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MONOCLONAL IMMUNOGLOBULINS IN CONGENITAL TOXOPLASMOSIS

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SUMMARY

Monoclonal immunoglobulins in serum and cerebrospinal fluid (CSF) were found in newborns with congenital toxoplasmosis. The M-components were of IgG-class and of both κ and λ type. The monoclonal proteins were found in the serum of newborns but not in the serum of their mothers. The monoclonal immunoglobulins were therefore selectively transferred or synthesized by the newborn. There was a local production or selective local accumulation of immunoglobulins in the cerebrospinal fluid. The M-components disappeared and the IgM level in serum and cerebrospinal fluid decreased after therapy. IgA was found to be elevated between 2–4 months of age. CRP was elevated in the first weeks after birth but afterwards returned to normal. The Dye test localized antibody activity to the site of the M-components in the electrophoresis of both serum and cerebrospinal fluid. The Dye test antibodies of mothers' sera also showed restricted heterogeneity with about the same electrophoretic localization as in the children's sera. Rheumatoid factors were found in serum and CSF of newborns with congenital toxoplasmosis, but not in serum of their mothers.

INTRODUCTION

Monoclonal immunoglobulins occur in patients with multiple myeloma and Waldenström's macroglobulinaemia. They have also sometimes been detected in other disorders such as malignant lymphoma (Laurell, Laurell & Waldenström, 1957), epithelioma (Osserman, 1958), collagen disease (Laurell, Laurell & Waldenström, 1957), liver disease (Osserman, 1958), sarcoma, malaria (Hällén, 1966) and chronic infections (Kappeler *et al.*, 1961, Riva, 1964). They are also seen in apparently healthy adults (Waldenström, 1961) and in increasing frequency with age (Axelsson, Bachmann & Hällén 1966, Hällén, 1963). Raised serum levels of IgM, and sometimes of IgA on the basis of a polyclonal increase of the immunoglobulins are found in congenital infections (Remington & Miller, 1966, Soothill, Hayes & Dudgeon,

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1966, Stiehm, Amman & Cherry, 1966). This paper describes monoclonal immunoglobulins in newborns with congenital toxoplasmosis. The M-components were characterized, the immunoglobulins and CRP of serum and cerebrospinal fluid quantitated in repeated samples, the CSF/serum immunoglobulin quotients determined, the Dye test activity localized in the electrophoretic pattern of sera and CSF and rheumatoid factors investigated in sera and CSF.

MATERIAL AND METHODS

Serum samples were obtained at intervals from four newborns with congenital toxoplasmosis and from three of their mothers. In one (A.N.) of these children CSF was sampled on various occasions. The samples were kept at −20°C until analysed. A normal serum pool of 400 adult blood donors was used as reference.

Anti-human IgG. Cohn Fraction II (Kabi 1064 γ-globulin RbG was further purified by DEAE-cellulose chromatography (Peterson & Sober, 1956) and used for immunization of rabbits. The antisera were pooled and absorbed with light chains isolated from reduced and alkylated normal IgG. The specificity of the antiserum was checked by immunelectrophoresis and Ouchterlony gel diffusion.

Specific rabbit anti-human IgA, IgM, κ chains, λ chains and α_2-macroglobulin, respectively, were obtained from Professor C.-B. Laurell, General Hospital, Malmö, Sweden.

Anti-human albumin. Rabbits were immunized with purified albumin (Kabi, Stockholm, Sweden) in Freund's complete adjuvant. The antisera obtained were specific, as judged from testing in immunelectrophoresis and Ouchterlony gel diffusion.

Anti-human CRP. C-reactive protein was purified according to the description of Hokama & Riley (1963). The preparation with Freund's complete adjuvant was used for immunization of rabbits. The antiserum was absorbed with normal serum and was specific when tested on immunoelectrophoresis and Ouchterlony gel diffusion.

Quantitation of specific proteins was performed using the single diffusion in tube technique of Oudin (1952), as modified by Bachmann & Laurell (1965). For small serum samples electrophoresis in agarose gel containing antibody (Laurell, 1966) (IgG, IgA, light chains of type κ and λ and C-reactive protein) and single radial immunodiffusion (Mancini, Carbonara & Heremans (1965) (IgM) were used.

Agarose electrophoresis was performed in 0·6% agarose (Miles-Seravac Pty Ltd, Maidenhead, Berks, England), barbital buffer pH 8·6 with 0·05 M Ca-lactate and 10 V per cm (Laurell, 1965).

Preparative electrophoresis in agarose 0·6%. A 0·7-cm thick block of 0·6% agarose was used and electrophoresis performed in barbital buffer of pH 8·6 and 10 V per cm. The sample was mixed with agarose to the same concentration (0·6%). Agarose slices (0·5 cm) with the separated proteins were cut and frozen at −80°C over night. The fractions were afterwards thawed and the protein solution thereby released.

Immunoelectrophoresis was performed according to Scheidegger (1955) in 0·6% agarose.

Crossed antigen–antibody electrophoresis was performed as described by Laurell (1965).

Double diffusion in gel was according to Ouchterlony (1958).

Total protein concentration of cerebrospinal fluid was determined by the Folin-Ciocalteu method (Lowry *et al.*, 1951).

Reduction and alkylation of IgG was according to Fleichman, Pain & Porter (1962).

Latex fixation test of human fraction II (*Hu F II*) was according to Singer & Plotz (1956). Rapid slide (Hyland Division, Travenol Laboratories S.A., Brussels, Belgium).

Sensitized sheep cell (*SSC*) *agglutination test* according to Waaler (1940) and Rose *et al.* (1948) was adapted to a micro-titre system.

Dye test (*Toxoplasma gondii*) according to Sabin & Feldman (1948) was performed in the laboratory of Dr Siim, Statens Seruminstitut, Copenhagen, Denmark.

CASE REPORTS

The patient A.N. a girl born on 6 April, 1969, was the second child of an apparently healthy mother. The child was born 6 weeks before term. Birth weight 2260 g. Delivery was uncomplicated. On the third day of life the infant developed severe jaundice and was referred to the Department of Pediatrics, University Hospital, Lund, for exchange transfusion. Physical examination revealed hepatosplenomegaly. The circumference of the head was pathologically increased.

Laboratory findings. Serum bilirubin 27·4 mg/100 ml. Haemoglobin 13·1 g/100 ml. Red blood cell count 4·7 million/mm^3. Leucocytes 3200/mm^3 with 2% band formed neutrophils, 22% segmented neutrophils, 17% eosinophils, 1% basophils, 45% lymphocytes, 8% monocytes and 2% myelocytes. Total protein concentration of the cerebrospinal fluid 860 mg/100 ml. Cell counts in the cerebrospinal fluid 566 per 10 mm^3. Dye test titres (Sabin & Feldman, 1948) of serum and cerebrospinal fluid were 1/6250 and 1/1250, respectively. Titres of complement fixing antibodies to *Toxoplasma gondii* were 1/30 for serum and 1/15 for cerebrospinal fluid. The mother's serum showed a Dye test titre of 1/6250 and complement fixing antibody titre to *Toxoplasma gondii* of 1/60. A bone marrow smear from the child was normal.

Encephalography showed stenosis of the aqueduct, and ventriculography demonstrated severely dilated ventricles and porencephaly frontally. Examination of the eyes revealed extensive changes of the type seen in congenital toxoplasmosis. Blood exchange transfusion was performed three times, on 19, 20 and 21 April, 1969. Treatment with pyrimethamine and sulphonamides was started on May 3. On June 13 the patient was operated upon with insertion of a Spitz-Holter shunt because of her hydrocephalus.

Another three newborns with congenital toxoplasmosis from the Children's Hospital of Gothenburg, Sweden, were also investigated. Sera were kindly supplied by Dr Ragnar Norrby, Institute of Medical Microbiology, University of Gothenburg, Sweden.

The patient C.J. a girl born on 21 April, 1968, 4 weeks before term, was the first child of an apparently healthy mother. Birth weight 2420 g. Length 47.5 cm. Delivery was uncomplicated. The patient was found to be microcephalic with head circumference of 30 cm and with skin bleedings, jaundice, hepatomegaly, splenomegaly and bad reflexes.

Laboratory findings. Serum bilirubin 17·1 mg/100 ml. Haemoglobin 13·6 g/100 ml. Red blood cell count 4·7 million/mm^3. Thrombocytes 35,000/mm^3. Leucocytes 13,200/mm^3 with 9% band shaped neutrophils, 25·5% segmented neutrophils, 2% eosiniphils, 0·5% basophils, 46·5% lymphocytes, 3% monocytes, 6·5% myelocytes, 3% metamyelocytes, 1% plasma cells and 3% blast forms. Total protein concentration of the cerebrospinal fluid 1000 mg/100 ml. Cell counts in the cerebrospinal fluid 34/mm^3. Dye test according to Sabin-Feldman showed a serum titre $>1/250$. After separation on Sephadex G-200 the 19S peak Dye test was negative and 7S peak Dye test titre was 1/2000. The test titre of serum of the mother

was $\geqslant 1/8000$ and complement fixing antibody titre of serum from the mother was 1/16. X-ray examination of the skull showed intracranial calcifications. Examination of the eyes revealed microphthalmus and a retrolenticular mass in the right eye. X-ray of the chest showed enlargement of the heart. Blood exchange transfusion was performed on 21 April, 1968. On the third day of life the patient had convulsions and blood glucose was measured 10 mg/100 ml. Blood transfusion was given on 30 April because of anaemia. Haemoglobin decreased to 4·5 g/100 ml. The patient died on the 17th day of life. Mice inoculated with autopsy material from the lung, brain and spleen showed a significant increase in the Dye test (Sabin & Feldman) titre.

The patient D.K. was a boy born on 24 June, 1968 and the second child of a 22-year old mother who had a vaginal bleeding in the 4th month of gestation and a bad cold in the 6–7th month of gestation. The patient was born 3 weeks before term. The cord was wound twice around the neck. The patient was pale and hyptonic at the time of delivery. Apgar score 1. Oxygen treatment was used. Birth weight 2550 g. Length 59 cm. Circumference of the head 37 cm. Separated cranial sutures. Bulging fontanelles. Hyperreflexibility, hypertonicity and sun set phenomenon were noted. The patient had convulsions within the first hours of life.

Laboratory findings. Haemoglobin 16·8 g/100 ml. Leucocytes 15,400/mm^3 with 12% band neutrophils and 46·5% segmented neutrophils, 1·5% eosinophils, 36% lymphocytes, 2·5% monocytes, 1·5% myelocytes.

The Dye test according to Sabin-Feldman gave a titre of 1/4000 and complement fixing antibodies to a titre of 1/64 in the serum of the child. A Dye test titre of 1/8000 and complement fixing antibody titre of 1/128 were obtained using the serum of the mother. X-ray examination of the skull revealed intracranial calcifications. Eye examination: changes in fundi.

Treatment with sulphonamides was given from 28 June to 25 July, 1968 and pyrimethamine from 28 June to 10 July, 1968. After treatment the patient developed microcephalus instead of hydrocephalus. Two months later decreases were noted in the titres of the Dye test (1/2000) and of the complement fixing antibodies (1/8).

The patient T.N. a boy born on 25 July, 1967, and probably 5 weeks before term, was the first child of a 17-year old mother. Birth weight 2/070 g. Length 43 cm. Delivery was uncomplicated. The child had cutaneous haemorrhages, but otherwise the neonatal period was uncomplicated.

Laboratory findings. Haemoglobin 21·3 g/100 ml. Thrombocytes 56,000/mm^3. Leucocytes 9,000/mm^3 with 3% band neutrophils and 7·5% segmented neutrophils, 0·5% eosinophils, 70% lymphocytes, 17% monocytes, 1% myelocytes and 1% metamyelocytes. Sabin & Feldman's Dye test of serum from the child gave a titre of 1/4000 and serum from the mother gave a titre 1/8000. Antibody was distributed approximately as follows: 1/3 19S and 2/3 7S in the child and 1/5 19S and 4/5 7S in the mother. Complement fixing antibodies to *Toxoplasma gondii* were detected in the serum from the child to a titre 1/32 and in the serum from the mother to a titre of 1/64.

X-ray examination of the skull revealed intracranial calcifications. Eye examination: microphthalmia, cataract and extensive choroiditis.

Treatment with sulphonamides from 19 October to 22 November, 1967 and pyrimethamine from 26 October to 22 November, 1967. Follow-up examination of the patient showed that he could walk at 25 months of age, he was blind, deaf and mentally retarded.

RESULTS

Characterization of monoclonal immunoglobulins

Electrophoresis of the first serum samples of patient A.N. revealed the presence of two narrow bands in the slow γ-region (Fig. 1). The concentration of the two monoclonal bands taken together was about 0·2 g/100 ml. The most cathodal band was typed by crossed antigen–antibody electrophoresis as IgG kappa (in γ_4-position) and the more anodal band was typed as IgG lambda (in γ_3-position) (Fig. 2). Six weeks after the beginning of treatment with pyrimethamine and sulphonamides, the M-components of serum of patient A.N. were no longer demonstrable.

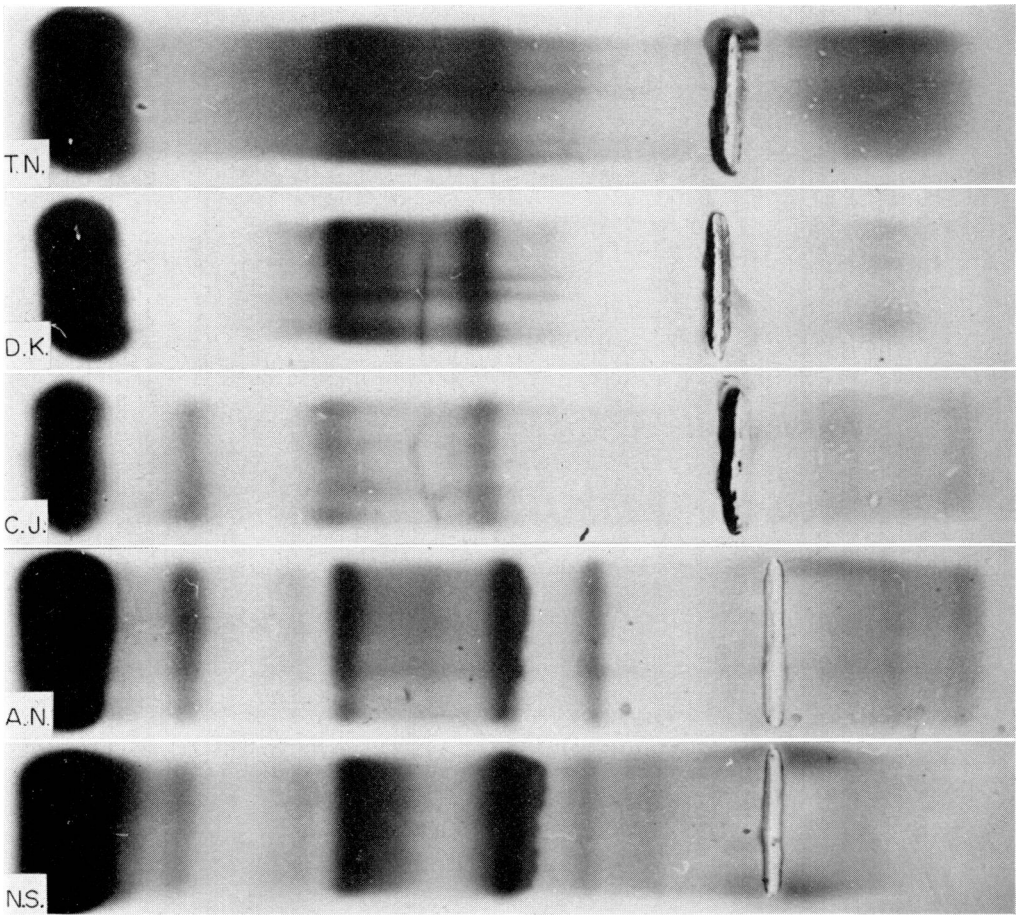

FIG. 1. Electrophoresis in agarose of sera from newborns with congenital toxoplasmosis and normal serum (NS) as reference.

Agarose gel electrophoresis of the mother's (A.N.) serum revealed no M-components corresponding to those of the child. This was also shown with the crossed antigen–antibody electrophoresis (Fig. 2) comparing the child's serum with the mother's serum.

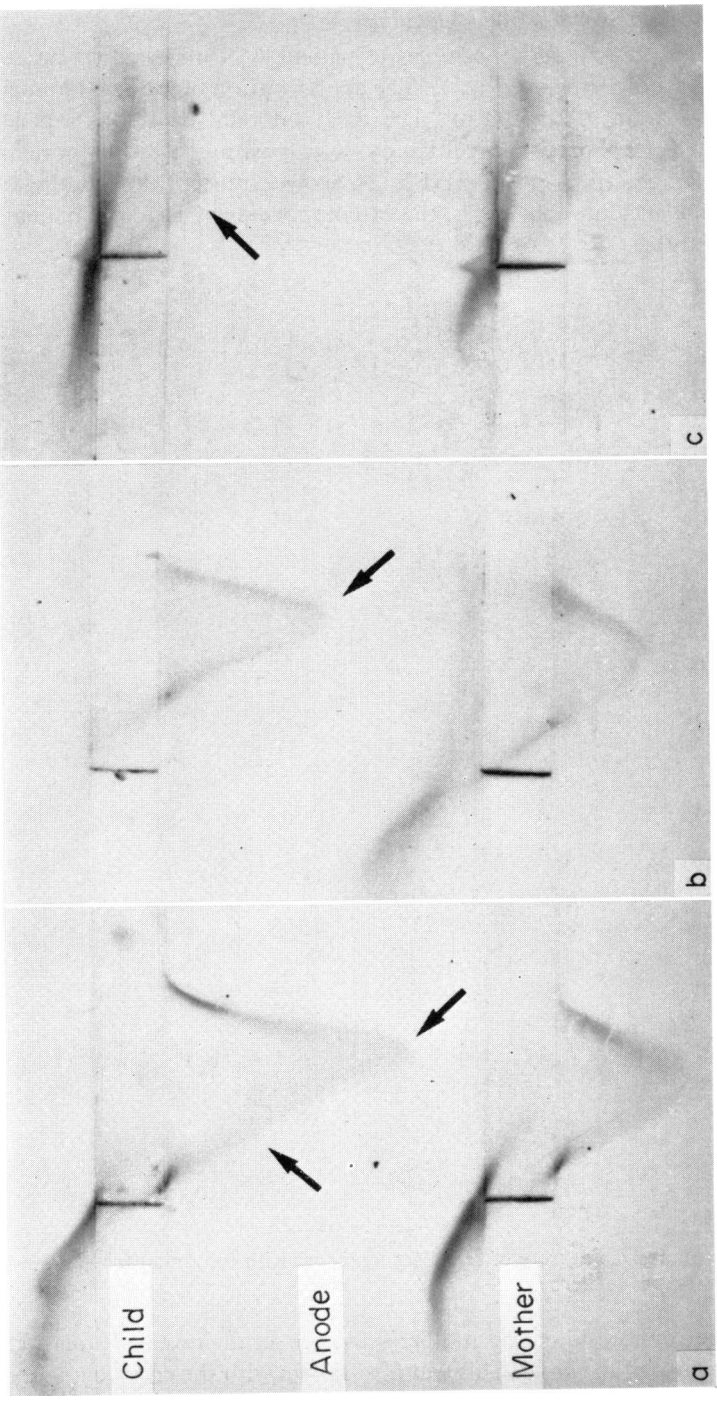

FIG. 2. Antigen–antibody crossed electrophoresis of (a) serum-IgG, (b) -kappa- and (c) -lambda-light chains, respectively, of child and mother A.N. M-components are indicated by arrows.

Cerebrospinal fluid of patient A.N. demonstrated on crossed antigen–antibody electrophoresis one IgG-κ peak corresponding to the serum γ_4-peak. In the γ_3-position IgG-λ precipitation bands were seen on crossed antigen–antibody electrophoresis and immunoelectrophoresis. There was a deficiency of anodal IgG. Investigation of five times concentrated

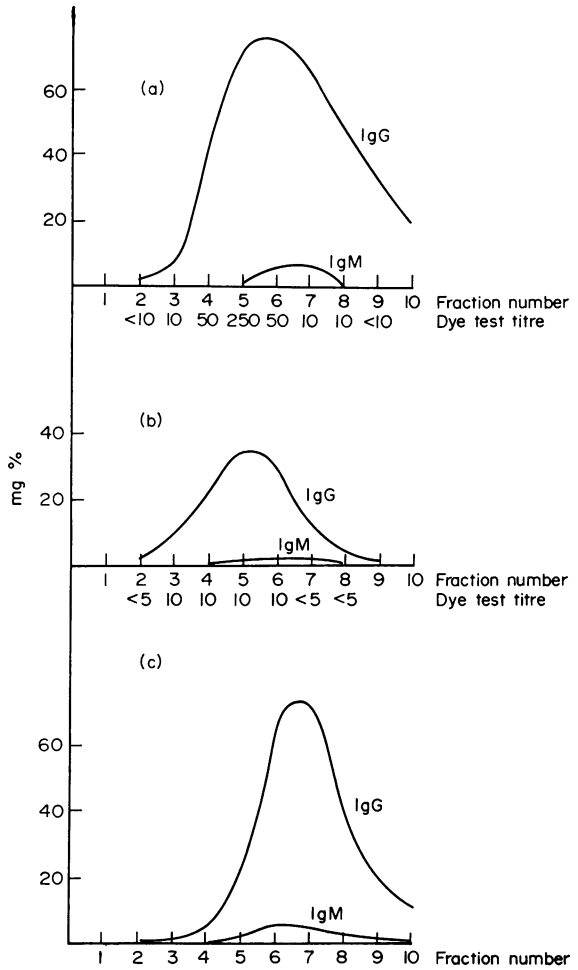

FIG. 3. Content of IgG, IgM and Dye test (Sabin & Feldman, 1948) titre of fractions of sera from preparative agarose electrophoresis. Fraction 1 nearest the cathode. Sera of the patients (a) A.N. and (b) C.J. and (c) normal serum (pool of 400 blood donors) were run in the same way. Note the displacement of IgG to the cathode in the patients' sera.

CSF (collodion bags) by quantitation of IgG in fractions from preparative electrophoresis in agarose (performed in the same way as the sera in Fig. 3) proved that IgG was confined to fractions 4–6 (fractions 3 and 7, <1 and <8 mg/100 ml, respectively).

Patient C.J. and D.K. also showed sharply outlined bands in the slow γ-region of agarose electrophoresis (Fig. 1). The concentration of the M-components was about 0·2 g/100 ml. The most cathodal bands were typed as IgG-κ (in the γ_4-position) of both sera. Patient C.J.

also showed an IgG-λ band in the γ_3-position. The anodal γ-globulin was deficient in both sera. Patient C.J. died on the 15th day of life. In the last serum sample the IgG level had decreased but the M-component was still distinct and unchanged on agarose electrophoresis and crossed antigen–antibody electrophoresis.

TABLE 1. Quantitative measurements, by the Oudin technique, of the serum immunoglobulins in A.N. Blood exchange transfusion was performed on 19, 20 and 21 April after the first sample had been obtained. Treatment with pyrimethamine and sulphonamides started on 3 May

Serum sample number	Date	mg/100 ml IgG	IgA	IgM
S1	19/4–69	798	48	52
S2	26/4–69	N.D.	30	72
S3	5/5–69	1008	10	79
S4	9/5–69	998	12	43
S5	19/5–69	1019	14	36
S6	24/6–69	662	22	17
S7	24/7–69	504	40	26

N.D. = not done.

TABLE 2. Quantitative measurements, by the Oudin technique, of the immunoglobulins of consecutive samples of cerebrospinal fluid from the patient A.N.

CSF number	Date	mg/100 ml IgG	IgM	IgM	Total protein
CSF1	21/4–69	231	2	7	N.D.
CSF2	2/5–69	252	2	8	600
CSF3	5/5–69	79	2	15	700
CSF4	7/6–69	22	0·2	1	N.D.
CSF5	9/7–69	9	<0·2	<0·6	125

N.D. = not done.

Crossed antigen–antibody electrophoresis of serum from patient T.N. with congenital toxoplasmosis showed abnormally high concentrations of cathodically located IgG-κ and IgG-λ proteins. A broad diffuse increase in the slow γ-region was noted on electrophoresis but there were no distinct bands (Fig. 1). Serum from one patient, 18-day old, with congenital rubella, showed γ-globulin with normal electrophoretic dispersion as did normal cord sera. Sera from jaundiced newborns after blood exchange transfusion also showed a diffuse normal

dispersion of IgG without distinct bands in the electrophoretic pattern. The electrophoretic dispersion of γ-globulin from the mothers of the patients D.K. and T.N. was normal.

Quantitation of IgG, IgA, IgM and CRP

Serum IgM and serum IgA were increased as judged from the values of Berg (1969) in the sample of patient A.N. obtained before blood exchange transfusion on the third day of life (Table 1, S1). In patients T.N. and D.K. in whom no blood exchange transfusion was performed, serum IgM were elevated in the first serum samples obtained at one and 4 weeks of age, respectively. IgA was elevated in serum samples taken between 2–4 months of age of all three living patients (Tables 1, 3) (Berg, 1969). After the beginning of treatment of patient A.N. the serum IgM decreased (Table 1, S3–S7, Table 3). The serum IgG of the newborns with congenital toxoplasmosis was within the normal range except in patient C.J. where the IgG level was low. The serum immunoglobulin levels of the mothers were within the normal range.

TABLE 3. Quantitative measurements by the Laurell technique (IgG, IgA and CRP) and the Mancini technique (IgM) of serum immunoglobulins and CRP of patients with congenital toxoplasmosis

Patient birth date	Age	mg% IgG	IgA	IgM	CRP	Monoclonal immunoglobulins
C.J.*	9 days	630	<0·5	146	2·7§	IgG-K
68 04 21	15 days	473	<0·5	154	4·5	IgG-L
D.K.†	1 day	N.D.	<0·5	N.D.	2·3	IgG-K
68 06 24	1 mth	735	23·8	154	<0·5	
	2 mths	683	69·3	116	<0·5	
T.N.‡	8 days	1418	<0·5	108	1·1	
67 07 25	3 mths	714	47·5	65	<0·5	
	4 mths	714	43·6	77	<0·5	

* Blood exchange transfusion 21 April, 1968 and blood transfusion 30 April, 1968.
† No blood transfusion. Treatment with sulphonamides 28 June–25 July, 1968 and pyrimethamine 28 June–10 July, 1968.
‡ No blood transfusion. Treatment with sulphonamides 19 October–22 November, 1967 and pyrimethamine 26 October–22 November, 1967.
§ Normal values of fifty blood donors ranged from 0·1–1·1 mg%.

C-reactive protein (CRP) in serum was initially shown elevated in all patients and was normalized within the earliest months (Table 3). CRP of CSF was not measurable by the method used.

In cerebrospinal fluid from patient A.N. the concentration of IgG, IgA and IgM were elevated in the first samples. After the beginning of treatment and operation IgG, IgA and IgM gradually decreased (Table 2) and IgA and IgM were normalized.

CSF/serum quotients

CSF/serum concentration ratios of IgG, IgA, IgM, albumin and α_2-macroglobulin were

increased (Table 4) (Schultze & Heremans, 1966). However the ratio of the IgM was higher than that of α_2-macroglobulin despite their similar molecular weights. Furthermore the albumin CSF/serum quotient was low compared with the IgG and IgA CSF/serum quotients (Table 4).

TABLE 4. Comparison of the levels of albumin, α_2-macroglobulin and the immunoglobulins, measured by the Oudin technique, in cerebrospinal fluid (CSF3) and serum (S3) of the patient A.N.

	Percentages of concentrations in a normal serum pool				
	Albumin	IgG	IgA	IgM	α_2-macroglobulin
Cerebrospinal fluid (CSF3)	5·25	7·5	1·2	19·5	5·25
Serum (S3)	65	96	5	102	115
CSF/serum quotient	0·08	0·08	0·24	0·19	0·05

TABLE 5. Rheumatoid factors of the patients under study

Sample	Sample obtained at age	Latex test	SSC test titre
A.N. (serum)	2 mths	+	<1/8
A.N. (CSF)	25 days	(+)*	1/16–1/32 positive
Mother A.N.		−	N.D.
C.J.	9 days	+	1/32 positive
D.K.	1 mth	+	1/8
Mother D.K.		−	1/8
T.N.	8 days	−	N.D.
T.N.	3 mths	−	1/8
M.P.†	12 mths	−	1/8

* Undiluted
† Patient with healed congenital toxoplasmosis.
N.D. = not done.

Location of Dye test (Sabin-Feldman) activity in the electrophoresis

Investigation of fractions from preparative electrophoresis in agarose of all samples investigated (namely serum and CSF from child A.N., serum of mother A.N. and serum of child C.J.) localized Dye test activity to the γ_3-γ_4 region.

In serum from patient A.N. Dye test antibodies were found maximally in fraction 5 (Fig. 3). In CSF from patient A.N. and serum from patient C.J. the Dye test antibodies was found only in the slow γ-region, as was the IgG. In mother A.N. maximum Dye test activity 1/50 was found in fractions 4, 5, 6, with normal distribution of IgG.

Absorption experiments

Fractions of the γ-region from a preparative agarose electrophoresis of serum from patient

A.N. were tested after absorption four times with concentrated toxoplasma antigen obtained from Dr Siim, Copenhagen), but there was no loss of IgG or Dye test activity.

Rheumatoid factor tests

Human γ-globulin (F II) Latex fixation test proved positive in 1/20 dilutions of serum from patients A.N., C.J. and D.K. and positive in undiluted cerebrospinal fluid from A.N. (Table 5). No agglutination was found in serum from T.N. or the mothers of A.N. or D.K. One patient one year of age with healed congenital toxoplasmosis showed no agglutination of human γ-globulin.

The sensitized sheep cell agglutination test was positive to a titre of 1/32 in serum from patient C.J. and in cerebrospinal fluid to a titre of 1/16–1/32 from patient A.N. Other sera were negative in the SSC-test.

DISCUSSION

The appearance of plasma cells in the human foetus in congenital toxoplasmosis and congenital syphilis provides indirect evidence of humoral antibody synthesis during intrauterine life (Silverstein & Lukes, 1962). In congenital infections, IgM and sometimes also IgA are increased in cord serum (Soothill *et al.*, 1966, Stiehm *et al.*, 1966). High IgM levels at birth have been found in congenital toxoplasmosis (Remington & Miller, 1966, Remington, Miller & Brownlee, 1968). The finding of highly increased IgM and IgA levels on the third day of life in serum from the prematurely born patient A.N. suggests that the IgM and IgA antibody synthesis were induced *in utero* by the infection. Under normal conditions only IgG molecules pass the placental barrier (Brambell, 1958) and the foetus synthesizes only small amounts of IgG (Mårtensson & Fudenberg, 1965). The level of IgG in the patients were within the normal range or low for the degree of prematurity (Berg & Nilsson, 1969). However, the electrophoretic mobility of the IgG molecules showed abnormalities with part of the IgG being of homogeneous monoclonal type. Serum samples from the patients' mothers contained no corresponding monoclonal immunoglobulins. The monoclonal immunoglobulins might therefore have been selectively accumulated by the foetus or they may have been synthesized by the foetus or newborn as IgM and IgA synthesis had started *in utero*.

Monoclonal proteins developed in the γ-region of serum from horses hyperimmunized with pneumococcal vaccines (Van der Scheer, Wyckoff & Clarke, 1940) and in New Zealand rabbits immunized with streptococcal group specific vaccines (Osterland *et al.*, 1966). A transition of polyclonal to monoclonal immunoglobulins has also been observed in minks with Aleutian disease, caused by virus (Porter, Dixon & Larsen, 1965). Studies on isolated human specific antibodies to dextran, levan, techoic acid, blood group A substance and tetanus toxoid (Mannik & Kunkel, 1963, Edelman & Kabat, 1964, Allen, Kunkel & Kabat, 1964, Yount *et al.*, 1968) have revealed restricted heterogeneity of the antibodies. The electrophoretically homogeneous immunoglobulins of the patients might reflect a similar degree of restricted antibody synthesis after stimulation by mainly one parasitic agent. Although hypergammaglobulinaemia of the diffuse polyclonal type is one of the typical features of longstanding bacterial and parasitic infections, M-components have been reported in adults with such conditions as malaria (Hällén, 1966) (for recent review see Michaux & Heremans, 1969). Monoclonal immunoglobulins were noted in serum from the children but not in serum from their mothers. The M-components might also reflect a restricted intense antigenic

stimulus *in utero*. In the patient C.J. that died from the infection all IgG was found in the γ_3-γ_4-region. M-components have also been found in one patient with congenital syphilis (Aiuti, Ungari & Serra, 1968). Otherwise M-components are extremely rare in childhood. They have been found in one patient with Swiss type agammaglobulinaemia after thymus transplantation (Harboe *et al.*, 1966) and transiently in one patient with Wiskott-Aldrich syndrome (Dalloz *et al.*, 1965) and transiently in CSF in one patient with pseudomonas meningitis (Hochwald & Thorbecke, 1964).

High immunoglobulin levels were also found in the CSF. The immunoglobulin concentration and CSF/serum ratios suggested a local production in CSF or selective local accumulation of IgG, IgA and IgM in CSF. In the CSF IgG confined to the γ_3-γ_4-region suggested a local production of IgG. After treatment with pyrimethamine and sulphonamides and operation the high immunoglobulin levels in cerebrospinal fluid became normal and the M-components disappeared. The serum IgM also decreased after treatment. As in tertiary syphilis, high IgM levels in cerebrospinal fluid has been suggested as a sign of activity of the disease (Oxelius, Rorsman & Laurell, 1969).

In malaria (Hällén, 1966), as in our patient A.N. with congenital toxoplasmosis, the serum M-components disappeared on treatment and are possibly the response to antigenic stimulation by the parasite.

CRP has been regarded as a parameter of activity of infectious diseases. All newborns with congenital toxoplasmosis investigated showed high CRP values in the neonatal period.

Dye test antibodies were found in the area of the M-components of the electrophoretically separated sera. However, it is unlikely that the M-components were exclusively Dye test antibodies. Dye test activity of about the same titre in the mother's sera was also located to the same region of the electrophoretic pattern but here no M-components were noted. Neither were we able to adsorb Dye test activity or γ-globulin to *Toxoplasma gondii* antigen. However, in the absorption experiments the amounts of Toxoplasma antigen used may have been too small.

It is known that patients with infectious and parasitic diseases may have rheumatoid factors in their sera. The incidence of rheumatoid factors is also high in dysproteinaemias and paraproteinaemias (Bartfeld, 1969). In all three patients with supposed active infection of *Toxoplasma gondii* rheumatoid factors were found and rheumatoid factors were also found in the cerebrospinal fluid.

The human foetus becomes immunocompetent quite early in foetal life with evidence of functional cellular (Playfair, Wolfendale & Kay, 1963) and humoral immunity (Van Furth, Schmit & Hijmans, 1965). However, we do not know whether this ability is limited when stressed by a transplacental infection. The role of maternal antibodies in inhibiting the antibody response in prenatal and neonatal life (Greengard & Bernstein, 1935, Möller, 1964) and the importance of the antigenic experience of the newborn (Lodinova, Jouja & Lane, 1967) in handling infectious agents should be considered. The findings of M-components and rheumatoid factors in the newborns but not in the mothers are major differences between these groups in their immune response to *Toxoplasma gondii* infection.

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