology compared with virus isolation at this age. Similarly, in acute enterovirus infections, such as meningitis, IgM antibody response in the acute-phase serum was often deficient (Table 2). The study of Bell et al. (1) has already underlined the possibility of negative IgM antibody response in sera sampled within the first 2 days of hospitalization in aseptic meningitis.

In the opposite situation (positive IgM serology without virus isolation), two explanations may also be invoked. First, the relative difficulty of obtaining some serotypes of EV in cell culture (e.g., CA9) is well known, especially, as in this study, when only two different lines are used for virus isolation. Moreover, the neutralizing effect of antibodies (mainly specific IgA) present in the gut was recently emphasized (24). Second, we lack documented information about the mean duration of the IgM response. A few cases from our experience showed a persistence of the IgM antibodies for several months; similarly, other studies reported IgM persistence for up to several years (2, 5, 13). Thus, it is very difficult to precisely define the effective beginning of an EV infection on the basis of a single positive serum for IgM serology, as illustrated by the low correlation observed between the presence of IgM antibodies in the first serum specimen and the rise of total antibody titer on two successive serum specimens (Table 4). These findings can also explain why the seasonal recrudescence of EV infections in summer and autumn was not obvious when studied by IgM serology (Fig. 1).

Although a second serum specimen was available from 153 out of the 314 patients, IgM serology was not performed at this stage for the following reasons. (i) The aim of the study was to evaluate IgM antibodies on acute-phase serum specimens as a mean of rapid diagnosis of EV infections. (ii) Little diagnostic information could be expected from the IgM serology on convalescent-phase serum specimens: either no difference was noted in the total neutralizing antibody titers between acute and late serum specimens and there was no reason to observe a variation of IgM antibod-

TABLE 3. Relation between virus isolation and IgM neutralizing antibody assay on acute-phase serum with regard to type specificity

No. of positive types per serum specimen by IgM serology[a]	No. of cases with IgM antibody against a serotype:		No. without virus isolation
	Corresponding to the isolated type	Different from the isolated type	
1	4	9	61
2	3	2	10
3	1	1	1
4	1	0	0

[a] Six serotypes were tested for each serum specimen (see Materials and Methods).

TABLE 4. Correlation between early data (virus isolation and IgM serology on acute-phase serum) and total rise in antibody titer on paired serum specimens for the routine diagnosis of EV infections

Virus isolation/IgM serology	No. with rise in antibody titer (>2) on paired sera/ no. tested (%)
−/−	0/87 (0)
+/−	12/34 (35.3)
−/+	3/9 (15.8)
+/+	4/13 (30.8)

ies, or a significant rise in antibody titer was detected and IgM antibodies were likely to contribute to this rise (as it was actually noted in several cases) but were of little interest for diagnostic purposes.

Another feature pointed out by this study is the poor specificity of the IgM serology on acute-phase serum specimens with regard to the serotype isolated by cell culture (Fig. 2) and the relatively high rate of polyspecific responses exhibited by IgM serology (Table 3). Besides the possibility of coinfections with two different serotypes, another explanation lies in the expression of an anamnestic response to a serotype implicated in a previous EV infection a few months or years earlier, which is a well-known fact in infections with other families of viruses, such as *Myxoviridae* (16). In fact, young children with fewer EV infections give a more specific response than do older children or adults (18). Heterotypic responses were mainly observed with immunogenic serotypes such as CA9 and E7 (Fig. 2). The problem of heterotypic IgM antibody responses in EV serology has been recognized for many years and arises with all of the available techniques: neutralizing antibody and 2-mercaptoethanol treatment (21), immunodiffusion (22), indirect (4) and capture (1, 9) enzyme-linked immunosorbent assays, indirect (23) and capture (19) radioimmunoassays, countercurrent immunoelectrophoresis (15), and immunoblotting (14, 20). Thus, it was not surprising to observe a high rate of heterotypic response with our IgM neutralizing antibody assay, especially since we tested sera taken at an early stage of the patients' hospitalization.

The poor correlation between the data provided by virus isolation or rise in antibody titer (or both) and the IgM neutralizing antibody assay indicates that this test is of limited value for the purpose of a rapid diagnosis, especially in young people and in the course of acute EV infections, such as meningitis. However, associations between previous infection with EV and chronic pathology have been increasingly suspected for a wide range of diseases (e.g., immunodependent diabetes mellitus [7, 9], cardiac diseases [6, 10], postviral fatigue syndrome [3, 24], glomerulonephritis [8], etc.). The IgM neutralizing antibody assay described in this paper may prove useful for investigating such pathological conditions for which large numbers of serum specimens are required. Complementary studies on the duration of the IgM antibody response would also reveal interesting features about the physiopathology of these chronic EV infections.

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