IDIOPATHIC CD4+ LYMPHOCYTOPENIA: A COMMON SYNDROME IN ARAB CHILDREN UNDER INVESTIGATION FOR IMMUNODEFICIENCY

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Abstract — The case definition for the syndrome of idiopathic CD4+ T-lymphocytopenia (ICL) includes a heterogeneity of disorders. As yet there have been very few published reports of children who meet the WHO/CDC criteria and it is unclear whether ICL is an acquired or inherited disorder. Children referred to a paediatric immunodeficiency unit between 1991 and 1993 for investigation of immunodeficiency were included if they met the above criteria. Six unrelated children from various locations in the Persian Gulf region met the criteria for ICL. The parents of all six patients were related. These patients emphasise the heterogeneity of the syndrome and that low CD4+ counts may be present in early childhood. In view of the early onset of symptoms ICL may be congenital in some patients. parental consanguinuity suggests an autosomal recessive mode of inheritance.

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INTRODUCTION

The human immunodeficiency virus (HIV) infects and depletes CD4+ T-lymphocytes and is the major infective cause of secondary immunodeficiency. Increasing numbers of adults are being reported with CD4+ T-lymphocytopenia but without evidence of HIV infection (1-13). An accepted case definition for a syndrome of idiopathic CD4+-T-lymphocytopenia (ICL) includes a heterogeneity of disorders (14-16). These patients have other features characteristic of HIV-induced AIDS including normal or raised immunoglobulin levels and many have opportunistic infections but had been healthy in earlier life. This pattern is consistent with an acquired immunodeficiency possibly as a result of infection with an unknown agent (17), though available evidence makes this unlikely (14). Most reported cases of ICL have been adults, however, the duration of CD4+lymphocytopenia is unknown. The very small number of children reported with ICL may be partially due to the higher normal levels of CD4+ lympohocytes in childhood making it more difficult to reach the criterion of $<0.3\times10^9$/L (18). Low CD4+ counts are a feature of several inherited immunodeficiencies presenting in childhood but most have either a defined genetic cause or are accompanied by low CD8+ and immunoglobulin levels. We identified six children from a defined ethnic background who fulfilled the criteria for a diagnosis of idiopathic CD4+ T-lymphocytopenia.

MATERIALS AND METHODS

Study Population

Patients were identified from children referred over a period of two years for investigation of possible immunodeficiency who met the criteria for ICL agreed upon by the CDC and the WHO: (10)

a. persistently low CD4+ lymphocytes ($<0.3\times10^9$/L or < 20% of total)

b. No evidence of HIV by serology

c. No other known cause for the immunodeficiency

d. Presence of opportunistic infections

One patient (No.3) has been previosuly described in another series of immunodeficient children (19).

Virological Testing

Serum samples were tested for antibodies to HIV 1,2 with an ELISA test (Bioelisa HIV 1+2, Biokit SA - Barcelona, Spain). HIV p24 antigen in serum was tested using the Coulter ICD prep. HIV-1 PCR was performed, using the Amplicor HIV-1 test kit (Roche Diagnostic Systems.) Antibodies to HTLV-1 and 2 were also screened for by ELISA.

Immunological Testing

For lymphocyte subpopulation analysis whole blood was incubated with directly conjugated monoclonal antibodies; the red cells lysed with FACS lysing solution

(both Becton Dickinson) and fluorescence measured on a FACSCAN flow cytometer (Becton Dickinson). Lymphocyte function was estimated by measurement of stimulation index and delta DPM units in three standard tests: Phytohaemagglutinin (PHAS) using whole blood (PHA from Murex Diagnostics Ltd), Candida and PPD stimulation on ficoll separated mononuclear cells. Serum IgG, A and M levels were determined using polyclonal antibodies (Atlantic, Scarborough, Maine, USA) on a centrifugal fast analyser. Serum IgG subclasses were measured by single radial immunodiffusion assay using monoclonal antibodies obtained from Unipath, Bedford, UK. Serum isohaemagglutinin titres were determined by agglutination of standard erythrocytes in saline solution and compared with published values. NitroBlue Tetrazolium Test (NBT), C3, C4, and total haemolytic complement levels were measured by standard methods. Adenosine Deaminase (ADA) and Purine Nucleoside Phosphorylase (PNP) deficiency were excluded as previously described (20,21).

RESULTS

Six patients were identified during the period 1991-1993, who had no evidence of previously defined congenital immunodeficiencies, but fulfilled the criteria for ICL. Their demographic and clinical characteristics are shown in Table 1. Five children were products of first cousin marriages and the parents of the sixth child were also related. The parents had no symptoms of immunodeficiency but patient 3 had a sibling who had died from infection and was reported to be IgA deficient. No known HIV risk factors were present except blood transfusions given to patients 1 and 6 but after the onset of symptoms. Five of the patients remained clinically stable for three years on regular immunoglobulin therapy and porphylactic antimicrobials, but with persistently low CD4+ counts. Patient 1 had continued infections and eventually died of liver failure due to sclerosing cholangitis.

The results of virological studies and lymphocyte enumeration are also shown in Table 1. No evidence of infection with HIV 1,2 or HTLV1,2 was found. The total lymphocyte counts were low - median 116, range 0.80-1.98$\times 10^9$/L. CD4+ counts were particularly low, and ranged from 0.07-0.27 with a median of 0.12$\times 10^9$/L. CD8+ counts were normal or near normal in four patients, but were markedly depressed in two cases. CD4/CD8 ratios were always low and ranged from 0.17-0.78.

The rest of the immunological results are shown in Table 2. Proliferative responses to mitogens and antigens were generally reduced. Serum IgG, A, and M were within normal limits in all cases except patient 3, who had elevated concentrations of IgG, and low levels of IgA. Low levels of IgG2 were noted in four patients and low levels of IgG3 in three patients. IgG antibody production was confounded by blood or immunoglobulin transfusion in most patients. IgM antibody production (isohaemagglutinin titre) was relatively well preserved. NBT test, C3, C4, and total haemolytic complement levels were normal.

Table 1. Clinical, Laboratory and Demographic Characteristics of Six Patients with Idiopathic CD4+ T Lymphocytopenia

Patient /Sex	Age at study/ presentation	Origin/Con-sanguinuity	HIV antibody HIV 1 PCR P24 antigen	Lymphocytes* CD4** 10^9/L-% CD8#	CD3 (T-cells)% CD 16 (NK-cells)% CD19 (B-cells)%	IgG g/L IgA.g/L IgM g/L	(normal range)	Clinicala features
1.F	7 years/ 2 years	South West Coast of Iran 1st. cousins	Negative 1+2 negative ND	1.26 0.11 - 16 0.63-42	49 6 13	15.0 1.84 0.55	(5.0-16.0) (0.5-2.4) (0.5-1.8)	Candidiasis, salmonella enteritis and osteomylitis, herpetic keraatitis, eczema, chronic suppurative otitis media, molluscum contagiosum, cryptosporidiosis sclerosing cholaangitis, failure to thrive.
2.F	17 months/ 4 months	Oman 1st. cousins	negative 1+2 negative negative	0.80 0.12-15 0.15-19	18 27 49	11.7 0.43 0.60	(3.0-10.9) (0.2-0.7) (0.6-2.1)	Candidiasis, cryptosporidiosis, disseminated BCG infection, recurrent chest infection, severe failure to thrive.
3.F	9 years 8 years	Kuwait cousins	negative 1+2 negative negative	1.06 - 0.27 - 25 0.55 - 52	72 17 4	25.3 0.11 0.71	(5.4-6.0) (0.5-2.4) (0.5-1.8)	E-coli urinary tract infections, mycobacterium fortuitum, chronic diarrhoea, severe failure to thrive.
4.M	15 months/ 3 months	Oman 1st. cousins	negative 1+2 ND negative	1.26 - 0.10 - 8 0.50 - 40	42 17 37	10.6 0.60 0.93	(0.3-0.9) (0.2-0.7) (0.6-2.1)	Candidiasis, salmonella enteritis, cryptosporidiosis, recurrent cutaneous and chest infections (bronchiectasis), severe failure to thrive
5.M	3 years/ 6 months	UAE 1st. cousins	negative 1+2 ND negative	0.82 - 0.07 - 9 0.32 - 39	77 2 18	7.1. 1.7 0.83	(3.7-15.8) (0.3-2.) (0.5-0.8)	Candidiastis, herpes, pneumococcal sepsis, failure to thrive cryptosporidiosis, pneumocystis carinii
6.M	3.5 years/ 7 months	Saudi Arabia 1st. cousins	negative 1+2 negative ND	1.98 - 0.18-9 1.03-52	57 10 33	4.2 1.73 0.34	(3.7-15.8) (0.3-2.0) (0.5-2.0)	Recurrent salmonella enteritis, pseudomonas otitis, respiratory infections, rickets, severe failure to thrive.

Normal range (10^9/L)

* Total lymphocytes < 6 years 2.8 - 5.1 > 6 years 2.0 - 2.7

** CD4 < 6 years 1.0 - 1.8 > 6 years 0.7 - 1.1

\# CD8 < 6 years 0.8 - 1.5 > 6 years 0.6 - 0.9

Table 2. Other Immunological Investigations.

Patient	Blood group	Isohaemagglutinis*	PHA	Candida	PPD	IgG subclasses mg/dl (normal range)	
1	B + ve	Anti A 1:8	SI 3.5**	SI 17.1	SI 3.9	1 = 580	(280-900)
			δ 3813***	δ 4195	δ 774	2=22	(59-151)
						3=23	(38-78)
						4=6	(7.5-26)
2	O + ve	Anti A 1:32	SI 2.2	SI 7.29	SI 2.3	1 = 84	(222 - 600)
		Anti B 1:512	δ 1509	δ 10265	δ 2125	2=112	(40 - 120)
						3 = 30	(9 - 45)
						4 = 6	(8 - 25)
3	B + ve	Anti A 1:32	Not tested	Not tested	Not tested	1 = 290	(280 - 900)
						2 = 47	(59 - 251)
						3 = 14	(38 - 78)
						4 = 17	(826)
4	AB ++ ve	Not applicable	SI 1.47	SI 0.06	SI 0.49	1 = 760	(231 - 599)
			δ 2869	δ 1346	δ 2190	2 = 88	(40 - 120)
						3 = 104	(9 - 45)
						4 = 18	(8 - 24)
5	O + ve	Not done	SI 10.6	Not tested	Not tested	1 = 440	(303-729)
			δ 6715			2 = 20	(40 - 188)
						3 = 23	(15-37)
						4 = 18	(6-33)
6	A + ve	Anti B 1:2	SI 55.7	SI 13.1	SI 3.0	1 = 144	(303 - 729)
			δ10672	δ 65096	δ 11107	2 ≤ 3	(40 - 188)
						3 = 12	(15-37)
						4 = 32	(6-33)

* Normal range > 1:8 ** SI *** d

DISCUSSION

Six children under investigation for immunodeficiency over a period of two years met the criteria for ICL. The first adults with ICL were described in 1989 (1) and the condition was the focus of much interest at the 8th International AIDS Conference in 1992, particularly because of the possibility of a new immunodeficiency virus causing the condition (14). Though ten children were under investigation by CDC in 1993 (4) there are very few published reports of children (22,23) who meet the CDC/WHO criteria (10). The data reported on adults (1,13) indicates that ICL is rare, is probably not new, is not caused by any recognised virus and there has been no consistent isolation of novel tranmissible agent (17). It seems to be a heterogeneous condition (16), different to HIV disease, though some individuals with ICL have been reported who have had high risk exposure to HIVI (3,9) or may even have cleared the virus after infection (24). A minority of adults have an inherited immunodeficiency (15) and the same is likely to be true for children.

All six children in this study had profound depression of their CD4+ T-lymphocyte counts and significant opportunistic infections. There was no evidence that a known or unknown transmissible agent was responsible, the cases were not clustered, coming from different parts of the Persian Gulf region. The predominant course was not one of a progressive clinical and immunological deterioration as seen in HIV infection. Five of the patients remained clinically stable on regular immunoglobulin therapy and prophylactic antibimicrobials and all six had persistently low but stble CD4+ counts.

The patients did not fulfill criteria for diagnoses of previously defined immunodeficiencies, e.g. common variable immunodeficiency or severe combined immunodeficiency. Patients with these latter conditions generally have more profound defects in B-cell development and hypogammaglobulinaemia. Most of our patients had normal levels of immunoglobulin making a diagnosis of primary immunodeficiency unlikely. We also excluded ADA deficiency which has been described as a cause of ICL in adults (15) and PNP deficiency which causes a syndrome of T-lymphocyte deficiency with preserved immunoglobulin production in children (21).

The poor proliferative responses to mitogens and antigens were probably a reflection of the severe CD4+ T-lymphocytopenia, though intrinsic lymphocyte abnormalities are also a possibility (23). The IgG subclass deficiencies were probably because of poor B and T-cell interaction and link these patients to another series of patients predominantly from the Persian Gulf

(15). The latter group were characterised by hypergammaglobulinaemia that masked major abnormalities of IgG2 and antibody production and patient 3 in included in both reports. Both groups of patients had high levels of consanguinity, this is suggestive that these patients suffer from one or more recessively inherited congenital immunodeficiencies affecting lymphocyte function. This is supported by the occurrence of a probable immunodeficiency in a sibling of patient 3.

CD4+ T-lymphocytes are higher in children compared to adults and individuals with counts $<0.3\times10^9$/L because they fall at the lower end of the normal range should be vanishingly rate (18). Low CD4+ counts may be a normal finding in certain ethnic groups, however this has not been reported in Arab populations and is not found in most patients referred to us from the Persian Gulf. ICL is not confined to children from Arab populations since we have identified patients from Europe and sub-Saharan Africa who meet the diagnostic criteria. Patients with the level of immunodeficiency described in this report may be over represented in referrals from abroad to a specialist centre. Children with more minor immunodeficiency might not warrant referall, whereas those with more severe immunodeficiency may die before transfer can be arranged. However, ICL is probably not as rare in children as the lack of published reports suggest, and may be relatively common form of primary immunodeficiency in some ethnic groups.

It is unlikely that these patients have infections caused by a previously described immunodeficiency virus but probably suffer from one or more genetic immunodeficiencies. The case definition for ICL was constructed in response to patients who had clinical features and immunological investigations consistent with HIV infection but were negative for all tests for the virus. This report emphasises that the case definition can include children with probable inherited congenital immunodeficiency and adds to the hetergeneity of disorders which fall within it. It thus has limitations as a form of classification of immunodeficiency which is better classified on the basis of defined molecular defects or pathogens. It has, however, proved useful in focusing attention on previously rarely described conditions of unknown pathogenesis as are seen in these patients.

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