Paraproteinaemia and Unusual Dys- γ -globulinaemia in a Case of Wiskott-Aldrich Syndrome

An Immunochemical Study

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The increased susceptibility to infection in the Wiskott-Aldrich syndrome (WAS) has led to a number of immunochemical studies, with somewhat varied results. Kildeberg (1961) found hyperimmunoglobulinaemia with a raised level of β_{2A} (IgA) and of β_{2M} (IgM) and especially of a γ -globulin where the precipitation line was prolonged and indicated the presence of γ -globulin with the mobility of both β_1 and a slow α_2 . Amiet (1963) reported raised levels of all three immunoglobulins in his patients. West, Hong, and Holland (1962) described a large increase in IgA in 2 cases suffering from WAS, whereas the concentration of IgM in one of them was at a low normal level. Palmgren and Lindberg (1963) found a reduced IgM, IgA being normal or slightly raised. Cooper, Chase, St. Geme, Krivit, and Good (1964) examined 15 cases, recording low values of IgM and normal IgA and IgG. Gugler, Ammann, and Rossi (1965) and Ungari, Aiuti, and Iacovacci (1966) observed reduced levels of IgM. Stiehm and Fudenberg (1966) studied 3 cases, noting in all a conspicuous increase of IgA, in one a slightly raised level of IgG, and in 2 a lowered level of IgM. Dalloz, Castaing, Nezelof, and Seligmann (1965) reported a 5-month-old child with WAS, and a temporary paraproteinaemia.

Immunochemical studies were carried out in one of two brothers, both of whom were suffering from WAS. In the serum of the older boy, who died before reaching the 14th year, paraproteinaemia of the IgG-L type was identified as well as a striking increase in the level of IgA, a clearly reduced IgM, and a normal IgD.

Case Report

The patient, a boy, was born in August 1952. His younger brother, born in December 1960, suffered from the same disease and died at the age of 2½ years. Some clinical data of this case were published elsewhere (Houštěk, Hrodek, Rádl, and Libánská, 1966), but no immunoglobulin studies could be made at that time.

From birth he had loose stools, often with blood. At the age of 3 months petechiae were present, and later there was recurrent haematuria. At 2 months, eczema appeared. An increased susceptibility to infection was manifested by many serious affections: conjunctivitis, blepharitis, corneal ulcer, and stomatitis alternated with pharyngitis, otitis, sinusitis, bronchopneumonia, and staphylococcal sepsis. He had herpes zoster on two occasions, and a cellulitis of the leg was followed by staphylococcal osteomyelitis of the tibia. At the age of 11 years, in the course of x-ray examination, a shadow in the right chest adjacent to the heart was observed. It was thought to be a tumour, but in view of the poor general condition of the patient it was not possible to investigate it further, and, after a course of radiotherapy, the shadow disappeared.

In the course of the last 16 months of his life an unusual and progressive neurological disorder developed with lesions of the III, IV, V, VI, VII, IX, X, XI, and XII cranial nerves. CSF and the EEG showed only minor deviations from the normal. He died aged 13½ years.

Laboratory findings. Hb was normal or slightly low. Leucocyte count ranged from normal up to 20,000 cells/mm.³, with a shift to the left. A marked eosinophilia up to 36% was often found. The number of lymphocytes was normal in the first month of life and generally remained normal, but tended to fall in periods of infection. The thrombocyte count was always reduced, ranging from 4000 to 65,000/mm.³ Repeated tests for antibodies against thrombocytes were negative. The bone-marrow examination revealed 2% mature plasma cells.

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The blood group was A₁ Rh positive. The titre of anti-B antibodies was reduced (1:8). The serum complement was 28 U (average normal value 35 U). Antibodies against tetanus and diphtheria were measured in 1953 and gave abnormally low values; repeated examination in 1963 gave unmeasurably low values. Smallpox vaccination was carried out at 2 years old, with a positive result after intradermal revaccination. BCG vaccination was given in the postnatal period; the Monrad test was positive until 11 years, and the Mantoux test was then negative.

The karyotype was examined by cultivation of lymphocytes from the peripheral blood and showed polyploidy in an unusually high percentage of mitoses. In one mitosis an exceptionally large chromosome was observed, corresponding to the third pair, according to the Denver classification.

Necropsy confirmed the clinical diagnosis. Among other findings was early cirrhosis of the liver of unknown aetiology. The thymus was characterized by premature involution (8 g. instead of 26 g.). In the mucosa of the duodenum a reticular lymphosarcoma, 1.3 × 1 × 0.3 cm., was present, composed of uniform large cells containing hyperchromatic nuclei with mitoses; between the cells were numerous reticular fibrils. Histology of the brain showed subacute encephalitis, localized predominantly in the grey matter and in the basal ganglia. In the myocardium there were minute circumscribed areas of necrosis and fibrotic degeneration. In the postero-basal segment of the lower lobe of the right lung, where at the age of 11 years a 'tumour' had been observed and irradiated, a solitary cylindrical bronchiectasis was found, with chronic purulent inflammation but no indication of malignancy.

Examination of serum proteins. The serum proteins were tested by biuret reaction. Paper electrophoresis was by Masopust and Homolka's (1960) technique. Electrophoresis on agar and immunoelectrophoresis by micro-method have been described elsewhere (Rádl, 1966). Semi-quantitative immunoprecipitation was by the Roth method (1962).

The following antisera were employed. Polyvalent antisera: horse antihuman serum: Sevac Prague 358/62, Pasteur Inst. Paris 223. Rabbit antihuman serum: Sevac 10/63 and rabbit antiserum against the patients serum proteins 61/64 and 66/64 (prepared by author).

Monovalent antisera: Rabbit anti-IgG Sevac 1-08/64, rabbit anti-IgM Sevac 1v/64, horse anti-IgM Sevac 1-08/64, rabbit anti-IgA Sevac 1v/64, rabbit anti-IgA Ned. Roode Kruis No. 2205, rabbit anti-Fab fragment of IgG Sevac 11v/62, rabbit anti-IgD 86/65 (own make—Rádl, 1966), rabbit anti-Bence Jones protein type K (I) No. 97/65 and anti-Bence Jones protein of type L (II) No. 101/65 (Rádl, 1966).

Results

The abnormal character of immunoglobulins was first suspected when the homogeneous fraction (the M-component) was detected in the slowest part of γ -globulins in electrophoresis, both on paper and on agar (Fig. 1). The immunoelectrophoretic analysis of serum proteins when using buffers with a low ionic strength ($\mu = 0.03$) revealed only a faint line of IgM as compared with normal serum, and a thicker and elongated IgA line. The precipitation line IgG had a normal shape except

FIG. 1.—Paper electrophoresis (veronal buffer, pH 8.6, $\mu = 0.1$) of the patient's serum in 1964 and 1966, compared with normal serum (M-component indicated by arrow).

FIG. 2.—Immunoelectrophoresis of the patient's serum in veronal-acetate- CaCl_2 buffer, pH 8.6, $\mu = 0.03$. Well of origin: undiluted serum from the patient (1); serum after incubation with 0.1 M cysteine (2); serum diluted 1 : 2 with physiological solution (3); serum diluted 1 : 2 after incubation with cysteine (4); serum diluted 1 : 4 with physiological solution (5); serum diluted 1 : 4 after incubation with cysteine (6). Horse immune serum Sevac 358/62 (A) and monovalent anti-IgG Sevac (B) in channels. Paraprotein lines indicated by arrows.

for its anodic part, from which another much thicker line branched off with the mobility of β_1 -globulin. Moreover, some of the protein precipitated in the well of origin, and did not migrate in agar even after incubation of the serum with cysteine (Fig. 2). Only by increasing the ionic strength of the buffer to 0.06 was the migration of this protein achieved, and it formed with the antiserum No. 358/62 a second abnormal line at the cathodic end of IgG. By using specific antisera the presence of paraprotein of the IgG-L type was confirmed (Fig. 3). The amount of paraprotein was estimated by evaluating the M-component in paper electrophoresis. The absolute values tended to decrease during the last two years of the patient's life (Table). During the last two months of life the M-component in the serum was undetectable by paper electro-

phoresis; by contrast, paraprotein in the serum was still identifiable by immunoelectrophoresis on the last day of life.

The semiquantitative immunoprecipitation showed the persisting low values of the serum IgM ($\frac{1}{4}$ th of the values normal at that age). The concentration of IgA rose from 10 to 15 times more than normal values. However, the precipitation line IgA retained a 'normal' character. It did not form an abnormal bow and reacted with antisera against both types of L-chains. The IgD level was within normal limits. The analysis of the blood serum of the patient's parents was normal.

The urine, sweat, CSF, and stool extract of the patient were also tested. In the CSF a paraprotein precipitation line was evident 1 month before death. The urine showed no reaction to protein

TABLE
Serum Protein Electrophoresis and Liver Function Tests

	Protein- aemia (g./ 100 ml.)	Paper Electrophoresis (Relative %)						M-component		Thymol turbidity (MacLag- gan units)	Takata	Weltmann % CaCl ₂
		Albumin	α_1	α_2	β_1	β_2	γ	%	(g./ 100 ml.)			
Normal ..	7.2	61.0	5.0	7.5	9.0		16.0	None	None	<7.0	—	0.05
Standard ..	± 0.44	± 3.0	± 0.74	± 1.05	± 1.25		± 2.10					
1961 ..	5.6	43.3	9.3	12.4	8.7	9.0	17.3	?	?	1.0	—	0.09
23.6.1962 ..	6.2	44.5	7.2	12.3	10.6	8.6	16.8	?	?	12.0	—	0.09
25.6.1963 ..	4.8	49.1	5.5	9.2	8.6	8.3	19.3	?	?	5.9	—	0.07
5.3.1964 ..	7.3	45.0	5.8	9.5	14.2		12.4	13.1	0.95	NE	NE	NE
11.5.1964 ..	7.4	40.0	6.2	10.5	18.3		8.5	16.5	1.22	28.2	—	0.06
13.10.1964 ..	6.6	49.3	5.1	9.2	7.9	6.0	9.5	13.0	0.86	16.5	—	0.05
12.4.1965 ..	6.7	50.0	6.2	11.1	12.6		11.1	9.0	0.60	7.5	—	0.04
20.7.1965 ..	7.0	49.0	5.0	8.0	16.8		11.8	9.4	0.66	18.6	+	NE
25.1.1966 ..	5.6	49.8	6.2	10.5	17.6		15.9	*	*	14.5	—	0.05

? = Data not available; NE = not examined; * = not demonstrated by paper electrophoresis; — = negative; + = positive.

FIG. 3.—Immunoelectrophoresis of patient's serum compared with normal serum (1), veronal buffer, pH 8.6, $\mu = 0.06$. Patient's serum undiluted (2 and 4) and diluted 1 : 4 with physiological solution (3 and 5). Polyvalent horse immune serum Sevac 358/62 (A), monovalent anti-IgM Sevac (B), anti-IgA Ned. Roode Kruis No: 2205 (C), anti-IgG Sevac (D) in channels. Paraprotein lines indicated by arrows.

FIG. 4.—Immunoelectrophoresis of patient's serum (above) and stool extract (below). Horse polyvalent serum Sevac 358/62 (A) and anti-IgA Sevac (B) in channels.

with sulphosalicylic acid; and when concentrated 50 times, only a faint albumin line was shown by immunoelectrophoresis. In the concentrated sweat only albumin was detected. In repeated assays of stool extract only a single pronounced precipitation line was evident; this had the shape and mobility of the serum IgA and reacted with the specific antiserum against IgA (Fig. 4).

Discussion

Both the clinical and the laboratory findings proved conclusively that the boy suffered from WAS. However, the case shows certain peculiarities when compared with other reported cases. First, there are pronounced qualitative and quantitative changes in the serum immunoglobulins (paraproteinaemia of the type IgG-L and an extreme rise of IgA); further, the relatively advanced age of the patient; and finally the finding of a reticular lymphosarcoma localized only in the duodenal mucosa.

These facts provide several questions for discussion. In the first place, were the changes in immunoglobulins due to WAS, or were they caused by another concomitant disease? With regard to the problem of aetiology of the paraproteinaemia, it is quite evident that our patient did not have myeloma. Repeated bone-marrow examination was negative for myeloma cells, x-ray films of the skeleton were normal, and necropsy showed no sign of myelomatosis. Nor was there any other form of generalized malignant proliferation in the lympho-plasma-reticular system, as is sometimes associated (facultatively, Spengler, Roulet, Ricci, Schnider, Schoop, Kappeler, and Riva, 1961) with paraproteinaemia. The finding of the small reticular lymphosarcoma in the duodenal mucosa does

not adequately explain the production of paraprotein, which lasted for at least two years and showed a tendency to decrease during the last 16 months of life. On the other hand, it does support the view that malignancy of the reticuloendothelial system is often associated with WAS (ten Bensel, Stadlan, and Krivit, 1966; Krivit, 1965; Chaptal, Royer, Jean, Alagille, Bonnet, Lagarde, Robinet, and Rieu, 1966). Peterson, Cooper, and Good (1965) made similar observations in other diseases with immunological deficiency.

In this case the character of the paraproteinaemia could be 'reactive' (Rádl and Masopust, 1964; ten Berge, 1965), as was described in many cases with long protracted chronic diseases, e.g. osteomyelitis, tuberculosis, chronic pyelonephritis, or chronic cholangitis. In some subjects an abnormal reactivity (probably genetic) may develop because of chronic irritation of the reticuloendothelial system, a disturbance in the mechanisms regulating the synthesis of immunoglobulins, and this may result in the formation of paraproteins.

Why the paraproteinaemia tended to diminish terminally remains obscure. In the case described by Dalloz *et al.* (1965) paraproteinaemia disappeared completely under similar circumstances. Complete exhaustion and breakdown of the defence mechanism of the body is a possible explanation.

Finally, paraproteinaemia is a rare finding in children (Dik and Beetstra, 1959; Stoop, Ballieux, and Weyers, 1962; Dalloz *et al.*, 1965) so it is surprising that it was found in two out of four cases of WAS.

The very high IgA serum level is not characteristic of WAS, as it is well recognized as the main constituent of the immunoglobulins in external secretion (Tomasi, Tan, Solomon, and Prendergast, 1965). Its high level in the serum and stool

extract of our patient may be due to the enhanced antibody production present in existing infections. Raised IgA has been described in Laenec's cirrhosis (Fahey, 1965; McKelvey and Fahey, 1965), and in our case an incipient cirrhosis of unknown aetiology was found.

In previous studies of the immunoglobulins in WAS, the most constant finding has been the low level of IgM (Chaptal *et al.*, 1966; Gugler *et al.*, 1965; Ungari *et al.*, 1966), frequently accompanied by a low titre of isohaemagglutinins (Amiet, 1963; Cassimos, Anastasea-Vlahou, Kattamis, and Kanavakis, 1960; Gugler *et al.*, 1965; Kildeberg, 1961; Palmgren and Lindberg, 1963; Ungari *et al.*, 1966). Palmgren and Lindberg (1963) found a low titre of antibodies against H antigen and none against O antigen after vaccination with antigens of typhoid and paratyphoid B. In contrast to this, Cooper *et al.*'s patients (1964) produced early 19S antibodies against the flagellar and the somatic antigens of salmonella, in spite of the low level of IgM. In addition, they found lymphopenia, pathological changes in the architecture of the lymphatic tissue, and failure to develop delayed skin hypersensitivity. Nevertheless, the changes in immunoglobulins that we found and that are in agreement with those described by other writers, show that in WAS there is a disorder not only of cellular defence but also of humoral defence mechanisms. This is supported by the failure to form circulating antibodies after vaccination (Palmgren and Lindberg, 1963; Gugler *et al.*, 1965).

There still remain several unsolved questions. The neurological disorder in the final phase of the disease could be connected with the presence of readily precipitating paraprotein in the serum and CSF. Similar symptoms have been described in Waldenström's macroglobulinaemia and in myeloma (Wuhrmann and Märki, 1963). The finding of an unusually large chromosome might also be related with paraproteinaemia (Siebner, Spengler, Bütler, Heni, and Riva, 1965) or with malignancy of the lympho-plasmo-reticular system. Finally, the possible effects of therapeutic irradiation at the age of 11 years has to be taken into account.

Summary

A boy with the Wiskott-Aldrich syndrome is described who died at the age of 13½. The main findings were: paraproteinaemia of the type IgG-L; a greatly raised IgA level and a low IgM level in the serum; an almost selective presence of protein in the stool extract, similar in its antigen behaviour to serum IgA; an unusually large chromosome in one mitosis of a peripheral lymphocyte; and the

post-mortem finding of a localized reticular lympho-sarcoma in the duodenal mucosa.

It is concluded that the Wiskott-Aldrich syndrome falls into the group of diseases with hereditary immunological deficiency, and that both cellular resistance and humoral resistance are disordered.

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