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Observations on levels of immunoglobulin M in confirmed cases of *Trypanosoma rhodesiense* infection in the Lambwe Valley

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By means of established single-diffusion techniques, immunoglobulin M (IgM) levels in the blood and cerebrospinal fluid of patients with confirmed Trypanosoma rhodesiense *infections were studied at the time of parasitological diagnosis, while the patients were being treated with suramin and melarsoprol, and during post-treatment surveillance. During the 3-year study period the number of diagnosed cases in which there was a raised level of IgM in the blood varied considerably, depending on whether diagnosis was made early or late in the course of the infection. In some cases the blood IgM levels returned to normal during preliminary courses of treatment with suramin and in others by the end of a 1-month course of treatment with melarsoprol, while in some other cases they did not return to normal for about 2 years. Similar results were obtained for IgM in cerebrospinal fluid, except that if IgM could be detected at the start of treatment with melarsoprol it was still detectable at the end of treatment. However, cerebrospinal IgM usually disappeared after 2 years. The results indicate that IgM levels are a useful indicator in the diagnosis of the more chronic cases.*

An elevated concentration of immunoglobulin M (IgM) in the blood is a valuable indication of *Trypanosoma gambiense* infection in man (Mattern et al., 1961, 1964; Binz et al., 1968). W. H. R. Lumsden (unpublished data), Cunningham et al. (1966), and Cornille & Hornung (1968) have reported similar observations in *T. rhodesiense* infections. This communication describes observations made on IgM levels in the serum and cerebrospinal fluid (CSF) of confirmed cases of *T. rhodesiense* sleeping sickness in the Lambwe Valley endemic area of South Nyanza District, Kenya. During the period 1968–70 the prevalence of infection was low; only 56 cases were detected either at the diagnostic centres serving a population of 10 000 people who were exposed to the risk of infection, or in surveys confined to localities, having a total population of 6 000, where the risk was greatest (Watson, 1972). Whenever possible, the main emphasis was given to the study of IgM levels at the time of diagnosis, during the course of treatment, and during a post-treatment period of surveillance.

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MATERIALS AND METHODS

Although 56 cases of sleeping sickness were diagnosed between 1968 and 1970 (Watson, 1972), the present writer was able to study IgM levels in the blood of only 49 patients and in the CSF of only 40 patients. The intention was that whenever a blood film was prepared for microscopic examination, a blood sample for IgM determination should taken at the same time on filter paper; initially, however, this objective was not always achieved.

For various reasons the full sequence of investigations for blood IgM was completed in only 26 patients and for CSF IgM in only 20 patients. Most of the infections were diagnosed at the outpatient department of Homa Bay hospital, about 50 km from the Lambwe Valley field station; not infrequently treatment was begun before the initial specimens for immunological study were collected. Moreover, because of the shortage and lack of continuity of hospital staff associated with the opening of the Homa Bay hospital in 1968/69, it was impossible to ensure the regular collection of material, even while patients were in hospital, during that period.

Determinations of blood IgM levels were made on dried blood blots collected on Whatman No. 4 filter paper by means of the single-diffusion technique of Cunningham et al. (1967), with the slight modification that 1.5% agar was used for preparing test plates. Areas of precipitation on the test plates 6.0 mm or larger in diameter were considered to indicate a raised level of IgM in the blood. Determinations of CSF IgM levels were made by means of the single-diffusion technique of Fahey & McKelvey (1965), a buffer solution of pH 7.2 and a 1.5% agar solution being used. A ring of precipitation with CSF indicated an abnormal IgM level.

The results given by test and control samples of blood and CSF (control specimens being obtained from uninfected persons resident within the sleeping sickness endemic area) were compared with those given by a standard serum (obtained from Iochum, Lausanne, Switzerland) containing a high concentration of IgM.

RESULTS

IgM levels in the blood of confirmed cases of sleeping sickness at the time of diagnosis

The results of this investigation are presented in Table 1. Altogether, 6 patients (12.3% of those examined immunologically) had a normal blood IgM level at the time trypanosomes were first detected. This apparent anomaly might be accounted for by the diagnosis having been made early in the infection before the IgM response was fully developed. In one case the blood IgM rose to an abnormally high level before treatment could be started; in four others it became abnormal during the early stages of treatment. The sixth case was diagnosed on the first day of illness, trypanosomes being found in a chancre; treatment was begun immediately and since the patient was moved out of the area no further IgM investigations were undertaken.

Table 1. IgM levels in the blood of confirmed sleeping sickness cases at the time of diagnosis; Lambwe Valley, 1968–70

Year	No. of cases examined for raised IgM	No. and percentage of cases with:			
		Raised IgM		Normal IgM	
		No.	%	No.	%
1968	23	22	95.6	1	4.4
1969	16	15	93.7	1	6.3
1970	10	6	60.0	4	40.0
totals	49	43	87.7	6	12.3

While in 1968, and again in 1969, only 1 case of sleeping sickness did not show a raised IgM level at the time of diagnosis (5% and 6% of the annual totals, respectively), 4 out of 10 cases in 1970 had a normal IgM level when they were first diagnosed. The most probable explanation for this difference lies in the earlier diagnosis of the disease in 1970; by that time the people in the area were making greater use of diagnostic facilities available at the Lambwe Valley field station.

Associated with this feature in 1970 was a change in the clinicopathological picture; by that time glandular involvement was much more frequently encountered, and this was also attributed to earlier diagnosis. In 1968, 1969, and 1970 the proportions of cases with glandular involvement were 20%, 30%, and 70%, respectively. The relationship between glandular involvement and a raised IgM level is shown in the following tabulation.

Stage of infection	*No. of cases*	
	Normal IgM	*Raised IgM*
chancre	1	0
trypanosomes in glands	4	11
no trypanosomes found in glands	1	32

IgM levels in the blood of sleeping sickness cases during treatment

It was not possible to undertake immunological studies of patients throughout their stay in hospital until late 1968, and at about that time the treatment of cases was standardized. Treatment regimes using suramin and melarsoprol for early and late cases, respectively, have been described by Watson (1972).

All the 26 patients for whom complete IgM levels were recorded throughout the course of their treatment were in the late stage of sleeping sickness. Treatment therefore consisted of a preliminary course of suramin (0.25 g given as a test dose, followed by 2 doses of 1.0 g spread over 1 week), followed by 3 3-day courses of melarsoprol at intervals of 1 week.

Only one-quarter of the cases in which the IgM level was raised initially showed a fall to normal levels during the very short course of treatment with suramin. In the few instances in which the IgM level was normal before treatment began, it had reached an abnormal level by the end of the short course of treatment, as shown in the following tabulation.

IgM level before treatment		*IgM level after treatment with suramin*	
		normal	*raised*
raised	22	6	16
normal	4	0	4

The findings suggest that in those patients in whom the IgM level was normal before treatment (i.e., in the earlier stages of infection), a massive stimulus had already been initiated and IgM production could not be arrested immediately by the small quantity of suramin administered. Conversely, amongst those infections that had reached a more advanced stage, the stimulus for IgM production had less impetus and in the presence of antibodies, chemotherapy was more readily able to interrupt the process.

The effect of melarsoprol on IgM levels is shown in the following tabulation.

IgM level after treatment with suramin and before treatment with melarsoprol		*IgM level at the completion of treatment with melarsoprol*	
		normal	*raised*
raised	20	12	8
normal	6	6	0

In the 4 patients who had normal IgM levels at the time of diagnosis, but raised levels during treatment with suramin, the IgM levels had returned to normal by the end of the course of treatment with melarsoprol, i.e., 1 month later. The rise and fall of IgM levels in these patients was therefore rapid.

In 8 cases (30% of the total), however, the IgM level remained raised from the time of diagnosis to the completion of treatment, a few of them showing a temporary, but unsustained, fall to normal levels during that period. This group contained all four patients who experienced some degree of reaction to melarsoprol during treatment. The other four cases had been very difficult to diagnose originally, having a very light or even periodic parasitaemia, which suggested chronic infection.

IgM levels in blood during post-treatment surveillance periods

Follow-up surveillance for evidence of recovery or relapse was undertaken periodically amongst the patients after their discharge from hospital. For these purposes both parasitological and immunological examinations were made. The results of immunological investigations (Table 2) show that, in all cases examined, IgM levels did not return to normal until more than 2 years after the patients were discharged from hospital.

Table 2. IgM levels in the blood of cured cases of sleeping sickness during post-treatment surveillance periods; Lambwe Valley, 1968–70

Time after patient's discharge from hospital (months)	No. of cured cases examined	No. and percentage of persons with raised IgM levels	
		No.	%
4–9	15	3	20.0
10–15	16	3	18.7
16–24	26	2	7.6
25–32	9	0	0.0

IgM levels in the CSF of confirmed cases of sleeping sickness at the time of diagnosis

In trypanosomiasis investigations, lumbar puncture (LP) is undertaken for purposes of either assessment or diagnosis. During the investigation reported here, assessment LP was carried out when the disease had already been diagnosed by direct examination of blood or glandular fluid or by animal inoculation tests. The objective was to determine whether or not the central nervous system had been invaded. A diagnostic LP was carried out when, after a prolonged search, trypanosomes could not be found, but where there was strong evidence to suggest that the patient was suffering from sleeping sickness. CSF cell and protein values are very important even in cases in which trypanosomes are not discovered. It had been intended to estimate the IgM levels in CSF simultaneously with other examinations, but this was not always possible during the earlier studies. IgM levels in the CSF of 40 patients at the time of diagnosis are presented in Table 3.

Table 3. IgM levels in the CSF of confirmed sleeping sickness cases at the time of diagnosis; Lambwe Valley, 1968–70

Year	No. of cases examined for raised IgM	No. and percentage of cases with:			
		Raised IgM		Normal IgM	
		No.	%	No.	%
1968	14	12	85.7	2	14.3
1969	16	13	81.2	3	19.8
1970	10	7	70.0	3	30.0
totals	40	32	80.0	8	20.0

The decrease between 1968 and 1970 of the proportion of cases found to have IgM in the CSF at the time of diagnosis suggests again that the cases were being discovered earlier in the course of the disease. The CSF showed abnormalities (i.e., an increase in the number of cells or in the amount of protein, or the presence of trypanosomes) in 7 of the 8 cases in which there was no CSF IgM.

A very simple relationship between the presence of IgM in the CSF and the abnormality of the latter is shown in the following tabulation.

	No. of cells and amount of protein in CSF [a]	*IgM in the CSF*	
		none	*present*
normal	1	1	0
abnormal	38	7	31

[a] In one of the 40 patients the CSF was not examined for cells and protein.

IgM levels in the CSF of patients with sleeping sickness during treatment

The CSF was examined both before and after the course of treatment with melarsoprol in only 20 patients. The results obtained were as follows:

IgM in CSF before treatment		*IgM in CSF after treatment*	
		none	*present*
none	5	4	1
present	15	0	15

In no case where IgM was present in the CSF before treatment began had it disappeared by the time therapy was completed. CSF IgM therefore appears to be more persistent than blood IgM.

IgM levels in CSF during post-treatment surveillance

Surveillance of cases undertaken periodically after treatment showed that CSF IgM gradually disappeared over a period of about 2 years (Table 4).

Table 4. IgM levels in the CSF of cured cases of sleeping sickness during post-treatment surveillance; Lambwe Valley, 1968–70

Time after patient's discharge from hospital (months)	No. of cured cases examined	No. and percentage of persons with raised IgM levels	
		No.	%
0–3	5	4	80.0
4–9	7	3	42.8
10–15	9	2	25.0
16–24	10	3	30.0
25–32	2	0	0.0

DISCUSSION

The production of blood IgM, stimulated by a trypanosome infection, takes some time to develop. The most acute forms of *T. rhodesiense* infection occurring in areas where diagnostic centres are reasonably available to the population will occasionally be detected before the IgM level in the blood rises above normal. The evidence presented above suggests that trypanosome infections associated with a normal IgM level can be explained on the basis of early diagnosis and that they had no other significance.

At the other end of the spectrum, with infections that were classed as *T. rhodesiense* but more closely resembled *T. gambiense* sleeping sickness, the blood IgM was invariably found to be raised when the diagnosis was first made. Thus, in clinical practice it was found that a raised blood IgM level in a patient attending voluntarily from the endemic area was a particularly useful indicator for making a final diagnosis in the most chronic forms of the disease.

In some patients, including those with the more chronic forms of the disease and those experiencing melarsoprol reactions, a very long time may elapse after a cure has been achieved before the blood IgM level returns to normal; nevertheless, the level does return to normal, although this may not be achieved for 2–3 years.

Changes in the CSF indicating involvement of the central nervous system in cases of trypanosomiasis were defined as more than 3 cells/mm^3 or more than 25.0 mg of protein per 100 ml of blood. During investigations in the Lambwe Valley, involvement of the central nervous system had occurred to some degree in 7 cases (18% of those investigated) before IgM could be detected. It appears that, as in the blood, there is a period following infection before the production of CSF IgM becomes apparent.

IgM persists in the CSF much longer than it does in the blood. This difference seems to support the view that CSF IgM is synthesized *in situ* and has its own particular characteristics; for example, in contrast to blood IgM, CSF IgM lacks isohaemagglutinating properties.

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RÉSUMÉ

OBSERVATIONS SUR LES CONCENTRATIONS D'IMMUNOGLOBULINE M DANS DES CAS CONFIRMÉS D'INFECTION À *TRYPANOSOMA RHODESIENSE* DANS LA VALLÉE DE LA LAMBWE

Utilisant une technique d'immunodiffusion, l'auteur a étudié les concentrations d'immunoglobuline M (IgM) chez des patients atteints de trypanosomiase avérée à *Trypanosoma rhodesiense* dans la vallée de la Lambwe, de 1968 à 1970. Les examens, portant à la fois sur le sang (49 malades) et sur le liquide céphalo-rachidien (LCR) (40 malades) ont été effectués au moment où le diagnostic a été posé, pendant et après le traitement.

Durant les trois années de l'étude, la proportion des cas présentant une augmentation des titres sanguins d'IgM au moment du diagnostic a diminué de 95,6 à 60,0%, en raison probablement de la précocité croissante du diagnostic. Chez 12% des malades, la teneur en IgM était normale au moment du diagnostic, mais les titres se sont élevés pendant la période précédant le traitement ou pendant celui-ci.

Le traitement a comporté l'administration de suramine, puis de mélarsoprol. Dans un quart des cas ainsi traités, les titres sanguins d'IgM sont revenus à la normale pendant la cure préliminaire par la suramine. Chez les quelques malades présentant des teneurs normales avant le début du traitement, les titres se sont élevés à la fin du traitement par la suramine, mais ont repris des valeurs normales pendant le traitement par le mélarsoprol.

Dans 30% des cas, les concentrations sanguines d'IgM sont restées élevées pendant toute la durée du traitement par le mélarsoprol. La moitié des malades de ce groupe ont présenté des réactions adverses au médicament; les autres étaient des sujets chez lesquels le diagnostic avait soulevé des difficultés. Les examens pratiqués après le traitement pendant la période de surveillance ont montré que le retour à la normale de l'IgM sanguine exigeait au moins deux années.

Les examens de LCR ont donné des résultats similaires. En trois ans, la proportion des malades chez lesquels le LCR renfermait de l'IgM s'est abaissée de 85,7 à 70,0%. Dans certains cas où la recherche de l'IgM était restée négative, d'autres anomalies du LCR ont été notées: augmentation du nombre des cellules et du taux de protéines, présence de trypanosomes. Chez un malade à LCR initialement normal, l'IgM est apparue pendant le traitement par le mélarsoprol. Lorsque l'IgM était décelée dans un LCR avant le traitement, on la retrouvait à la fin de celui-ci et elle ne disparaissait progressivement qu'après deux ans ou plus.

Selon l'auteur, la mise en évidence de concentrations sanguines élevées d'IgM chez des sujets se présentant spontanément à la consultation est un indice précieux qui peut conduire au diagnostic dans les formes les plus chroniques de la maladie.

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