

Autoimmunity and regulatory T cells in 22q11.2 deletion syndrome patients

To the Editor,

Chromosome 22q11.2 deletion syndrome (22q11DS), also known as DiGeorge syndrome or velocardiofacial syndrome, is one of the most common genetic disorders. 22q11DS is associated, with various degrees of thymic dysplasia, related immunodeficiency, and autoimmune disorders. Reports of autoimmunity diseases in patients with 22q11DS have been sporadic and the frequency reported so far is around 10% (1–3). Recently has been described a higher frequency (23%) of autoimmune diseases in a large cohort of patients with 22q11DS (4). A crucial role in establishing and maintaining self-tolerance and immune homeostasis in humans is mediated by regulatory T cells (Treg), defined as CD4⁺ CD25^{high}-CD127^{low} FOXP3⁺.

A decreased frequency of Treg (5, 6) or a reduction in absolute counts of Treg has been reported in patients with 22q11DS (7). An extensive analysis of immune status of ten asymptomatic children affected by 22q11DS showed an increase in memory/activated phenotype of Treg (8).

We evaluated the clinical and immunologic assessment in a cohort of patients with 22q11DS, with the aim to understand the presumed implication of regulatory T cells in the onset of autoimmune manifestations.

Data of 50 patients (28 males and 22 females) were collected in the University Department of Pediatric Bambino Gesù Children Hospital, Rome. The mean age was 6 years (median 5 years, range 0–17 Years) Clinical diagnosis of 22q11DS was confirmed by cytogenetic or molecular methods. Informed consent to collect samples was obtained from patients' family members.

Analyses of Tregs were available in 25 of 50 patients and in 22 age-matched healthy donors (HD).

Peripheral blood mononucleated cells (PBMC) were isolated and surface-stained with anti-human CD3, CD4, CD25, CD127, CD8, CD16, CD56, CD19, CD45RA, and CD45RO (all from BD Pharmingen, San Diego, CA, USA). For Tregs analysis, the samples were further processed with eBioscience Fixation/Permeabilization solution (eBioscience, San Diego, CA, USA) and the anti-human transcription factor FOXP3 Alexa488 (clone 259D; Biolegend, San Diego, CA, USA) according to the eBioscience intracellular staining protocol.

Cells were acquired with a FACSCanto II flow cytometer (Becton Dickinson Biosciences, San Jose, CA, USA) and analyzed with FLOWJO software (TreeStar Inc, Ashland, OR, USA).

In brief after gating on lymphocytes by dimensional parameters, we first identified the CD4⁺ subset; in this subset, we gated on cells with CD127^{low}/intermediate expression and CD25 positive; and in this latter gate, we evaluated the positivity for the anti-human transcription factor FOXP3. The frequency of Treg was estimated as the percentage of CD25⁺ CD127^{low} FOXP3⁺ cells among CD4 positive cells.

Statistical analysis was performed using GRAPHPAD PRISM software (Graphpad Prism software, San Diego, CA, USA).

The nonparametric Mann–Whitney test for independent samples was used for comparisons between groups and Fisher's exact test in the analysis of contingency tables.

In Table 1 are reported the data of 50 patients with 22q11DS. One patient (n. 26), presenting with a picture of severe combined immunodeficiency (SCID), has undergone thymus transplantation at the age of 7 months. At 8 years of age, a T-cell defect and a reduced frequency of Treg were observed and he was excluded from our study.

Lymphocytes counts, CD3⁺ T cells, and CD4⁺ T cells subsets were reduced in percentage and absolute counts in the majority of the patients, and CD4⁺ CD45RA⁺ subset was decreased in 14 of 35 patients, in which these data were available (40%). The frequency of CD19⁺ B cells and NK cells were normal or moderately increased in most of them (9). The absolute counts of Treg cells were significantly decreased in all patients analyzed (8–88 cells/ μ L, $p < 0.003$) compared with age-matched healthy donors, but with a normal frequency distribution (Fig. 1A). The absolute counts of Tregs decline significantly in all age groups, while their frequencies do not differ compared with healthy donors (Fig. 1B). Furthermore,

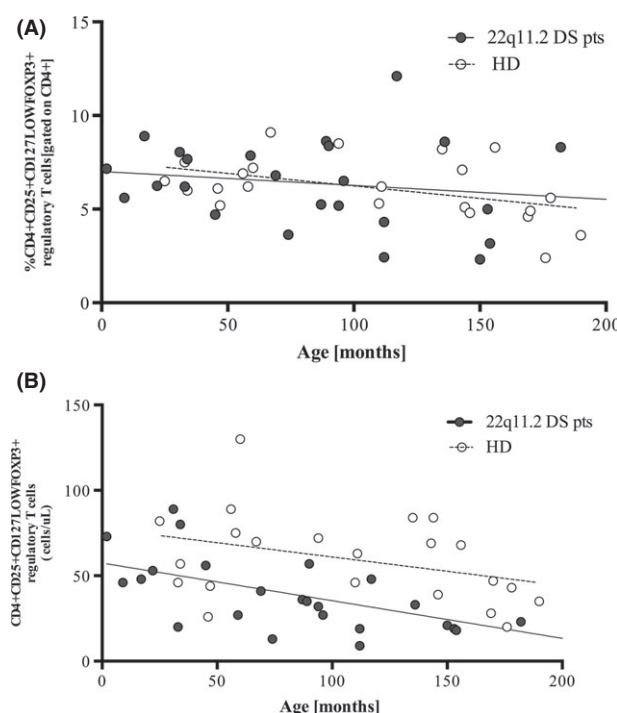


Figure 1 Tregs relative frequencies (A) and absolute counts (B) of Tregs in the peripheral blood of patients and age matched healthy controls. The absolute counts of Treg were obtained indirectly by multiplying the percentage of Treg and the absolute counts of lymphocytes.

Table 1 Patient's clinical and immunologic data

Patient	Age	Recurrent infections	Autoimmune diseases	Lymphocytes, cells/l*	CD3+, cells %*	CD4+, cells %*	CD8+, cells %*	CD19+, cells %*	CD16+ CD56+, cells %*	CD45RA on CD4+, cells %*	CD4+ CD25+ CD127low FOXP3+, cells %
1.	1 months, 18 days	RRI, B	–	2880↓	54.9	35.6	18.2	29.2	15.3	78.9	7.16
2.	8 months, 9 days	–	–	2460↓	51	33.1	14.6	34.3	13.1	90.9	5.6
3.	1 year, 5 months	RRI	–	2510↓	45.5↓	21.5↓	16.7	35.4↑	18.5	49.8↓	8.9
4.	1 year, 10 months	RRI, B, Pn	–	3460↓	50.4↓	24.4↓	23.6	26.7	22.3	55.3↓	6.25
5.	2 years, 7 months	A, RRI	–	4970	49.3↓	22.2↓	20.8	31.6	17.8↑	66.7	8.05
6.	2 years, 9 months	RRI	–	1940↓	44.9↓	17.0↓	19.8	31.6	23.1↑	55.9	6.2
7.	2 years, 10 months	RRI	–	3620	59	28.8	31.4↑	23.3	14.4	60.4	7.67
8.	3 years, 9 months	RRI	JIA	2700	69.4	44.3	20	16.9	13.7	55.8	4.71
9.	4 years, 11 months	–	–	1860↓	50.0↓	18.6↓	21.2	27.9	19.3↑	57	7.86
10.	5 years, 9 months	–	–	2500	63.5	23.9↓	28	23.8	11.9	33.1	6.8
11.	6 years, 2 mo	B, Pn	–	1350↓	–	27.4↓	–	–	20.6	–	3.64
12.	7 years, 3 months	–	–	1720↓	61.6	40	15.9↓	27.2↑	8.4	52	5.25
13.	7 years, 5 months	B, Pn	JIA, T	2270	38.3↓	17.8↓	17.8↓	30.8↑	30.1	27↓	8.63
14.	7 years, 6 months	B, O, EBV	–	2960	58.5↓	23.0↓	29.3	19.9	20.7	39.6↓	8.38
15.	7 years, 10 months	RRI	–	1810↓	53.5↓	33.8	17.2↓	21.9	24.1	50	5.19
16.	8 years, 9 months	–	–	2370	46.9↓	17.8↓	22.8	21.7	29.6	53.9	6.51
17.	9 years, 4 mo	O, B, Pn	AT	1100↓	–	18.2↓	–	–	8.7	–	4.31
18.	9 years, 4 months	RRI, B	–	1520↓	78.1↑	51.5↑	16.6↓	14.2	6.9	73.6	2.43
19.	9 years, 9 months	EBV	P	1280↓	52.3↓	31	14.9↓	27	18.9↑	44.5↓	12.1
20.	11 years, 4 months	–	–	1510↓	48.7↓	25.6↓	22.2	27.3↑	21.6	39.8↓	8.6
21.	12 years, 6 months	–	–	3370	56.4	27.4↓	16.5↓	19.6	22.7↑	29.9↓	2.31
22.	12 years, 9 months	–	–	1320↓	66.5	28.3↓	31.3	18.5	14.3	–	5
23.	12, 10 months	–	CD	1620	68.5	34.8	25.2	14.4	16.6	–	3.17
24.	15 years, 2 months	O, B	–	970↓	45.08↓	28.44↓	12.58↓	18.38	36.1↑	15↓	8.31
25.	17 years, 1 months	O, TP	P	1660	62.1	34.3	22.7	13.4	23.3↑	66.5↑	6.1
26.	8 years, 2 months	Pn, S	AT, AH, AHA	610↓	76	41.6	31.2	3.7↓	17.2↑	10↓	3.4

Table 1. (Continued).

Patient	Age	Recurrent infections	Autoimmune diseases	Lymphocytes, cells/l*	CD3+, cells %*	CD4+, cells %*	CD8+, cells %*	CD19+, cells %*	CD16+ CD56+, cells %*	CD45RA on CD4+, cells %*	CD4+ CD25+ CD127low FOXP3+, cells %
27.	6 years	Pn, B	–	2150	69.7	35.3	29.2	19.5	10.3	–	–
28.	7 years, 4 months	–	–	1310↓	65.6	29.8↓	30.5	13.5	20.5↑	–	–
29.	1 months	–	–	7660	42.6↓	27.4↓	12.417	17	37.6↑	78.5	–
30.	3 years	–	–	1280↓	53.9↓	32.9	18.6	25.3	17.5↑	–	–
31.	14 years, 10 months	–	–	1780	49.5↓	36.7	10.8↓	26.3↑	23.3↑	–	–
32.	5 years	–	–	3030	42.0↓	23.8↓	14.6↓	22	34.8↑	–	–
33.	1 years, 1 months	–	–	1210↓	28.0↓	15.0↓	9↓	28	37↑	60↓	–
34.	7 months	–	–	9490↑	61.7	58.0↑	15.5	31.7	3.2	63.8↓	–
35.	4 years	–	–	2310	58	29	17	20	19↑	72.4	–
36.	3 years, 6 months	–	–	2100↓	45.7↓	18.9↓	15.2↓	42.2↑	10.1	–	–
37.	1 years, 1 months	–	–	2010↓	37.2↓	25.5↓	9.8↓	30.9	27.8↑	58.8↓	–
38.	13 years, 8 months	–	–	1270↓	72	34	33	15	10	46.5	–
39.	2 years, 5 months	–	JIA	3421	60	35	20	25	6	–	–
40.	3 years, 2 months	–	–	1400↓	45↓	15.0↓	21	30	19↑	86.7↑	–
41.	9 years, 5 months	–	–	1515↓	59↓	27.0↓	28	26	15	–	–
42.	3 years, 6 months	–	–	4141	58	34	19	28	7.7	52.9↓	–
43.	1 years, 5 months	–	–	3644	45↓	33	10↓	24	18↑	–	–
44.	12 years, 4 months	O, A, B	–	1720	46.4↓	26.6↓	16.2↓	19.7	32.6↑	38.3	–
45.	10 years, 11 months	–	–	1540↓	54.0↓	25.9↓	27.1	20.4	24.4↑	–	–
46.	4 years, 10 months	A, O, TP, RRI	–	2400	45.0↓	31	12.5↓	36↑	17	57	–
47.	1 year	–	–	2642↓	55	43	15	20	16↑	86	–
48.	2 years, 2 months	RRI, B	AT, AHA	3596	42.0↓	30	10↓	35↑	5	–	–
49.	8 months	–	–	3782	32.0↓	22.0↓	17	28	37↑	59.1↓	–
50.	6 years	–	–	1115↓	58	24.0↓	22	20	9	62.5	–

RRI, recurrent respiratory infections; B, bronchitis; Pn, pneumonia; A, adenotonsillitis; O, otitis; EBV, persistent EBV infection; TP, tympanic perforation; S, sepsis; JIA, juvenile idiopathic arthritis; T, thyroiditis; AT, autoimmune thrombocytopenia; P, psoriasis; CD, celiac disease; AH, autoimmune hepatitis; AHA, autoimmune hemolytic anemia.

The relative frequencies are referred to lymphocytes; ↑ values above and ↓ values below the normal reference range.

*Normal values from Shearer, JACI, 2003.

there is not statistical correlation with frequency or absolute counts of Tregs and autoimmune diseases in the analyzed patients.

Nine of fifty patients (18%) had an autoimmune disease (thrombocytopenia, hemolytic anemia, arthritis, thyroiditis)

with a mean age of presentation of 3 years of age (range 1–5 years).

We observed recurrent and severe infections in 16 of 50 patients (32%); in this group, there is a higher frequency of autoimmunity (44%, 7 out of 16) compared with those without

recurrent infections (6%, 2 of 34). The association between infections and autoimmune manifestations is statistically significant ($p = 0.003$).

Our data confirm the reduction in absolute counts of Treg in 22q11DS children compared with healthy controls (7). This quantitative decrement, more evident as the age increases, might compromise the efficacy of their suppressive role and contribute to the development of autoimmune phenomena.

We observed a high prevalence (18%) of autoimmunity, mostly thrombocytopenia, anemia, and arthritis with an earlier onset, according to recent reports (3, 4, 10). Although our preliminary results did not show any association between Treg and autoimmunity, a larger cohort should be analyzed to better define the Treg role in autoimmunity in these patients. Treg subsets should be also analyzed because an impairment of naïve versus a memory/activated phenotype could compromise their functionality. Indeed, in one patient (pt13), with a picture of relapsing arthritis and severe autoimmune thyroiditis, we detected an important reduction in naïve CD4 T cells and an increased frequency of effector Treg subset (CD4⁺ CD25^{high} CD127⁺ CD45RA⁺ FOXP3^{high}) (data not shown).

Treg lymphocytes are essential in the control of the peripheral tolerance, but other factors could influence the development of autoimmune manifestations, like recurrent infections and the reduction in the naïve T-cell compartment. We found a statistically significant association ($p = 0.003$) between infections and

autoimmunity, suggesting that recurrent infections might contribute to the breakdown of immune tolerance.

In this context, we recommend to follow carefully 22q11DS children with severe recurrent infections and autoimmune manifestations; in these patients, antibiotic prophylaxis could help to control the immune activation status. Moreover, hematologists and rheumatologists should be alerted to the fact that autoimmune diseases are the most frequent in patients with 22q11DS, with tendency to recur more frequently than in children without the deletion.

The identification of prognostic markers in patients with increased risk of autoimmunity could be helpful for choosing the more appropriate prophylactic/therapeutic approach.

Silvia Di Cesare¹; Pamela Puliafito¹; Paola Ariganello¹;
Genni Enza Marcovecchio¹; Martina Mandolesi¹; Rossella Capolino²;
Maria Cristina Digilio²; Alessandro Aiuti^{3,4,5}; Paolo Rossi¹ &
Caterina Cancrini¹

¹Department of Pediatrics, University of Rome Tor Vergata and Bambino Gesù Children's Hospital, Rome, Italy; ²Department of Medical Genetics, Bambino Gesù Children's Hospital, Rome, Italy; ³San Raffaele Telethon Institute for Gene Therapy (TIGET), Division of Regenerative Medicine, Stem Cells, and Gene Therapy, IRCCS San Raffaele Scientific Institute, Milan, Italy; ⁴Pediatric Immunohematology and Bone Marrow Transplantation Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy;

⁵Vita-Salute San Raffaele University, Milan, Italy

E-mail: Di.cesare@med.uniroma2.it

DOI:10.1111/pai.12420

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