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Immunoglobulin M and G responses measured by immunofluorescence in patients with Lassa or Marburg virus infections

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Immunoglobulin M antibodies can be measured by indirect immunofluorescence in sera of patients suffering from Lassa fever or Marburg virus disease 4–7 days after onset of illness. Titres reach a peak 1–2 weeks later. These antibodies disappear, or titres decrease considerably, 1–2 months after onset of illness. Antiviral IgG antibodies can be detected at the same time as, or a little later than, IgM antibodies, but they persist much longer. None of the three patients discussed in this paper who died of Lassa fever developed IgG antibodies and only one developed IgM antibodies.

Lassa fever and Marburg virus disease are severe systemic illnesses with high fatality rates. Lassa fever is endemic in two regions of West Africa, namely the eastern part of Sierra Leone and north-west Liberia, and central and north-east Nigeria. Early recognition of the disease is of great importance because patients require strict isolation and vigorous supportive treatment. Only two sporadic outbreaks of Marburg virus disease have been recognized, but the illness was extremely severe in both instances.

Specific laboratory diagnosis of Lassa fever is currently based on isolating the virus from whole blood or serum, which takes at least 2–3 days. Using either material, virus-specific antigen can be detected by the indirect fluorescent antibody (IFA) test (*1*). Similar early detection of antigen in blood and serum of patients suffering from Marburg virus disease has recently been reported (*2*).

Many patients wait several days after the onset of symptoms before seeking medical attention, at which time the detection of specific immunoglobulins may permit a diagnosis to be made immediately. In this report we describe the use of the IFA test to measure IgM viral antibodies in human sera. We also investigated the specificity of this reaction. The IgG viral antibody response was assayed at the same time.

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MATERIALS AND METHODS

Antigen preparation

The following virus strains were used: Lassa virus, strain J, isolated in 1976 from a human serum specimen collected in Sierra Leone; and Marburg virus, patient 2 (*2*), from a heart muscle specimen collected in 1967 in the Federal Republic of Germany.

The third Vero tissue culture passage of each virus strain served as seed virus for the antigen preparation. About 10^2 $TCID_{50}$ of each agent was added to individual polystyrene flasks containing confluent 150 cm^2 monolayers of Vero cells. Eagle's minimal essential medium (MEM) with bovine fetal serum (20 ml/litre) was used as maintenance medium. The infected cells were trypsinized (using a solution containing 0.5 g of trypsin and 0.2 g of EDTA per litre) after about 30% of the cell sheet had become infected (day 3 for Lassa virus, day 4–5 for Marburg virus). The cells were resuspended in 5 ml of BA-VBS (barbital buffer, pH 7.4, with 2 ml of bovine serum albumin per litre added). The cell suspension was exposed to ultraviolet (UV) light (1500 μW per cm^2) for 20 minutes for virus inactivation. An equal volume of BA-VBS was added, and one-tenth of this suspension was used for safety testing in Vero cells. The remaining suspension was dropped onto polytetrafluoroethylene-coated microscope slides with 12 wells. The slides were then dried in air, fixed in acetone for 10 minutes, and stored at −70°C until used. Because the Marburg virus was not completely inactivated after exposure to UV light, all tests involving this antigen were performed in the Center

for Disease Control's Maximum Containment Laboratory, a P-4 facility for handling highly pathogenic agents.

Pretreatment of sera

All sera from Lassa fever patients were collected between 1972 and 1976 from persons who had contracted the disease in Nigeria, Sierra Leone, or Liberia. The sera from Marburg virus patients were obtained from survivors of the 1975 outbreak in South Africa (*3*).

To test for false-positive IgM staining caused by rheumatoid factor, we absorbed a portion of each serum specimen with glutaraldehyde cross-linked human IgG prepared according to Avrameas & Ternynck (*4*). We also absorbed the IgG antibodies in sera with staphylococci containing protein A, because IgG antibodies can mask the detection of specific IgM antibodies in unfractionated serum samples. The preparation of the bacterial absorbent was described by Knez et al. (*5*). The quality of the reagents and the volume needed for optimal absorption of IgG antibodies and rheumatoid factor were evaluated by radioimmunoassay using vaccinia virus and several human sera. We obtained good results when we mixed 0.5 ml of a 10% suspension of *Staphylococcus aureus* (Cowan I) with 0.4 ml (2.3 mg) of a suspension of glutaraldehyde cross-linked IgG. This mixture was sedimented, after which the pellet was washed once with phosphate-buffered saline (PBS), pH 7.4, and resedimented. A volume of 0.3 ml of a 1:4 serum dilution was added to the pellet and incubated at room temperature for 1 hour with constant shaking. After centrifugation, the supernatant was retained for IFA testing.

Fluorescent staining procedures

Staining procedures have already been described in detail (*1*). Commercially prepared[a] sheep anti-human IgM and IgG conjugated with fluorescein-isothiocyanate was used as second overlay. Evans blue, at a final concentration of 1:2000, served as counterstain. Untreated and pretreated sera were always evaluated concurrently, and all tests were done in duplicate. Positive and negative control sera were included in each experiment. All tests with infectious serum were performed in the Maximum Containment Laboratory.

[a] The conjugates were obtained from Wellcome Reagents Division, Greenville, NC, USA. Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the US Department of Health, Education, and Welfare.

RESULTS

IgM and IgG antibody response after Lassa virus infection

Lassa virus-specific antigen appeared as a bright yellow-green halo on the membrane of infected cells and as small condensed aggregates in the cytoplasm.

Used in the tests were 39 sera collected from 26 patients during the first 4 months after onset of symptoms. Specific Lassa virus diagnosis in all instances was based on isolating the virus and/or on a fourfold or greater rise in antibody titre to Lassa virus antigen but not to lymphocytic choriomeningitis virus antigen.

Table 1 summarizes the number of sera with IgM and IgG antibody response at weekly intervals after onset of illness. IgM antibodies developed within the first week after infection and seldom persisted beyond 1 month; IgG antibodies appeared at the same time or a little later. Not only did treatment of the sera not reduce the IgM antibody titre, but 3 additional sera showed an IgM antibody titre after treatment. This finding indicates that the IgM antibody titre is a measure of specific IgM and is not a false-positive reaction caused by rheumatoid factor.

Table 1. IgM and IgG antibodies in patients after Lassa fever infection

Week	Number of sera	Virus isolated	IFA-positive sera		
			Untreated		Treated
			IgM	IgG	IgM
1	8	7	5	1	5
2	5	5	4	4	4
3	10	2	10	10	10
4	9	0	8	9	9
5–8	3	0	0	3	1
9–16	4	0	0	4	1

The individual antibody titres of 13 patients whose sera were collected during the first 2 weeks after onset of illness are shown in Table 2. All but one serum sample yielded Lassa virus. IgM antibodies were demonstrated first on day 4 and IgG antibodies on day 5 after onset of illness. It is of interest to note that all patients who survived the infection developed IgM and IgG antibodies by day 5 whereas none of the 3 who died developed IgG antibodies and only 1 of them developed IgM antibodies.

Table 2. Development of IgM and IgG antibodies in Lassa fever patients

Patient	Days after onset	Virus isolated from serum	IFA titre[a]		Remarks
			IgM	IgG	
1	3	+	<4	<4	
2	4	+	4	<4	
3	4	+	<4	<4	
4	4	+	8	<4	
5	5	+	4	<4	
6	5	+	8	32	
7	7	+	8	<4	patient died
8	7	–[b]	<4	<4	patient died
9	9	+	4	256	
10	9	+	8	256	
11	11	+	64	512	
12	11	+	16	64	
13	12	+	<4	<4	patient died

[a] All titres are given as reciprocals of serum dilutions.
[b] Lassa virus isolated from pleural fluid.

Complete sets of sera with which to demonstrate a rise in titre of antibody and subsequent decline for individual patients were not available, but Table 3 shows the decline of the IgM antibody titre in 4 Lassa fever patients. The IgM antibody titre after treatment of each serum is also shown. The rapid decline of IgM antibodies is best seen in patient 18, who after an excellent initial IgM antibody response had such a low antibody level 3 months after infection that it could be detected only after the serum had been pretreated. The serum of patient 15 with a low initial IgM antibody titre had no detectable antibody 6 weeks after onset of illness.

As expected, pretreatment with protein A-positive *S. aureus* lowered the IgG antibody titres fourfold to eightfold.

Table 3. Duration of IgM antibodies in Lassa fever patients

Patient	Days after onset	IFA titre[a]		
		Serum untreated		Serum treated
		IgM	IgG	IgM
15	16	4	64	4
	22	<4	64	4
	33	<4	128	4
	43	<4	64	<4
18	19	64	256	64
	23	16	512	32
	28	32	512	32
	82	<4	128	4
20	20	32	128	32
	97	<4	128	<4
24	23	16	256	16
	39	<4	128	<4

[a] All titres are given as reciprocals of serum dilutions.

IgM and IgG antibody response after Marburg virus infection

Immunofluorescence was similar to that described for Lassa virus antigen, except that intracytoplasmic viral aggregates were usually much larger.

Serial serum specimens from two Marburg virus patients were tested (Table 4). As was true with Lassa virus infection, IgM antibodies developed during the first week of illness and declined rapidly at the end of the first month. Pretreatment of the sera did not change the IgM antibody titre significantly. IgG antibodies reached a peak titre a month after infection and decreased to a medium or low level 1–2 years after infection.

Table 4. IgM and IgG antibody titres after Marburg virus infection

Patient	Time after onset	Virus isolated	IFA titre[a]	
			IgM	IgG
2M	5 days	+	<2	<2
	7 days	+	4	4
	14 days	–	32	32
	19 days	–	16	64
	27 days	–	4	256
	8 months	NT	<4	64
	9½ months	NT		32
	2 years	NT		8
3M	2 days	+	<2	<2
	7 days	+	8	4
	12 days	–	32	16
	26 days	–	8	128
	4 months		<4	128
	9½ months	NT		128
	1 year	NT		32

[a] All titres are given as reciprocals of serum dilutions.
NT = not tested.

DISCUSSION

Specific IgM antibodies can be demonstrated in the serum of Lassa fever patients during the acute phase of illness. Since most sera obtained later than the third day after onset of symptoms were positive, this procedure should allow a specific diagnosis to be reached for many patients within hours of initial medical consultation. As patients who died of Lassa fever failed to produce detectable antibodies, more work is needed to determine the time that must

elapse after onset before we can safely conclude that a negative Lassa IgM (or IgG) antibody test rules out Lassa fever. Furthermore, we have as yet no information on antibody evolution in Lassa fever patients who have only a mild clinical illness; admission to most West African hospitals is limited to patients with truly severe disease.

Although our observations on human Marburg virus infections were much more limited, generally similar data were obtained. Thus we propose that rapid IFA screening be used if the differential diagnosis includes one or more of the viral haemorrhagic fevers caused by such agents as Lassa, Marburg, Ebola, Rift Valley, or yellow fever viruses.

Pretreatment of sera to remove substances that could cause false-positive IgM antibody reactions (*5, 6*) may be necessary in individual cases, but our results indicate that such substances are not regularly induced by Lassa virus infection. In fact, tests following pretreatment with *S. aureus* containing protein A revealed small amounts of anti-Lassa IgM in 3 instances in which no antibody had been detected in the untreated sera.

It is noteworthy that many sera from Lassa fever patients contained both readily detectable virus and immunofluorescent antibodies. This phenomenon has not been observed in human infections with the related arenavirus, Machupo, which causes Bolivian haemorrhagic fever (*7, 8*). In patients with this disease, antibodies are rarely detectable for at least 2 weeks after onset of symptoms, which eliminates IFA as a diagnostic tool during the acute phase of illness. Work is in progress in West Africa to determine whether Lassa fever patients display circulating antigen–antibody complexes and, if so, whether the presence of such complexes is correlated with certain clinical manifestations or with the overall severity of the disease or with both.

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RÉSUMÉ

RÉPONSE EN IMMUNOGLOBULINES M ET G CHEZ LES MALADES ATTEINTS D'INFECTIONS À VIRUS LASSA OU MARBURG: MESURE PAR LES ÉPREUVES D'IMMUNOFLUORESCENCE

La présence d'anticorps IgM spécifiques peut être décelée par l'immunofluorescence indirecte (IF) vers le milieu de la première semaine après le début de la maladie dans le sérum des malades souffrant de fièvre de Lassa. L'exécution de l'épreuve IF prenant moins de deux heures, un diagnostic spécifique devrait donc, dans la plupart des cas, pouvoir être posé peu après la consultation initiale. Les anticorps IgG apparaissent en même temps ou un peu plus tard que les anticorps IgM; ils n'ont été trouvés chez aucune des trois personnes qui ont succombé à l'infection due au virus Lassa, et l'une seulement de celles-ci était porteuse d'anticorps IgM. D'autres études seront cependant requises pour parvenir à des conclusions sur la réponse en anticorps des diverses classes chez les patients qui succombent à la maladie. Le titre des anticorps IgM a baissé rapidement au cours des deux semaines après le début de l'infection alors que les anticorps IgG ont manifesté une plus grande permanence.

Pour éliminer la possibilité d'une réaction faussement positive due au facteur rhumatoïde, les sérums ont été préalablement traités par absorption avec des IgG humaines liées au glutaraldéhyde. D'autre part, les IgG pouvant masquer la présence d'IgM spécifiques, les sérums analysés ont également été absorbés avec *Staphylococcus aureus* contenant une protéine A. Dans les épreuves opérées ensuite, les titres d'IgM n'ont pas subi de réduction significative; et de surcroît elles ont permis de déceler la présence de faibles taux d'IgM anti-Lassa dans trois sérums pour lesquels les épreuves antérieures avaient donné des résultats négatifs.

Les épreuves pratiquées sur deux séries de sérums prélevés chez deux malades souffrant de l'infection à virus Marburg ont donné des résultats analogues à ceux obtenus pour les infections à virus Lassa. Si le diagnostic doit trancher entre plusieurs des fièvres hémorragiques virales, il est donc impératif de pratiquer rapidement les épreuves IF.

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