ORIGINAL ARTICLE

Antibody deficiency and autoimmunity in 22q11.2 deletion syndrome

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Background: Although severe T cell immunodeficiency in DiGeorge anomaly is rare, previous studies of humoral function in these patients have found no antibody abnormalities but have not examined the response to polysaccharide antigens. Isolated cases of autoimmunity have been reported. Several patients with 22q11.2 deletion attending our immunology clinic suffered recurrent sinopulmonary infection or autoimmune phenomena.

Aims: To investigate humoral immunodeficiency, particularly pneumococcal polysaccharide antibody deficiency, and autoimmune phenomena in a cohort of patients with 22q11.2 deletion.

Methods: A history of severe or recurrent infection and autoimmune symptoms were noted. Lymphocyte subsets, immunoglobulins, IgG subclasses, specific vaccine antibodies, and autoantibodies were measured. Subjects were vaccinated with appropriate antigens as indicated.

Results: Of 32 patients identified, 26 (81%) had severe or recurrent infection, of which 13 (50%) had abnormal serum immunoglobulin measurements and 11/20 ≥ 4 years old (55%) had an abnormal response to pneumococcal polysaccharide. Ten of 30 patients (33%) had autoimmune phenomena; six (20%) were symptomatic.

Conclusions: Humoral immunodeficiency is more common than previously recognised in patients with 22q11.2 deletion. Normal T cell function and immunoglobulin levels do not exclude poor specific antibody responses. Patients should be referred for formal immunological assessment of cellular and humoral immune function.

DiGeorge anomaly, first described in 1968, is characterised by thymic hypoplasia, hypocalcaemia, cardiac outflow tract defects, and facial dysmorphism, but shows wide clinical variability. The majority of patients are heterozygous for a deletion at 22q11.2, a finding also seen in Schprintzen (velocardiofacial) and occasionally Opitz (G/BBB) syndromes. Patients with similar clinical features, but with a deletion in 10p have also been described. Initially, DiGeorge anomaly was associated with severe T cell immunodeficiency,¹ although recent studies have confirmed that this is rare.² Previous studies of humoral function in patients with 22q11.2 deletion syndrome have found no antibody abnormalities,³ recurrent infection with only minor immunoglobulin abnormalities,⁴ or selective polysaccharide antibody deficiency in one family.⁵ An increased incidence of IgA deficiency has also been described.⁶ An indication of further humoral dysregulation has been suggested, with isolated reports of autoimmunity in patients with heterozygous 22q11.2 deletion.^{7,8}

Previous studies of immune function have focused on patients with severe T cell immunodeficiency, or humoral dysfunction, but have not specifically examined the response to polysaccharide antigens. The lack of studies of antipolysaccharide antibody responses in 22q11.2 deletion may have been because such responses are seen as T cell independent. As 22q11.2 deletion has been viewed as a T cell immunodeficiency, such studies may have seemed irrelevant. Original studies looking at the T independent polysaccharide response were in mice. There is increasing evidence to suggest a T cell requirement for this response at least in man.⁹ Several patients with 22q11.2 deletion who attend our paediatric immunology clinic suffered recurrent sinopulmonary infection or autoimmune phenomena. We therefore looked for evidence of humoral immunodeficiency, particularly pneumococcal polysaccharide antibody deficiency, and autoimmune phenomena in an unselected cohort of patients with 22q11.2 deletion.

METHODS

Patients with 22q11.2 deletion were routinely referred for an immunological assessment from regional genetics, paediatric cardiology, as well as general paediatric services. Referral was made either because of the diagnosis of 22q11.2 deletion, or because a patient with 22q11.2 deletion was suffering from recurrent infection. Some children being seen in the immunology clinic were diagnosed with 22q11.2 deletion as part of the diagnostic work up. A history of recurrent infection and symptoms suggestive of autoimmune disease were noted, as well as immunisation history, and details of cardiac surgery. Severe infection was defined as radiologically proven pneumonia or invasive infection in a normally sterile site. Recurrent infection was defined as more than five episodes of sinopulmonary infection requiring antibiotics each year. Serum immunoglobulins and IgG subclasses were measured by rate nephelometry; specific antibodies against tetanus, Hib, and pneumococcus were measured by enzyme linked immunosorbent assay (ELISA); and serum antithyroid microsomal, antinuclear, reticulins, mitochondrial, smooth muscle, and gastric parietal cell antibodies were screened by indirect immunofluorescence. A low immunoglobulin level was defined as less than 2 standard deviations below the age related geometric mean. Low specific antibody levels were defined as a level below the lower limit of normal laboratory range.^{10,11} Where specific antibodies were low, subjects were vaccinated with tetanus toxoid, conjugated *Haemophilus influenzae* type b, tetanus, and 23 polyvalent pneumococcal polysaccharide vaccine as indicated; serum levels were remeasured four weeks later. An absent response was defined as a final level below the lower limit of normal laboratory range, a poor response as a less than fourfold rise in antibody level with a final level above the lower limit of normal laboratory range, post vaccination. Lymphocyte subsets were measured by flow cytometry using a Becton Dickinson FACScan (Becton Dickinson UK Ltd, Oxford) and analysed using

Table 1 Median lymphocyte numbers and immunoglobulin levels in 22q11.2 deletion patients with severe or recurrent infection

Patient (age)	Follow up (y)	Infection	CD3 (cells/ μ l)	CD4 (cells/ μ l)	CD8 (cells/ μ l)	CD19 (cells/ μ l)	IgM (g/l)	IgA (g/l)	IgG (g/l)	IgG subclass (g/l)	Sp Abs	Treatment
*1 (6 y)	6	URTI, OM	1321	811	533	391	1.0	1.51	5.97	N	Low PPS	Nil
2 (7 y)	6	Pneumonia, URTI	1293	725	400	945	1.02	<0.07	13.3	High IgG1	N	Co-trimoxazole
3 (12 y)	4	Recurrent pneumonia	378	258	109	92	0.19	<0.07	2.2	Low IgG2	Low Tet, PPS	IVIg
4 (9 y)	8	OM, otorrhoea	—	—	—	—	0.68	2.31	16.8	N	N	Nil
5 (12 y)	7	Pneumonia, URTI, OM	560	295	274	306	0.28	0.57	7.85	N	N	Co-trimoxazole
6 (22 y)	10	URTI	505	243	297	156	0.66	1.1	11.4	N	Low PPS	Co-trimoxazole
7 (4 y)	2	URTI, otorrhoea	2163	1260	808	1147	0.33	0.39	5.7	N	Low PPS	Co-trimoxazole
8 (12 y)	1	OM, mastoiditis	643	355	244	313	0.6	0.58	13.0	N	N	Nil
9 (8 y)	8	URTI, endocarditis	1615	1052	567	550	0.46	1.54	7.95	N	Low Tet, PPS	Co-trimoxazole
10 (0.5 y)	0.5	Septicaemia [†]	2725	1958	919	519	0.52	0.22	2.8	—	—	IVIg
11 (0.5 y)	0.5	Pneumonia [‡]	1048	816	265	367	0.53	0.19	4.02	N	—	Co-trimoxazole, IVIg
12 (5 y)	4	URTI	—	—	—	—	0.5	0.44	9.0	N	N	Co-trimoxazole
*13 (9 y)	7	URTI	1229	613	593	413	0.49	1.59	9.48	N	Low PPS	Nil
14 (7 y)	4	OM	3000	1248	1871	596	0.59	0.53	8.98	Low IgG2	Low PPS	Co-trimoxazole
15 (4 y)	4	LRTI	1542	971	550	762	0.49	0.27	5.25	N	N	Co-trimoxazole
16 (13 y)	6	LRTI Bronchiectasis	1264	1224	1010	581	0.86	<0.07	14.9	Low IgG2	Low Tet, PPS	Co-trimoxazole
*17 (17 y)	2	Pneumonia, sinusitis	778	439	310	233	1.2	1.87	8.61	N	N	Nil
18 (1 y)	1	Septicaemia	850	651	227	326	0.3	<0.07	5.0	N	N	Nil
19 (12 y)	2	Pneumonias	1224	629	437	297	0.77	1.7	10.6	N	N	Nil
20 (5 y)	5	URTI/LRTI	748	339	351	619	0.31	0.37	4.42	N	Low PPS	Co-trimoxazole
21 (6 y)	6	URTI, otorrhoea	799	382	309	765	0.89	0.73	7.4	N	Low PPS	Co-trimoxazole
*22 (4 y)	2	URTI, pneumonia	626	313	302	383	0.72	0.32	9.6	N	N	Co-trimoxazole
23 (9 y)	9	URTI	1582	831	582	985	0.54	1.83	12.1	N	N	Nil
24 (8 y)	2	URTI, pneumonia	821	397	316	558	0.37	0.93	7.15	Low IgG2	Low PPS	IVIg, co-trimoxazole
25 (5 y)	1	URTI	852	355	503	326	0.75	0.79	10.2	Low IgG1	N	Nil
26 (8 y)	1	Pneumonia, otorrhoea, mastoiditis	896	512	398	270	0.55	0.66	4.35	Low IgG1	N	Nil

URTI, upper respiratory tract infection; LRTI, lower respiratory tract infection; IVIg, intravenous immunoglobulin.

*Recurrent infection which resolved with increasing age.

[†]*Klebsiella oxytoca* septicaemia.[‡]*Pseudomonas aeruginosa* pneumonia.

Table 2 Positive autoantibodies and clinical symptoms in children with 22q11.2 deletion

Patient	Age (y)	Sex	Autoantibodies	Clinical symptoms
2	7	F	Persistent +ve RhF	Raynaud's phenomenon
3	12	F	Persistent +ve RhF	Evans syndrome
4	9	F	+ve GPC	Pauciarticular arthritis
5	12	F	Transient +ve ANA	Nil
6	22	F	+ve thyroid microsomal, +ve ANA, +ve cardiolipin, +ve AECA antibodies	Raynaud's phenomenon, vasculitis
7	4	M	+ve antiplatelet antibody	Thrombocytopenia
8	12	F	Nil	Urticaria, Raynaud's phenomena
9	8	M	Transient +ve GPC	Nil
13	9	M	Transient +ve SM antibody	Nil
27	4	M	Persistent +ve RhF	Nil

RhF, rheumatoid factor; GPC, gastric parietal cell; ANA, antinuclear antibody; AECA, antiendothelial antibody; SM, smooth muscle.

the SimulSET 3.0F programme (Becton Dickinson). Investigations were organised as part of routine clinical evaluation and care.

RESULTS

Thirty two patients were identified with a hemizygous 22q11.2 deletion between 1992 and 2001. Nineteen were male (age 0.67–13 years, median 6), 13 were female (age 1–22 years, median 9.5). Seventeen had undergone cardiac surgery and six others had normal cardiac anatomy. Median follow up was three years (range 0.5–10 years).

Twenty six patients (81%) gave a history of severe ($n = 14$) or recurrent ($n = 21$) infection, the majority of which was sinopulmonary and included recurrent radiologically confirmed pneumonia, mastoiditis, computerised tomography confirmed bronchiectasis, *Streptococcus sanguis* endocarditis in a child with congenital heart disease, pneumococcal septicaemia in a child who was not asplenic, *Klebsiella oxytoca* septicaemia, and recurrent otorrhoea (table 1). In four, the recurrent infections resolved with increasing age. Eleven patients (34%) aged 6 months to 13 years received daily prophylactic co-trimoxazole because of severe or recurrent infection. Five patients (16%) received intravenous immunoglobulin every three weeks, because of recurrent infection associated with low antibodies and poor responses to vaccines, or in two cases, severe infection owing to *Pseudomonas aeruginosa* pneumonia and *Klebsiella oxytoca* septicaemia respectively. One patient had bronchiectasis, proven on high resolution computed tomography scan, despite prophylactic antibiotics; the other two had chronic chest symptoms, radiological abnormalities, and recurrent breakthrough sinopulmonary infection despite prophylactic antibiotics. One patient died from *Klebsiella oxytoca* septicaemia. Ten patients (31%) gave a history of recurrent infection, but did not receive prophylactic antibiotics.

Of the 26 patients with recurrent infection, 13 (50%) had abnormalities in serum immunoglobulin measurements: one had an isolated low IgG, one had an isolated high IgG, two had an isolated low IgG2 subclass, two had an isolated low IgG1 subclass, two had an isolated low IgM, one had an isolated high IgA, two had an absent IgA and low IgG2 subclass, and one each had an absent IgA and low IgM, and a low IgG1 and absent IgA respectively (defined as ≤ 2 SD below age related reference range).

Of 27 patients investigated for a specific antibody response to pneumococcal polysaccharide antigen, 11 of 20 who were ≥ 4 years old (55%), when a response would be expected, had an absent or poor response. Ten of these received prophylactic co-trimoxazole or intravenous immunoglobulin; one other patient was asymptomatic. Three patients also had a poor specific antibody response to tetanus toxoid protein antigen.

Ten of 30 patients investigated (33%) had autoimmune phenomena. Three (30%) had transient positive autoantibodies only, one had persistent positive autoantibodies only, five (17%) had positive autoantibodies associated with autoimmune disease (one vasculitis and Raynaud's phenomenon, one seronegative pauciarticular arthritis, one Raynaud's phenomenon, one thrombocytopenia, one Evan's syndrome), and one autoimmune disease with negative autoantibodies (recurrent urticaria with Raynaud's phenomena) (table 2). All 10 had either low immunoglobulins or poor response to specific vaccine antigens.

Six patients (19%) had anatomically normal hearts, five with a history of recurrent infection. Only one, who was asymptomatic, had no abnormality in immunoglobulin levels, specific antibody responses, or autoimmune phenomena. Seventeen patients (53%) underwent cardiac surgery; two had no immunoglobulin abnormality and no infection, and a further four had no immunoglobulin abnormality but suffered from recurrent infection.

The median CD4 count was 696 cells/ μ l (range 210–1958), median CD8 count was 392 cells/ μ l (range 109–1858), and median CD19 count was 544 cells/ μ l (range 92–6942).¹² Twenty of 25 (80%) patients with recurrent or severe infection had low CD8 numbers (defined as ≤ 2 SD below age related reference range) compared with 8/9 (89%) patients with autoimmune phenomena. Sixteen of 25 (64%) had low CD4 numbers compared with 7/9 (78%) patients with autoimmune phenomena. Fourteen of 25 (56%) had low CD19 numbers compared with 2/9 (22%) patients with autoimmune phenomena.

Twenty seven of 32 patients (84%) had clinical symptoms related to humoral immunodeficiency, with abnormal serum immunoglobulin levels or poor specific antibody responses or autoantibodies with autoimmune phenomena.

DISCUSSION

Profound T cell immunodeficiency in patients with 22q11.2 deletion is rare, with an incidence of $<1.5\%$.² Humoral immunodeficiency has largely been overlooked and is either not found³ or reported as isolated familial polysaccharide antibody deficiency.³ Our study indicates that humoral dysfunction associated with recurrent infection or autoimmune phenomena is more common than previously recognised. Eighty four per cent of our patients had evidence of recurrent infection or autoimmune phenomena, and 81% had evidence of severe or recurrent infection. There was one infection related death owing to *Klebsiella oxytoca* septicaemia; other severe infections included recurrent bacterial pneumonias, pneumococcal septicaemia, mastoiditis, and *Streptococcus sanguis* endocarditis (in an anatomically abnormal heart).

Less immediately serious "minor" infections such as recurrent otorrhoea and upper respiratory tract infection, were

common and may lead to more serious sequelae in the long term. Fifty five per cent of our patients who were at an age where a response would be expected have poor or absent specific antibody responses to pneumococcal polysaccharide, the most common immunological abnormality in this cohort. Almost half of our patients have received prophylactic co-trimoxazole or intravenous immunoglobulin and are symptomatically improved. The effect is not simply secondary to cardiac surgery, as nine of our patients had not undergone surgery, and five of six with normal cardiac anatomy also suffered from recurrent infection. Many patients were referred routinely because of a diagnosis of 22q11.2 deletion, rather than because of symptoms, and so our high figures are unlikely to be simply because of reporting bias. It may be that there are two cohorts of patients being followed: those seen with infectious problems precipitating referral to a specialist unit, and another smaller cohort referred for assessment because immunodeficiency is recognised within the spectrum of abnormalities associated with 22q11.2 deletion. Even if this were the case, our findings, which have not previously been recognised, indicate that a high proportion of children with 22q11.2 deletion and sinopulmonary infection have an abnormal pneumococcal polysaccharide antibody response.

Autoimmune arthritis is more common in patients with 22q11.2 deletion, and autoimmune cytopenias have also been reported.^{7,8} Ten (31%) of our patients had evidence of autoimmune phenomena. Although in three this was simply a transient positive autoantibody titre possibly secondary to intercurrent infection, six patients (19%) had symptomatic clinical autoimmune disease. All patients with autoimmune phenomena had an associated abnormality in immunoglobulin levels or specific antibody response.

A number of conflicting observations have been previously made in patients with 22q11.2 deletion. The majority of our patients had low B, T helper, and T cytotoxic cell numbers when compared with age related normals, but lymphopenia does not necessarily lead to persistent or severe recurrent sinopulmonary infection. Many patients with no apparent T cell immunodeficiency have no thymus visible at cardiac surgery, although microscopic thymic tissue is likely to be present. The thymus is important in educating T lymphocytes.

Humoral immunodeficiency in DiGeorge anomaly may reflect aberrant T cell/B cell interaction. Increased T cell apoptosis is described in a patient with DiGeorge anomaly, possibly explaining in part the T lymphopenia seen in this condition.¹³ Decreased numbers of CD5+ cells, implicated in autoimmunity, have also been observed.¹⁴ However, neither of these findings explains the increased incidence of autoimmunity found in these patients, in whom an increase in CD5+ B cells or a decrease in T cell apoptosis (and thus an increase in autoreactive T cells) may be more intuitive. Low numbers of CD8+ cells have also been suggested as a cause of autoimmunity.¹⁵ However, no specific pattern was associated with either humoral dysregulation or autoimmune phenomena in this study. In particular, a reversed CD4/CD8 ratio has been reported more commonly in arthritis in patients with 22q11.2 deletion, but we did not find any specific pattern of cellular or humoral abnormality.

The mechanism for these abnormalities remains unclear. Low T and B cell numbers per se do not necessarily cause immunodeficiency, but poor T cell development in an abnormal thymus might explain such a high incidence of antibody abnormalities and the autoimmune phenomena. Perhaps these occur because autoreactive T cells are less likely to be deleted in 22q11.2 deletion patients as the thymus is critical for inducing apoptosis in autoreactive T cells. Thymic maldevelopment in 22q11.2 deletion syndrome may allow such cells to persist or lead to dysregulated T cell/B cell interaction. Alternatively, recurrent infection may provoke autoimmune phenomena as may occur in other immunodeficiency states such as common variable immunodeficiency.

In conclusion, while severe T cell immunodeficiency in patients with 22q11.2 deletion is rare, humoral immunodeficiency appears to be more common than has been previously recognised. Normal T cell function and normal immunoglobulin levels do not exclude poor specific antibody responses, and susceptibility to severe or recurrent bacterial infection. Patients diagnosed with 22q11.2 deletion in cardiac, genetic, otolaryngology, and other clinics should routinely be referred for formal immunological assessment of cellular and humoral immune function. Prophylactic antibiotics help reduce the frequency of infection. Some patients have prolonged transient humoral immunodeficiency, but in others it seems to be persistent and severe enough to warrant intravenous immunoglobulin replacement therapy. The response of these patients to conjugated pneumococcal vaccines should be investigated. Furthermore, many patients have evidence of immunodysregulation manifest by autoimmune antibodies, some with clinical disease, associated with other immunoglobulin abnormalities. Whether this is simply an observation of a dysregulated thymus, or a portent of future clinical disease, remains to be determined; careful clinical follow up will be required.

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