Four families with immunodeficiency and chromosome abnormalities

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SUMMARY Six children, with severe deficiency of some or all of the immunoglobulins and minor somatic abnormalities, had chromosomal abnormalities: (1) 45,XY,t(13q/18q), (2) 46,XY,21ps+, (3) two brothers 46,XY (inv. 7), (4) 45,X,t(11p/10p)/46X,iXq,t(11p/10p) and, (5) in addendum, 45,XX,—18; 46,XX, r18. The chromosome abnormalities were detected in B- as well as T-lymphocytes (as evidenced by using both PHA- and PWM-stimulated cultures) in all probands, but one was mosaic in PHA culture, although all his PWM-stimulated cells were abnormal. Chromosomal variants were also detected in relatives of three and immunodeficiency in relatives of two.

Only two firm associations between immunodeficiency and autosomal chromosome abnormality in phytohaemagglutinin (PHA) stimulated T-lymphocytes are recognised. Some patients with ataxia telangiectasia show general chromosome instability with many spontaneous breaks (especially of chromosome 14 and other D-group chromosomes) (Nelson *et al.*, 1975; Oxford *et al.*, 1975). IgA deficiency is sometimes associated with chromosome 18 abnormalities—deletions of short and long arm (Feingold *et al.*, 1969; Faed *et al.*, 1972), ring forms (Finley *et al.*, 1968), and large chromosomes (Yanagisawa, 1972). There are also reports of immunodeficient patients with other chromosomal abnormalities (Jacobs *et al.*, 1968; Kretschmer *et al.*, 1968; Calvani *et al.*, 1976), especially of the D-group. We studied the chromosomes in four immunodeficient children who had unusual facies; each had an abnormality.

As the immunodeficiency was of antibody we studied the chromosomes not only in PHA-stimulated lymphocytes (predominantly T-lymphocytes), but also in pokeweed mitogen- (PWM) stimulated lymphocytes (which include B-lymphocytes).

Methods

Immunoglobulins were measured by radial diffusion (Fahey and McKelvey, 1965). Lymphocyte thymidine uptake after PHA stimulus, E-rosette count, and surface immunofluorescence were by the method of Espanol *et al.* (1974). Chromosomal analysis of PHA-stimulated lymphocytes after colcemid treatment was by the method of Hungerford (1965).

For chromosomal analysis 0.05 ml PWM (Gibco, New York) was added to 5 ml cultures (Wybran *et al.*, 1973).

T- and B-enriched preparations of lymphocytes were prepared by rosetting techniques from Case 1 and a healthy donor, cocultured with PWM, and tested for cytoplasmic immunofluorescence for immunoglobulin (Hayward and Lawton, 1977).

Buccal smears for X-chromatin studies were prepared by a modification of the method of Klinger and Ludwig (1957). Hydrolysis in 1 mol/l HCL for 8 minutes was followed by staining for 10 minutes in 2% aqueous toluidine blue.

Family 1. Case 1, a boy, was investigated soon after birth because his maternal uncle had had life-long immunoglobulin deficiency (he died before the proband's birth). At birth Case 1 had limb swelling, deformed toe nails, spindle shaped fingers, microcephaly, and an unusual facies with fullness over the maxillary and mandibular bones. Immunoglobulin deficiency was found (see below) and replacement treatment was started, although he had had no obvious infection. At 15 months he developed convulsions, up to five a day. Several members of his

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father's family had had convulsions. Neutropenia was noted once. Levels of serum IgG, IgA, and IgM have been persistently very low. Lymphocyte count was normal with normal E-rosette count, and a high

percentage positive for surface immunoglobulin immunofluorescence (Table). Chromosome analysis of PHA-stimulated cells showed a normal/translocation mosaic; 3 of 10 cells studied were normal;

Fig. 1 (scale in microns). Anomalous chromosome pairs from probands 1, 2, and 3. Photomicrographs of the normal and abnormal member of each affected chromosome pair are shown, in each patient, together with a drawing and the standard international diagram of the banding pattern (Paris Conference, 1972). Translocated chromosome in proband 1 (a mosaic) was formed by fusion of chromosomes 13 and 18, after loss of their short arms by breakage at the centromere (arrowed). Proband 2 has large satellites on chromosome 21. In proband 3, the arrows pointing to the inverted chromosome 7 (inv. 7) indicate the apparent breakage points at which the order of the bands has been reversed.

Family 2. Case 2, a 9-month-old boy, was investigated for growth retardation, developmental delay, and repeated febrile illnesses with convulsions. He had an unusual facies, with a mongoloid slant to the eyes, hypertelorism, anteverted nares, and broad upper lip. He had hyperextensible joints and pigmented naevi on the occiput, thigh, and axilla. A cystogram showed bilateral ureteric reflux with parenchymal opacification of the left kidney. His head was asymmetric and a CAT scan showed cortical thinning, especially of the frontal lobes. There was evidence of respiratory and urinary infections.

Levels of serum IgG, IgA, and IgM were all very low. Salivary IgA and isohaemagglutinins were absent. Chromosome analysis of both PHA- and PWM-stimulated lymphocytes showed that all cells had a giant satellite on the short arm of chromosome 21 (Fig. 1). He has since had occasional mild respiratory infections while on prophylactic cotrimoxazole, and his convulsions are well controlled on sodium valproate; his psychomotor development is retarded.

His mother's immunological tests were normal, as were her chromosomes. Both his father and 4-year-old brother were well, but show the same chromosome aberration in all PHA- and PWM-stimulated cells. Although the brother is immunologically normal, his father has a low PHA response (Table).

Family 3. Case 3, a 4-month-old boy, was investigated for growth retardation and feeding difficulties. He had an unusual facies, with a low set left ear, anti-mongoloid slant to the eyes, and high arched palate. He was wasted and breathless. *Pneumocystis carinii* infection was diagnosed on lung aspiration and *Giardia lamblia* were found in the faeces. He had intermittent neutropenia and low levels of IgG and IgA. IgM was low but rose to normal. The anti-A isoagglutinin titre was 1/1. Salivary IgA was not detected. There was pericentric inversion of chromosome 7 on all cells on PHA and PWM cultures (this had previously been identified in PHA culture by Dr N. Gregson). He gained weight after treatment with immunoglobulin injections, co-trimoxazole, and metronidazole.

His mother had a low PHA response, but her other immunological tests and her chromosomes were normal. His paternal grandmother, father, and 4-year-old brother each showed the same chromosome abnormality. His father was well and immunologically normal. His brother had neutropenia on one occasion and has low levels of serum IgG and IgA. He was relatively well until recently apart from mild oral ulceration; this is now severe and immunoglobulin treatment has been started.

Family 4. Case 4, a 3-month-old girl, was investigated for growth retardation, prolonged hypocalcaemia (until 9 weeks old), and frequent infections: chronic oral and perineal candidiasis, chronic paronychia and pulp space infection, and septic arthritis of the hip. She had an unusual facies with micrognathia, high arched palate, and epicanthic folds. Pectus excavatum, widely spaced nipples, stridor, large umbilical hernia, hypotonia, anterior bowing of tibiae, and dystrophic nails were noted. Barium meal showed malrotation of the intestine.

Serum IgG and IgA were low and salivary IgA was not detected. E-rosette-forming cells, PHA response, and surface immunoglobulin immunofluorescence were normal. Of 20 cells studied, on PHA stimulus, 9 had a single X-chromosome (45, X) and 11 had an isochromosome for the long arms of X—that is she was a mosaic (Fig. 3). All lymphocytes showed an extra band on the short arm of chromosome 10, and a missing band from the short arm of chromosome 11. PHA and PWM cultures gave similar results. Of 300 buccal cells 16% showed Barr bodies (normal female range 15 to 30%); some were abnormally large. Her parent's chromosomes were normal; the mother's PHA response was low.

She has had no subsequent serious infections and serum calcium has remained normal without further treatment, but there is evidence of psychomotor retardation.

Discussion

The association of immunoglobulin deficiency with chromosome aberrations and abnormalities raises a number of possibilities. It may be coincidental as autosomal abnormalities are found in about 0.3% of newborn babies (Evans, 1977), and we are becoming increasingly aware that immunodeficiency is not rare. The occurrence of both in a single individual will be an occasional chance finding, but the detection by chance of a significant chromosomal abnormality or variation in all four probands we studied would be a very odd run of luck. It is possible that the structural and chromosomal abnormalities were causatively associated, and immunodeficiency, which brought them to our attention, was associated by chance.

The other possibilities for the association are that the chromosome abnormality may cause the immunodeficiency, or that the immunodeficiency may have led to abnormal handling of an intra- or extra-uterine viral infection by the child or the mother which might have caused the chromosome abnormality, or that both were caused by the same environmental factor, for instance by a virus infection (Soothill *et al.*, 1966). There is no history

Fig. 3 (scale in microns). Anomalous chromosome pairs from proband 4, presented in the same way as in Fig. 1. In addition to the normal X and isoXq (with its duplicated long arm), there are two abnormal autosomes which seem to be balanced for a translocation between chromosomes 10 and 11. Arrows indicate the apparent breakage points at which part of the short arm of an 11 broke off to become attached to the short arm of a 10.

of overt viral infection during pregnancy in any of these families. It is very likely that the mechanism of the associations may be different in the different families. In families 2 and 3 it is more likely that they were coincidental as the chromosome abnormality was detected in an immunologically and structurally normal member of both families, but one cannot exclude the possibility that the abnormalities resulted from varying expression of the chromosome defect. The neutropenia in association with inversion of chromosome 7 in Case 3 is interesting in view of the report by Rowley (1973) of neutropenia with deletions of this chromosome. The known involvement of X-chromosome with immunoglobulin concentration (Butterworth *et al.*, 1967; Rhodes *et al.*, 1969) gives added significance to the finding in Case 4.

Although the family tree (Fig. 2) in family 1

suggests X-linkage, neither the normal IgM in the uncle, nor the normal number of lymphocytes with surface immunoglobulin in the child is consistent with X-linked agammaglobulinaemia, but there probably are other forms of X-linked immunodeficiency. Coincidental chromosomal abnormality with such an immunodeficiency must be considered, but there is evidence in favour of a causative relationship. The mosaicism of the T-lymphocytes (with apparently normal T-cell function, including that of co-operation) but the consistent chromosomal abnormality in the PWM-stimulated cultures, many of which would be B-lymphocytes which were functionally defective *in vitro* and *in vivo* would be an additional coincidence if the relationship were not causal.

The chromosomes affected in Case 1 have been

previously associated with immunodeficiency—chromosome 18 with IgA deficiency (Feingold *et al.*, 1969; Faed *et al.*, 1972), and group-D chromosomes with IgM deficiency (Kretschmer *et al.*, 1968), and severe combined immunodeficiency (Calvani *et al.*, 1976). An association between swollen feet and chromosome 18 abnormality has been described (Vianna-Morgante *et al.*, 1976) without immunological data. Perhaps there was a generalised genetic predisposition to chromosome instability involving the C-group defect in the mother, the 13/18 in the child, and one which we can only guess at in the uncle, causing the immunodeficiency.

Cytogenetic study of both PHA- and PWM-stimulated cultures permits study of variation of mosaicism in different cell populations and organ-specific cell selection. Full interpretation of these relationships demands more data, but if such associations are found consistently, they may throw light on the chromosome distribution of genes which control immunity mechanisms, other than those already described.

Addendum

Since completing this paper we have observed a child with a ring 18-chromosome, who showed the somatic abnormalities and IgA deficiency similar to the child described by Finley *et al.* (1968). Our patient was mosaic for this abnormality (3 cells 45, XX, -18; 17 cells 46, XX, r 18).

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