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Antibody responses *in vivo* in chromosome instability syndromes with immunodeficiency

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SUMMARY

The primary IgG, IgM and IgA antibody responses to Helix pommatia haemocyanin (HPH) were defective in patients with ataxia telangiectasia (AT) and Nijmegen breakage syndrome (NBS). The results in patients with Bloom's syndrome (BS) were heterogeneous, but all showed abnormal kinetics of the IgG response. The secondary response to diphtheria, tetanus and polio vaccine was normal in patients with AT and BS, but disturbed in the patients with NBS. The abnormalities of antibody response of AT and NBS are similar, although more profound in NBS; BS is different.

Keywords immunodeficiency chromosome instability immune response

INTRODUCTION

Immunodeficiency and chromosomal instability are found in ataxia telangiectasia (AT), Bloom's syndrome (BS) and Nijmegen breakage syndrome (NBS) (German, 1972; Hecht & Kaiser McCaw, 1977; Weemaes *et al.*, 1981). These syndromes differ although they have some symptoms in common (Table 1). In all three the humoral immunity is disturbed. In AT IgA and IgE deficiencies are common, as are abnormalities of the IgG subclasses (Thieffry *et al.*, 1961; Ammann *et al.*, 1969; Rivat-Peran *et al.*, 1981; Oxelius, Berkel & Hanson, 1982). Both patients with NBS had IgA deficiency (Weemaes *et al.*, 1981). In BS serum levels of at least one class of immunoglobulin are decreased (Hütteroth, Litwin & German, 1975; Weemaes *et al.*, 1979). The disturbance in cellular immunity is variable in these disorders; results of *in vitro* studies differ widely, even within a group of patients (McFarlin, Strober & Waldman, 1972; Hütteroth *et al.*, 1975; Weemaes *et al.*, 1981).

There are a few studies of the *in vivo* immune response in patients with breakage syndromes. One reports normal antibody response to tetanus, diphtheria and polio vaccine, but in another the response was decreased (Eisen *et al.*, 1965; Peterson, Cooper & Good, 1966). Responses to typhoid, actinophage and tularemia antigen are often below average (Eisen *et al.*, 1965; Peterson *et al.*, 1966; McFarlin *et al.*, 1972). In four patients with BS a four-fold rise in antibody levels was demonstrated after challenge with a diphtheria, tetanus and polio vaccine booster (Weemaes *et al.*, 1979). However, these studies are mostly of the secondary late recall response. The response to two doses of bacteriophage ϕX174 in patients with AT is normal in some, but not in others (Wedgewood, Ochs & Davis, 1975); it has not been studied in patients with BS and NBS. One injection subcutaneously of Helix pommatia haemocyanin (HPH) elicits a strong primary humoral (in IgG, IgA and IgM) and cellular immune response in all normal volunteers (Weits *et al.*, 1978).

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Table 1. The characteristic symptoms of patients with the chromosomal breakage syndromes: AT, NBS and BS

	AT	NBS	BS
Cerebellar ataxia	+	−	−
Telangiectasia			
conjunctival	+	−	−
elsewhere	+	−	+
Intrauterine growth retardation	−	±	+
Stunted growth	+/−	+	+
Microcephaly	−	+	+
Mental retardation	+/−	+	−
Skin abnormalities			
sun sensitivity	−	+	+
café-au-lait spots	+	+	+
vitiligo	+	+	+
progeria	+		
freckles	−	+	−
Infections	+	+	+
Tendency to malignancy	+		+

We have determined the primary immune response HPH and the secondary response to diphtheria, tetanus and polio vaccine in patients with the breakage syndromes AT, NBS and BS.

MATERIALS AND METHODS

Serum was obtained by venipuncture from six children with AT, two with NBS (full study in only one) and five with BS (see Table 2 for ages and sex) and from children (age 2–12 years) with recurrent infections in which immunological investigation was indicated but other causes for the

Table 2. Serum immunoglobulin levels in the patients

			IgM	IgG	IgA
	Age	Sex	iu/ml		
AT					
AtH	17	F	161	215	2
MK	10	M	146	74	<1
LK	8	M	110	43	105
MS	9	M	201	168	<1
MaS	3	F	158	52	56
HC	6	F	73	98	<1
NBS					
MH	6	M	150	34	<1
HH	10	M	66	110	<1
BS					
AnP	17	M	67	92	152
MaP	8	F	48	58	46
SuS	14	F	46	82	52
StS	14	M	27	66	32
GC	3	M	38	55	32

infections were established afterwards. Patients and controls had been previously immunized according to the Dutch National vaccination scheme. They received 1 ml of absorbed diphtheria, B-pertussis, tetanus and polio vaccine at the age of 3, 4, 5 and 12 months and with 1 ml of absorbed DT-polio vaccine at the age of 4 and 9 years. After bleeding 1 ml of absorbed diphtheria, tetanus, inactivated polio vaccine (DT-polio vaccine) was given and blood was drawn after 2 weeks. Afterwards 1 ml of HPH was given subcutaneously, and blood was obtained 2 and 6 weeks after immunization.

Immunoglobulin concentrations were determined by an immunoturbidimetric method using a LKB reaction rate analyser (van Munster *et al.*, 1977). Tetanus and diphtheria antibody titrations were performed according to Ipsen (1943a, 1943b). Titres were measured from 0·01 to 5·12 au/ml. If values above 5·12 au/ml were found, serum was retitrated, starting from a dilution 1/10, which resulted in a titration range from 0·1 to 51·20 au/ml. Antibody to polio types 1, 2 and 3 were titrated according to Salk, Younger & Ward (1954); data are presented for polio type 1 only. Antibodies to HPH were determined with an indirect ELISA technique (De Graeff *et al.*, 1983).

RESULTS

Primary response

All pre-immunization anti-HPH titres were negative except some very low IgM titres in three controls (Fig. 1a).

In the seven controls IgM, IgG and IgA antibody titres were positive at week 2 (Fig. 1), and fell in all but one by 6 weeks. The peak response was somewhat lower than those in adults and the decline was sharper,

In the AT patients small rise in IgM antibody titres were observed in only two. In none were IgG and IgA antibodies detected (Fig. 1). The one patient with NBS studied had no response in any immunoglobulin classes (Fig. 1).

BS patients varied (Fig. 1). The siblings (SuS and StS) had normal IgM and IgA antibody responses. Their IgG response was normal after 2 weeks and rose even higher after 6 weeks. The siblings (AnP and MaP) had low IgM responses after 2 weeks but one was normal at 6; both had low IgG responses after 2 weeks but high–normal or high levels after 6 weeks; their IgA responses were low. The youngest patient (GC) had no IgM or IgA responses. IgG antibodies were detected only at 6 weeks.

There was no obvious association between the antibody responses and the serum immunoglobulin levels (Table 2). In the AT patients the serum IgM and IgG levels were normal despite the disturbance in antibody synthesis. The two patients with normal serum IgA levels showed no IgA antibody response at all. In all five BS patients the serum IgM level was low, including two with normal rise in IgM anti-HPH antibody titre. Three BS patients had low serum IgA levels but normal IgA responses whereas the patient with normal serum IgA (AnP) had a smaller rise in IgA anti-HPH antibody titre.

Secondary response

The antibody titre before and after revaccination with DT-polio vaccine in the controls showed a wide range (Fig. 2), similar throughout the age range. All six patients with AT had pre-existing antibodies to diphtheria toxoid, tetanus toxoid and poliomyelitis vaccine and a normal rise to revaccination (Fig. 2). One patient with NBS (MH) who was treated with immunoglobulin, showed no response to revaccination. His brother (HH) had antibody responses to diphtheria and tetanus toxoids which may be low in the normal range. His antibody response to polio was normal (Fig. 2). The patients with BS had normal responses (Fig. 2) to diphtheria and tetanus toxoids, and to polio, except AnP who had a small rise in antitoxin from a low initial level. SuS had a high titre of anti-polio type 1 before immunization but no rise in titre afterwards; her antibody response to polio vaccine 2 and 3 was normal, as it was in the other patients (data not shown).

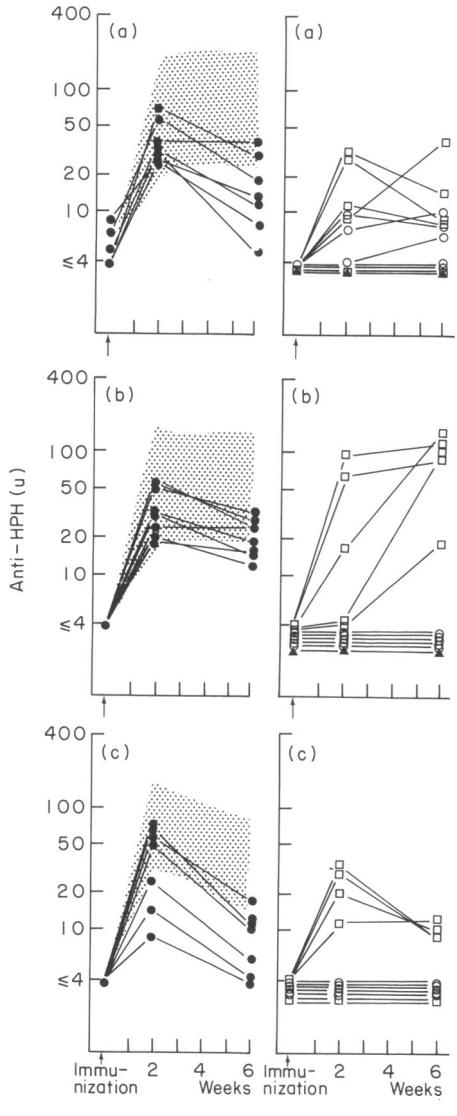

Fig. 1. (a) IgM, (b) IgG and (c) IgA antibody levels in patients with AT (○——○), with NBS (▲——▲) and BS (□——□) and in control children (●——●), before, and 2 and 6 weeks after immunization with HPH s.c. Antibody levels are expressed in units; a positive reference sample is ascribed the value as 100 u at a dilution of 1:100. The shaded area represents the normal range of adult controls (±s.d.).

DISCUSSION

The response to vaccination with HPH in the few control children at 2 weeks was somewhat lower in all three immunoglobulin classes than in adults and declined quicker, but the data are adequate for comparison with the patients. In all five patients with AT the primary response to HPH was defective for all immunoglobulin classes. HPH is a T cell-dependent antigen (Weits *et al.*, 1978) and in patients with AT the cellular immunity is disturbed. Gupta & Good (1978) and Gmelig-Meyling *et al.* (1980) described a deficit in T helper cells. In two patients with AT treated with serum thymic factor, the antibody response increased (Bordigoni *et al.*, 1982). A defect of regulatory T cells could

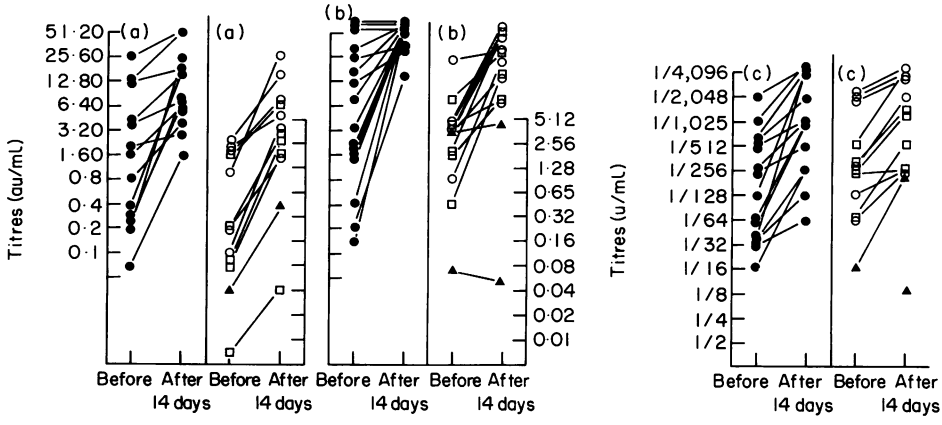

Fig. 2. Antibody titres to (a) diphtheria, (b) tetanus and (c) poliomyelitis type I in the patients and the control children (see Fig. 1 for symbols). Diphtheria and tetanus antitoxin titres are expressed in antitoxin units (au) per ml.

underlie the abnormality in antibody response to HPH in our patients. No IgA antibody formation was demonstrated in our patients with AT, even in those with a normal serum IgA level, this may be important in the sinopulmonary infections. The secondary response, as measured after a DT-polio booster was normal in patients with AT, so memory cells seem to be intact in our patients. However, defects in responses to DT-polio vaccine have been described by other authors (Eisen *et al.*, 1965; Peterson *et al.*, 1966) who did not outline the vaccination scheme used. The Dutch vaccination scheme is unusually intense, four immunizations in the first year and booster immunization at the ages of 4 and 9 years. This may explain the difference.

A progressive deterioration of the immune system with time is described in AT (Ammann *et al.*, 1969). We did not see this. In NBS the antibody responses are more abnormal than those in our patient with AT. No primary response to HPH was detected in the one child tested, and the secondary response was absent in the other. The T cell regulatory function in NBS is now under investigation.

The secondary response to DT-polio vaccine booster was normal in all patients with BS except in one for one antigen, despite the low serum IgG level in some patients.

The results of the primary response to HPH are heterogeneous in patients with BS. In two siblings (SuS and StS) the antibody titres in all three immunoglobulin classes were normal despite the low IgM and IgA serum levels. All other patients had abnormal responses, the youngest patient having the greatest defect. All patients showed abnormal kinetics of the IgG response in that the IgG titre was higher at 6 weeks. Transition from IgM to IgG antibody is prolonged in infants up to the age of 6 months (Stiehm & Fulgeniti, 1980). Tanaguchi *et al.* (1982) suggested on the basis of *in vitro* experiments that the immune disturbances in BS could result from arrested maturation of B cell differentiation and T cell regulatory function.

Chromosomal instability disorders have several characteristics in common (Table 1). The antibody responses of patients with AT resemble those of patients with NBS, as do other immunological and cytogenetic findings (German, 1972; Hecht & Kaiser McCaw, 1977; Weemaes *et al.*, 1981). However, the defect in antibody synthesis seems to be more profound in NBS. However, the immunological abnormalities in BS are different.

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