Immune Complexes in Serum and in Cerebrospinal Fluid in African Trypanosomiasis

CORRELATION WITH POLYCLONAL B CELL ACTIVATION
AND WITH INTRACEREBRAL IMMUNOGLOBULIN SYNTHESIS

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Abstract The possible occurrence of immune complexes (IC) in serum and in cerebrospinal fluid (CSF) has been studied in 36 patients with African trypanosomiasis (Trypanosoma brucei gambiense). In serum, very high levels of IC were detectable by the 125I-C1q-binding and by the conglutinin-binding assays with positive results in 94 and 87%, respectively, of untreated patients. Circulating IC were found in both early and late stages of the disease, without significant quantitative differences; their size was 15-25S. There was a significant negative correlation between C3 values and C1qBA. Our studies suggest that circulating IC occurring during trypanosomiasis may be the expression of a polyclonal B cell activation. Indeed, there was a significant correlation (P < 0.001) between the levels of circulating IC and either the levels of IgM (mean value 12.5±7.2 mg/ml) or with the levels of rheumatoid factor-like antiimmunoglobulin antibodies that were detected by solid phase radioimmunoassay in 74% of the patients.

IC were detected in 31 of 35 CSF samples, with a marked elevation in patients with definite involvement of the central nervous system as compared with earlier stages of sleeping sickness. The occurrence of IC in CSF was not related to an impairment of the blood-brain barrier as shown by analysis of CSF-serum albumin ratios. The level of IC in CSF did not correlate with the serum level and, therefore, circulating IC do not appear to cross efficiently an unimpaired blood-brain barrier. The analysis of IgG, IgM, and albumin concentrations in serum and CSF demonstrates a marked intracerebral immunoglobulin synthesis in patients with manifestations of meningoencephalitis. There was a correlation between CSF-C1q binding assay and this local IgG synthesis.

These data are consistent with a local formation of IC in CSF in patients with active meningoencephalitis. The results obtained in eight patients followed during therapy suggest that the presence of IC in CSF may be an indicator of a continuing central nervous system disease and that the quantitation of CSF-IC may be useful for monitoring patient care.

Introduction

The infection of man with Trypanosoma brucei gambiense is responsible for the development of sleeping sickness. It is still a serious health problem in many African countries (1). Two phases of the disease are generally recognized: first, there is a proliferation of trypanosomes in blood and in lymphoid tissues, then the parasites invade the central nervous system (CNS)\(^1\) and induce a meningo-encephalitis. A major feature of trypanosomiasis is an intense proliferation of lymphocytes, particularly involving B cells and leading to an increased polyclonal synthesis of immunoglobulins (2, 3). This occurs soon after infection. The development of cerebral lesions

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\(^1\)Abbreviations used in this paper: CNS, central nervous system; CSF, cerebrospinal fluid; C1qBA, 125I-C1q binding assay; IC, immune complexes; KgBA, conglutinin binding assay; Qalb, CSF-serum albumin ratio; RF, rheumatoid factor.

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been of the notion that trypanosomiasis, it has been shown that the host’s immune response plays a major role in the triggering of inflammatory foci (5). The present investigation has been undertaken in order to analyze the involvement of immune complexes in this disease. Circulating immune complexes (IC) were first studied in relation to the patient’s disease stage and to the expression of the polyclonal B cell activation. In particular, the correlation with the occurrence of rheumatoid factor (RF)-like antibodies was studied. IC were also found in CSF and their origin was questioned. The possibility of IC crossing from blood to CSF or of a local formation of IC within the CNS have been considered in relation to the functional state of the blood-brain barrier and to the local synthesis of immunoglobulins in CNS. The usefulness of the quantitation of CSF-IC for stage diagnosis and follow-up of patients with sleeping sickness has also been evaluated. Through these studies on African trypanosomiasis, it has been possible to approach two questions that are also relevant to other human diseases: (a) Does an intense polyclonal B cell activation generate circulating immune complexes in man? (b) Do IC cross an unimpaired blood-brain barrier?

METHODS

Patients. 82 serum samples and 34 cerebrospinal fluid samples from 36 patients with trypanosomiasis were collected in Zaire in the course of the regular activities of the Centre du Controle de la Trypanosomiase. In all the selected patients, the clinical diagnosis was confirmed by the microscopic demonstration of trypanosomes in blood, in lymph node aspiration fluid or in CSF. All these patients also had anti-trypanosome antibodies in serum. They were also submitted to a routine parasitological investigation. For some of our studies, the patients were classified according to the stage of the disease. Three different stages were considered: patients at stage I present hemolymphatic manifestations of the infection (enlarged lymph nodes, trypanosomes in blood, normal pattern of cerebrospinal fluid, no clinical sign of cerebral involvement); stage II is similar to stage I but trypanosomes appear in the CSF, stage III is characterized by neurological manifestations ("sleeping sickness") and abnormal CSF (trypanosomes, increased cell and protein content). Blood and CSF samples included in this study were collected for the routine diagnostic procedures. In particular, CSF samples were drawn for the routine detection of trypanosomes and not for the purposes of our investigation. Most of the serum and CSF samples were obtained before treatment. In a follow-up study, samples were also obtained during treatment and several months after treatment with melarsoprol (Arsobal, Phone-Poulenc, Vitry, France). Blood was drawn from patients and allowed to clot for 2 h. After centrifugation, the serum and CSF samples were frozen, stored, and shipped in liquid nitrogen. Control serum samples were obtained in Kinshasa from age-matched hospitalized Zairian patients suffering from schistosomiasis (eight), amebiasis (two), ascariasis (two), hookworms (two), lung cancer (one), and tuberculosis (two). Another group of control sera was obtained from 40 age-matched normal blood donors in Geneva. Five CSF samples were also obtained in patients from Geneva who had to undergo a myelography which requires prior removal of some CSF. These five CSF samples were considered as normal controls since no neurologic disease could be demonstrated in these patients. In addition, nine CSF and corresponding serum samples were obtained from patients hospitalized in Kinshasa for other parasitic diseases (malaria, schistosomiasis, filariasis).

Detection of immune complexes in serum and in CSF

Clq binding assay. The detection of immune complexes was performed in sera using the 125I-Clq binding test as described by Zubler et al. (6), but slightly modified as follows: in the first step, 50 µl of serum was mixed with 100 µl of Na2 EDTA pH 8.3 containing 0.4% Tween 20. For the detection of Clq binding material in CSF, 100 µl of CSF was mixed with 50 µl fresh normal human serum before adding 100 µl of Na2 EDTA pH 8.3, 0.4% Tween 20. After incubation at 37°C for 30 min, 50 µl of 125I-Clq and 500 µl 3.6% polyethylene glycol (DAB, 7, 6, 000 mol wt, Siegfried A.G., 4800, Zofingen, Switzerland) were added. The results were corrected by subtracting the percentage of precipitation obtained with a normal serum pool. The mean corrected value for 40 normal human donors was 0.6±0.45% (±1 SD).

Sera with high Clq-binding activity were treated with deoxyribonuclease I (DNase). 1 vol of serum was mixed with 1 vol of DNase (1 mg/ml in veronal-buffered saline, Worthington Biochemical Corp., Freehold, N. J.) and incubated for 3 h at 37°C. The enzymatic reaction was stopped by adding EDTA 0.2 M, pH 7.0 in borate buffer. Control sera were treated similarly except that the EDTA was added before the DNase. The digested and undigested material was tested for Clq binding activity.

Conglutinin-binding assay. The conglutinin binding assay was performed on serum according to Casali et al. (7, 8), using 125I-staphylococcal protein A as a labeled marker for the quantitation of conglutinin-bound IC. For the testing of CSF samples, CSF was diluted 1:2 in fresh normal human serum.

Detection of anti-trypanosome antibodies

Trypanosoma brucei brucei were obtained from lump 227 after a passage in irradiated mice. Trypanosomes were separated from erythrocytes on a DEAE column according to Lanham and Godfrey (9). Smears were prepared, dried, and fixed with ethanol. Serial dilutions of each serum or CSF sample were then tested for antibodies by indirect immunofluorescence using anti-IgG and anti-IgM conjugates (Nordic Immunological Laboratories, Tilburg, The Netherlands).
Quantitation of serum and CSF proteins

IgG, IgM, albumin, and C3 were quantitated by radial immunodiffusion using commercially supplied immunodiffusion plates (Behringwerke AG, Marburg/Lahn, West Germany). The protein content was determined by the Lowry technique (10).

Sucrose gradient ultracentrifugation

Serum and five CSF samples from patients with trypanosomiasis were subjected to ultracentrifugation in a 10–40% sucrose gradient (borate buffer, pH 8.3) using an SW 65 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The centrifugation was done at 50,000 rpm for 5 h.

Evaluation of the blood-brain barrier status and detection of an intracerebral immunoglobulin synthesis

The CSF-serum concentration ratios (Q) of albumin (alb), IgG, and IgM (Qalb, QlG, QlgM, respectively) were determined. The relative impairment of the blood-CSF barrier as reflected by an increased permeability to albumin was estimated from the Qalb values, as described by Tibbling et al. (11). The IgG index (QlG/Qalb) was determined according to Schliep and Felgenhauer (12), in order to evaluate the intensity of the local production of IgG in the CSF compartment. The intracerebral IgG synthesis was also calculated using Tourtellotte’s formula (13):

\[ \text{IgG synthesis (milligrams/100 ml)} = \frac{\text{IgG serum}}{369} - \left( \frac{\text{albumin serum}}{230} \right) \times \left( \frac{\text{IgG serum}}{\text{albumin serum}} \times 0.43 \right) \]

Solid-phase radioimmunoassay for anti-immunoglobulin antibodies (RF)

The assay for detecting anti-immunoglobulin antibodies using polyethylene tubes coated with rabbit IgG was described by Hay et al. (14) and was slightly modified for use in this study.

Statistical analysis

The data were analyzed using the Wilcoxon rank test, linear regression (r), and the Spearman correlation test (R).

RESULTS

Circulating immune complexes and C3 in human trypanosomiasis. A biological activity suggestive of the presence of immune complexes was investigated in serum from 36 patients, using the Clq binding assay (ClqBA) and the conglutinin binding assay (KgBA). There was an increased ClqBA in 94% of these patients, with a median value of 36% (Fig. 1). Values of KgBA were also increased in 81% of the patients with a median value of 64 μg eq aggregated human gamma globulin/ml (Fig. 1). In both ClqBA and KgBA, there was a very significant difference between the sleeping sickness group and either the group of other hospitalized African patients (P < 0.001) or the normal European control group (P < 0.001). There was a significant correlation between the results obtained with the two tests (R = 0.51, P < 0.001). The nature of the Clq binding material was further analyzed by ultracentrifugation of six serum samples on sucrose density gradient. The peak of ClqBA was found in 15–25S fractions (Fig. 2). The ClqBA was also measured in 10 samples, which were incubated in presence of DNase, and was not found significantly changed (undigested: 24.8%; digested: 26.3%). The levels of ClqBA and KgBA in serum were also analyzed according to the clinical stage at the time of diagnosis and of sample collection. At all stages, there was a significant increase in the values obtained in the two tests (P < 0.005). All patients at stage I were positive in both tests and there was no significant difference between the levels observed in the patients at various stages (Fig. 1).

In the 36 patients studied before treatment, serum C3 levels were measured. A significant decrease was observed in 67% of samples, with a mean value of 62.9±26.3% (Fig. 3). In other hospitalized African patients, the mean value was 105.4±33%. There was

![Figure 1](https://example.com/figure1.png)

**FIGURE 1** ClqBA (percent precipitated) and KgBA (micrograms equivalent aggregated human gamma-globulin per milliliter) activities in serum samples from patients with trypanosomiasis (○, stage I or II; ●, stage III) and from other hospitalized African patients. The normal range in European control group is indicated by the hatched area.
a significant negative correlation between C3 values and ClqBA ($r = -0.56, P < 0.005$) or KgBA ($r = -0.42, P < 0.025$). The decrease of serum C3 does not appear to be related to a severe impairment of protein synthesis since serum albumin levels were within the normal range (mean $43.2 \pm 9.5$ mg/ml).

Circulating immune complexes in relation to polyclonal B cell activation, rheumatoid factor, and anti-

trypanosome antibodies. African trypanosomiasis is known to be associated with a marked increase of immunoglobulin synthesis, with a polyclonal pattern (2). In 34 patients studied, high IgG and IgM serum levels ($34.8 \pm 13.6$ and $12.5 \pm 7.2$ mg/ml, respectively) were observed without any significant difference according to the stage of the disease. Circulating immune complexes were closely associated with the occurrence of polyclonal antibody synthesis. A very significant correlation was found between ClqBA ($R = 0.79, P < 0.001$) or KgBA ($R = 0.69, P < 0.001$) and serum IgM levels (Fig. 4a) and to a lesser degree between ClqBA and serum IgG levels ($R = 0.55, P < 0.001$). There was no correlation between KgBA values and IgG levels ($R = 0.32$, NS). One feature of this polyclonal antibody synthesis is the occurrence of RF-like anti-immunoglobulin antibodies. Using a solid-phase radioimmunoassay for IgM-RF, high levels of RF were obtained in 23/31 sera. There was a significant correlation between ClqBA ($R = 0.69, P < 0.001$) or KgBA values ($R = 0.61, P < 0.001$) and RF levels (Fig. 4b).

Anti-trypanosome antibodies were detected in all patients’ sera. There was no correlation between the titers of IgG or IgM anti-trypanosome antibodies and the IgG and IgM serum levels, nor with the levels of circulating IC (ClqBA and KgBA) (Table I).

Immune complexes in cerebrospinal fluid during African trypanosomiasis. Immune complexes were detected by the ClqBA in 31 of 35 cerebrospinal fluid samples in patients with trypanosomiasis ($P < 0.001$) (Fig. 5). There was no detectable Clq-binding material in the five “normal” CSF samples. Similarly, in nine CSF samples from hospitalized African patients with other parasitic diseases, there was no increase in ClqBA although the ClqBA was slightly elevated ($5-14\%$ precipitated) in the corresponding serum samples.

The KgBA was also performed on 32 of the CSF samples from patients with trypanosomiasis and elevated values were obtained in 45$\%$ ($P < 0.001$). In both ClqBA and KgBA a marked elevation of the
TABLE I
Anti-trypanosome Antibodies and Immune Complexes

<table>
<thead>
<tr>
<th>Stage</th>
<th>Patient</th>
<th>Anti-trypanosome antibodies</th>
<th>Serum IgG</th>
<th>Serum IgM</th>
<th>C1qBA mg/ml</th>
<th>KgBA ( \mu g ) eq agg-human gamma-globulin/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Eta.</td>
<td>IgG* 16</td>
<td>IgM* 4</td>
<td>37</td>
<td>13</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Mib.</td>
<td>40</td>
<td>320</td>
<td>56</td>
<td>14</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Mor.</td>
<td>40</td>
<td>320</td>
<td>44</td>
<td>31</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Nzu.</td>
<td>160</td>
<td>40</td>
<td>55</td>
<td>9</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Liz.</td>
<td>1,220</td>
<td>80</td>
<td>40</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>II</td>
<td>Gra.</td>
<td>40</td>
<td>640</td>
<td>42</td>
<td>13</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Mab.</td>
<td>40</td>
<td>320</td>
<td>19</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Mor.</td>
<td>160</td>
<td>320</td>
<td>23</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Nsi.</td>
<td>80</td>
<td>320</td>
<td>33</td>
<td>16</td>
<td>68</td>
</tr>
<tr>
<td>III</td>
<td>Abo.</td>
<td>20</td>
<td>80</td>
<td>42</td>
<td>14</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Tia.</td>
<td>20</td>
<td>80</td>
<td>39</td>
<td>18</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Mpa.</td>
<td>40</td>
<td>160</td>
<td>48</td>
<td>20</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Oke.</td>
<td>160</td>
<td>160</td>
<td>24</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Ngo.</td>
<td>320</td>
<td>160</td>
<td>38</td>
<td>18</td>
<td>59</td>
</tr>
</tbody>
</table>

* Reciprocal value of the last positive dilution, by indirect immunofluorescence using anti-IgG or anti-IgM conjugate.

CSF values was seen in patients with a definite involvement of the central nervous system (stage III) as compared with earlier stages (I and II) of sleeping sickness (C1qBA, \( P < 0.005 \); KgBA, \( P < 0.05 \)) (Fig. 5). The same CSF samples were analyzed for the protein content, the levels of IgG, IgM, albumin and C3, and for the serum protein pattern by immunoelectrophoresis. CSF samples with detectable trypanosomes (stage II or III) contained more proteins than normal CSF. There was a modest increase in the albumin concentration but IgG (1–49, median = 16 mg/dl) and IgM (0–25, median = 8 mg/dl) levels were strikingly elevated. In two of three CSF samples analyzed after sucrose density ultracentrifugation, a peak of heavy IgG (>20S) was identified (Fig. 6). Small amounts of C3 (0–1.4 mg/dl) were also detected in CSF, particularly at stage III of the disease (8/10 samples), whereas C3 was undetectable in control CSF samples. There was a significant correlation between CSF-IgG and CSF-albumin levels (\( r = 0.80, P < 0.001 \)) but not between CSF-IgM and CSF-albumin levels (\( r = 0.11, P = 0.30 \)).

![Figure 5](image-url)  
**Figure 5** Immune complexes were detected by the C1qBA (percent precipitable) and KgBA (micrograms equivalent human gamma-globulin per milliliter) in cerebrospinal fluid from patients with African trypanosomiasis. Comparison between patients at stage I or II (no cerebral manifestations) with those at stage III (meningo-encephalitis).

![Figure 6](image-url)  
**Figure 6** Sucrose density gradient fractionation of spinal fluid of one patient with African trypanosomiasis. The IgG and IgM concentrations in each fraction are indicated by interrupted and continuous lines, respectively. Fraction 8 corresponds to the top of the tubes.
**TABLE II**

Blood-Brain Barrier Function and Intracerebral IgG Synthesis in African Trypanosomiasis

<table>
<thead>
<tr>
<th>Patients</th>
<th>Stage</th>
<th>Qalb × 10⁻³&lt;sup&gt;**&lt;/sup&gt;</th>
<th>Impairment of CSF/blood barrier&lt;sup&gt;1&lt;/sup&gt;</th>
<th>QIgG × 10⁻³&lt;sup&gt;**&lt;/sup&gt;</th>
<th>QIgM × 10⁻³&lt;sup&gt;**&lt;/sup&gt;</th>
<th>IgG index&lt;sup&gt;§&lt;/sup&gt;</th>
<th>IgG synthesized in CNS mg/100 ml CSF&lt;sup&gt;**&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kiv.</td>
<td>I</td>
<td>3.00</td>
<td>None</td>
<td>0.29</td>
<td>0.1</td>
<td>0.1</td>
<td>-2.89</td>
</tr>
<tr>
<td>Eta.</td>
<td>I</td>
<td>3.27</td>
<td>None</td>
<td>0.27</td>
<td>0.1</td>
<td>0.08</td>
<td>-7.20</td>
</tr>
<tr>
<td>Ngu.</td>
<td>I</td>
<td>4.07</td>
<td>None</td>
<td>2.08</td>
<td>0.1</td>
<td>0.51</td>
<td>-6.34</td>
</tr>
<tr>
<td>Nsi.</td>
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<td>3.62</td>
<td>None</td>
<td>1.22</td>
<td>32.05</td>
<td>0.34</td>
<td>-3.91</td>
</tr>
<tr>
<td>Mak.</td>
<td>II</td>
<td>9.51</td>
<td>Slight</td>
<td>5.51</td>
<td>1.38</td>
<td>0.58</td>
<td>+1.36</td>
</tr>
<tr>
<td>Mab.</td>
<td>II</td>
<td>12.89</td>
<td>Slight</td>
<td>18.75</td>
<td>83.93</td>
<td>1.45</td>
<td>+28.11</td>
</tr>
<tr>
<td>Tia.</td>
<td>III</td>
<td>4.47</td>
<td>None</td>
<td>10.00</td>
<td>0.55</td>
<td>2.23</td>
<td>+27.49</td>
</tr>
<tr>
<td>Bus.</td>
<td>III</td>
<td>5.48</td>
<td>None</td>
<td>10.66</td>
<td>68.18</td>
<td>1.94</td>
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<tr>
<td>Bak.</td>
<td>III</td>
<td>5.65</td>
<td>None</td>
<td>6.17</td>
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<td>1.09</td>
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</tr>
<tr>
<td>Luz.</td>
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<td>6.86</td>
<td>None</td>
<td>5.68</td>
<td>20.69</td>
<td>0.83</td>
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<tr>
<td>Mpa.</td>
<td>III</td>
<td>7.18</td>
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<td>5.26</td>
<td>0.80</td>
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<tr>
<td>Bul.</td>
<td>III</td>
<td>8.40</td>
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<td>7.89</td>
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<tr>
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<td>3.12</td>
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<td>0.40</td>
<td>14.00</td>
<td>0.04</td>
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</tr>
<tr>
<td>Ndu.</td>
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</tr>
<tr>
<td>Min.</td>
<td>III</td>
<td>55.94</td>
<td>Severe</td>
<td>60.45</td>
<td>593.33</td>
<td>1.08</td>
<td>+50.28</td>
</tr>
<tr>
<td>Other hospitalized African patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bos.</td>
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<td>3.9</td>
<td>None</td>
<td>1.5</td>
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<td>0.38</td>
<td>-1.13</td>
</tr>
<tr>
<td>Mua.</td>
<td></td>
<td>7.0</td>
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<td>3.7</td>
<td>&lt;0.1</td>
<td>0.53</td>
<td>-4.36</td>
</tr>
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<td>Mag.</td>
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<td>3.7</td>
<td>None</td>
<td>1.6</td>
<td>&lt;0.1</td>
<td>0.43</td>
<td>-1.15</td>
</tr>
<tr>
<td>Ndi.</td>
<td></td>
<td>2.8</td>
<td>None</td>
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<td>&lt;0.1</td>
<td>0.50</td>
<td>-1.08</td>
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<tr>
<td>Mor.</td>
<td></td>
<td>2.7</td>
<td>None</td>
<td>1.1</td>
<td>&lt;0.1</td>
<td>0.41</td>
<td>-2.22</td>
</tr>
<tr>
<td>Kon.</td>
<td></td>
<td>2.5</td>
<td>None</td>
<td>1.3</td>
<td>&lt;0.1</td>
<td>0.52</td>
<td>-1.47</td>
</tr>
<tr>
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<td></td>
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<td>&lt;0.1</td>
<td>0.68</td>
<td>-0.45</td>
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<tr>
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<td>3.2</td>
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<td>4.2</td>
<td>&lt;0.1</td>
<td>1.31</td>
<td>+1.97</td>
</tr>
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<td>2.0</td>
<td>&lt;0.1</td>
<td>0.69</td>
<td>-0.11</td>
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<tr>
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<td>1.7–2.6</td>
<td>&lt;0.1</td>
<td>0.47–0.58</td>
<td>-1.98–0.66</td>
</tr>
</tbody>
</table>

Alb, albumin.

* Qalb, CSF/albumin; QIgG, CSF/IgG/IgG; QIgM, CSF/IgM/IgM.

1 According to (13), no impairment, Qalb = 7.2 × 10⁻³; slight impairment, Qalb = 7.7 – 14 × 10⁻³; moderate impairment, Qalb = 14 – 33 × 10⁻³; severe impairment, Qalb = 33 – 100 × 10⁻³. Normal values vary from 3.6±1.7 × 10⁻³ (at 17–30 yr) to 4.6±1.3 × 10⁻³ (at 41–50 yr).

§ IgG index, QIgG/Qalb.

<sup>**</sup> Concentration of CSF-IgG synthesized within the CNS. Calculated according to Tourtellotte’s formula:

\[
\text{IgG}_{\text{CSF}} = \left( \frac{\text{IgG serum}}{369} \right) - \left( \left( \frac{\text{albumin}_{\text{CSF}} - \text{albumin}_{\text{serum}}}{230} \right) \times \frac{\text{IgG serum}}{\text{albumin}_{\text{serum}}} \times 0.43 \right)
\]

= locally synthesized IgG/100 ml of CSF.
with trypanosomiasis, no or only slight impairment of the CSF blood barrier state was observed (Table II).

In contrast to the relatively moderate changes in the blood/CSF permeability for albumin, high CSF/serum ratios were observed for IgG (Q1gG) and/or IgM (Q1gM) in all patients at stage II and III (Table II). Low molecular weight (7S) IgM was investigated by immunochromatographic analysis of sucrose density gradient fractions in three CSF samples with a high IgM content. In all three, there was a spreading of IgM in 7 to 19S fractions; in one of them, two IgM peaks were identified in 7 and 19S positions, whereas the corresponding serum samples did not contain 7S IgM. These results were consistent with an intracerebral synthesis of IgG and IgM, which was further suggested by the analysis of the Q1gG/Qalb ratios, or “IgG index” according to Tibbling et al. (11) (Fig. 7). Indeed, this ratio, which does not vary with age in normal individuals (11), was markedly increased in 10/13 patients at stage II or III (Table II). In the remaining two patients, a high CSF/serum IgM ratio was observed. The concentration of locally synthesized IgG in CSF was calculated using Tournoulet’s formula (13). These values were in the normal range in the CSF samples collected at stage I, but were elevated in 10/13 CSF samples collected from patients at stage II or III. The 3/13 negative samples displayed an elevation of IgM and Q1gM suggestive of a local IgM synthesis. Correlations between, on one hand, the levels of circulating IC, detected by the ClqBA and the KgBA and, on the other hand, the levels of IC in CSF, were not significant (Fig. 8). Several CSF samples were more positive than the corresponding serum samples (Fig. 8). The correlation between the level of IC in CSF and the Qalb ratio was not significant but there was a correlation of CSF-C1qBA with the IgG index ($r = 0.50$, $P < 0.025$).

In the nine CSF samples from other African hospitalized patients studied, the IgG level was low (median 2.6 mg/dl) and there was no detectable IgM. There was no indication of an increased intracerebral IgG synthesis.

Evolution of circulating IC and CSF-IC during therapy. Eight patients were followed during the trypanocidal therapy. In three patients, there was a marked decrease of serum IgM (25 to 10; 20 to 0.3; 22 to 13 mg/ml) and of ClqBA (47 to 14; 40 to 2; 38 to 10%) during the first 3 wk, but only small changes were seen in the others. A significant decrease of the serum IgG level was seen in only one of these eight patients. In one patient with encephalitis, who had already suffered from three relapses of trypanosomiasis during the previous 2 yr, several samples of CSF and serum were collected serially. Although the ClqBA and KgBA decreased to normal values after 30 d, C1qBA remained elevated in CSF 18 mo after this course of treatment.

**FIGURE 8** Correlation analysis between the levels of immune complexes in serum and in corresponding CSF samples. C1qBA values are expressed in percent Clq precipitated; KgBA values in micrograms equivalent human gamma globulin per milliliter.

**DISCUSSION**

This study demonstrates the presence of circulating immune complexes in patients infected with trypanosomes. It confirms experimental data obtained in mice (5, 15) and preliminary human studies (16). The IC-like material that appears in most patients from the early stage of infection, can be detected using two different techniques with a significant correlation between the two sets of results. These two techniques, which were based on the reactivity of IC with C1q or of C3-coated IC with conglutinin, had previously been shown to detect a wide range of IC sizes (8). These data suggest that this material represents in vivo formed IC and this hypothesis is further supported by the significant negative correlation between ClqBA

**FIGURE 7** Graphical representation of the evaluation of blood-brain barrier function by CSF/serum albumin ratio (Qalb) and of IgG synthesis in CNS by CSF-IgG index (Q1gG/Qalb). The hatched area shows the normal limits (11). For symbols, see legend to Fig. 4.

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and levels of C3 in serum. The persistence of circulating IC during the entire course of the disease, even at stages when there is a very low parasitemia, suggests that parasitic antigens do not represent a main component of the IC. Besides, high levels of serum immunoglobulins were found in the patients studied. Recently, we demonstrated a variety of antibodies against hapten, hemocyanin, ovalbumin, DNA, and sheep erythrocytes in serum from these patients, suggesting an extensive expression of the B cell repertoire including the occurrence of anti-IgG antibodies similar to rheumatoid factor (manuscript in preparation). The highly significant correlation between the ClqBA and the level of RF would be consistent with an involvement of RF in the generation of circulating IC in this disease. This would be similar to the situation observed in mice undergoing a polyclonal antibody synthesis after injection of bacterial lipopolysaccharide. Indeed, it was found recently that in mice injected with bacterial lipopolysaccharide, there is an induction of circulating IC closely correlated with the occurrence of anti-immunoglobulin antibodies. Therefore, it is possible that IC appearing in trypanosomiasis are part of the expression of a polyclonal B cell activation.

A localization of IC in the CNS has been suggested by the demonstration of electron-dense deposits in the basement membrane of choroid plexus in patients with systemic lupus erythematosus and in NZBxNZW mice (17). In addition, deposits of IgG, bovine serum albumin, and C3 were shown in the choroid plexus of rabbits undergoing an acute IC disease (18). The latter model was associated with a slight alteration in the blood CSF barrier permeability, but IC were usually not detectable in the CSF. In experimental trypanosomiasis, a progressive involvement of the CNS was found parallel to a progressive appearance of electron-dense deposits in the endothelial and in the subependymal layers of the choroid plexus basement membrane (15). Our data demonstrate the presence of IC in CSF during the encephalitis stage of trypanosomiasis in man. We do not think that the occurrence of IC in CSF primarily results from a diffusion from serum to CSF through the blood brain barrier. First, IC are detectable in serum in large amounts in the few patients studied at the early stage of infection, without a concomitant appearance of IC in CSF. Secondly, at later stages, there is no correlation between the level of circulating IC and that of CSF-IC. In fact, most patients appear to have a normal or only slightly altered blood-brain barrier, which is probably efficient in preventing an appreciable crossing of IC from serum to CSF. However, one cannot entirely exclude the possibility that an active equilibrium may be involved in the observed picture of a blood-brain barrier for IC.

The appearance of IC in CSF at the encephalitic stage of African trypanosomiasis was closely associated with increased IgG and/or IgM levels in CSF. There was a dissociation between a moderate increase of the CSF/serum albumin ratio and high values of the CSF/serum IgG or IgM ratios. This leads to high values of "IgG index" which indicate that the majority of these immunoglobulins is synthesized within the CNS compartment (11). The quantitation of locally synthesized IgG in CSF was carried out using Tourtellotte’s formula (13), which can be used when the blood-brain barrier is not too altered (CSF/serum albumin ratios lower than 10 × 10⁻³) (19). This is the case in 14/16 of our patients (Table I). The elevation of intracerebral IgG synthesis is comparable with that observed in patients with subacute sclerosing panencephalitis and many reflect a similar reaction to the infectious process (19). This increased IgG synthesis, which is concomitant with the occurrence of trypanosomes in CSF, may reflect an immune response to trypanosome antigens, as suggested by the relatively high levels of anti-trypanosome antibodies in CSF, or to some autoantigens. It may also depend on an oligoclonal or polyclonal B cell activation in the CNS compartment.

Therefore, IC appearing in CSF are probably formed locally and may involve trypanosome antigens or autoantigens such as IgG or CNS antigens. A local formation of IC would be consistent with the observed correlation between the level of Clq-BA and the level of the IgG index. One should note that IC have also been demonstrated in CSF in patients with subacute sclerosing panencephalitis or multiple sclerosis (20).

The detection of IC in CSF during trypanosomiasis appears as a potentially useful marker for the early diagnosis of cerebral involvement in this disease and for the persistence of CNS infection during therapy. The significance of these results should also be considered in relation to the evaluation of the CNS involvement in systemic lupus erythematosus and other IC diseases.

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