

Immunologic defects in 22q11.2 deletion syndrome

Andrew McLean-Tooke, MRCP,^a Dawn Barge, PhD,^a Gavin P. Spickett, FRCP,^a and Andrew R. Gennery, MD^b

Newcastle-Upon-Tyne, United Kingdom

Background: 22q11.2 Deletion syndrome, the most common congenital chromosome deletion syndrome, is associated with developmental defects including cardiac abnormalities and hypoplasia or abnormal migration of the thymus. These patients have variable defects in T-cell immunity with an increased incidence of infection and autoimmune disease.

Objective: To investigate the immunologic constitution of children with 22q11.2 deletion syndrome.

Methods: We characterized the immunologic constitution of 27 children with 22q11.2 deletion syndrome and 54 healthy controls by flow-cytometric analysis of peripheral blood lymphocyte populations.

Results: Patients exhibited decreased T-cell numbers, although the normal age-related decrease in T-cell numbers was slower than in healthy children. There was a significant decrease in FoxP3⁺ natural regulatory T (nTreg) cells with a strong correlation between nTreg cells and recent T-cell emigrants from the thymus, suggesting a link between the nTreg cell population and thymic function. Although total B-cell numbers were unaffected, patients showed a significantly decreased proportion of memory B cells in the B-cell pool.

Conclusion: Lower nTreg cells in patients suggest that the generation and maintenance of these cells in children is related to thymic function. In addition to T-cell abnormalities classically seen in this syndrome, subtle defects in the B-cell compartment may also be seen. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: 22q11.2 Deletion syndrome, DiGeorge syndrome, lymphocyte count, autoimmunity, regulatory T cells, memory B cells

Chromosome 22q11.2 deletion syndrome describes a heterogeneous group of disorders that share a common genetic basis. 22q11.2 Deletion syndrome is the most common chromosomal deletion syndrome with an estimated incidence of 1:4000 live births.¹⁻³ Most patients with DiGeorge syndrome and velocardio-facial syndrome have hemizygous deletions of chromosome 22q11.2.¹ Other syndromes occasionally associated with this deletion include conotruncal anomaly face syndrome, Opitz/GBBB,

Abbreviations used

NK: Natural killer
nTreg: Natural regulatory T
RTE: Recent thymic emigrant

and CHARGE syndrome (coloboma, heart disease, atresia choanae, retarded growth and central nervous system development, genital hypoplasia, and ear abnormalities and/or deafness).⁴ When all patients with 22q11.2 deletions are considered together, cardiac malformations, speech delay, and immunodeficiency are the most common characteristics.⁵

Immune deficiency in 22q11.2 deletion syndrome occurs because of thymic aplasia or hypoplasia, may be variable, and does not correlate with phenotypic features.⁴ Patients with complete absence of thymus (<0.5% of patients) exhibit severe T-cell deficiency with severe combined immunodeficiency phenotype. The majority of patients with 22q11.2 deletion syndrome and immune defects exhibit mild to moderate deficits in T-cell counts. Immunodeficiency in these patients is not a result of absence of thymus but abnormal thymic migration.⁶ Patients may have microscopic nests of thymic epithelial cells that account for their ability to produce T cells. Studies of humoral deficiencies in 22q11.2 deletion have yielded conflicting results.⁷⁻¹⁰ Commonly IgG levels are lower than normal range at birth, increasing to normal values over time.¹⁰ B-cell counts are usually normal, although abnormalities in CD27⁺ memory B-cell subsets have been seen.¹¹ Antibody deficiencies may be seen, with 43% of patients in one study exhibiting evidence of antibody deficiency (IgA deficiency, IgM deficiency, IgG subclass deficiency, or specific antibody deficiency), with a significant correlation between the presence of recurrent infections and humoral abnormalities.¹¹ However, this is not a universal finding, with other studies showing relatively normal immunoglobulin levels and specific antibody responses.^{7,12,13}

Patients with 22q11.2 deletion syndrome exhibit an increased incidence of autoimmune disease including autoimmune cytopenias,¹⁴ autoimmune arthritis,¹⁵ and autoimmune endocrinopathy.¹⁶ The reason for this increase is unclear. Increased susceptibility to infections may predispose to autoimmunity through a variety of mechanisms including bystander activation, molecular mimicry, and release of sequestered antigens. Lymphopenia itself may play a role because of changes in peripheral T-cell expansion, resulting in alterations both qualitatively and quantitatively in peripheral T-cell responses. Thymic abnormality may result in impaired generation of natural regulatory T (nTreg) cells. nTreg cells are a thymic-derived population of CD4⁺ T cells that constitutively express CD25, the high-affinity α -chain of the IL-2 receptor. The transcription factor FoxP3 has been identified as

From ^athe Department of Immunology, Royal Victoria Infirmary, and ^bthe Department of Clinical Medical Sciences (Child Health), Newcastle University.

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Reprint requests: Andrew McLean-Tooke, MRCP, Specialist Registrar in Immunology, Department of Immunology, Royal Victoria Infirmary, Newcastle-Upon-Tyne, NE1 4LP, United Kingdom. E-mail: andymctooke@yahoo.co.uk.

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TABLE I. Frequency of clinical features seen in 27 patients with 22q11.2 deletion syndrome

Clinical feature	Frequency
Congenital heart disease	56% (15)
History of recurrent or persistent infection	89% (24)
Hospitalizing infection	37% (10)
Clinical autoimmune disease	15% (4)
Positive autoantibodies	34% (9)*
Hypocalcaemia	26% (7)
Cleft palate	37% (10)
Learning difficulties	86% (19)†
Dysmorphic features	100% (27)
Other malformations	34% (9)

Number of cases is given in parentheses.

*Nine of 25 patients analyzed.

†Nineteen of 24 patients because 2 patients were too young to assess, and for 1 patient, data were not available.

critical in the development of generation of these cells.¹⁷ nTreg cells play an important role in tolerance and development of autoimmune disease.¹⁸ Neonatal thymectomy in mice results in the development of autoimmune disease that can be prevented by inoculation of nTreg cells from nonthymectomized normal mice, suggesting that the thymus plays an essential role in maintaining this immunoregulatory T-cell population.¹⁹ One study of patients with DiGeorge syndrome demonstrated a significant decrease in the percentage CD4⁺CD25⁺ of total T cells compared with normal controls, most marked in infancy. However, no difference was observed in absolute counts or proportion of CD4⁺CD25⁺ T cells in patients with and without evidence of autoimmunity.²⁰ CD25 is expressed on recently activated T cells, and contamination of analysis with these cells could not be excluded.

In this study, we examine lymphocyte subsets in a cohort of pediatric patients with 22q11.2 deletion syndrome. We particularly focused in the T-cell pool on the CD4⁺-naive nTreg and recent thymic emigrant (RTE) compartments, and the memory B-cell compartments in the B-cell pool. Quantitative and qualitative maturation of the lymphocyte compartments in these patients is discussed.

METHODS

Blood samples

Peripheral blood samples were obtained from 27 patients (19 male, 8 female) diagnosed with 22q11.2 deletion by fluorescence *in situ* hybridization attending a pediatric immunology clinic. Clinical characteristics of the patients are shown in Table I. Control samples were collected from 54 (35 male, 19 female) apparently healthy, unrelated children undergoing general anesthetic for elective day surgery. Children with infection, inflammation, allergic disease, or other major comorbidity were excluded. Ethical approval for the study was obtained from the Local Research Ethics Committee and written consent obtained from all patients and/or parents.

Immunofluorescent staining

Four-color flow cytometry was performed by using a FACSCalibur (Becton Dickinson, Cowley, United Kingdom [UK]) on peripheral blood according to standard protocols. Reagents used were allophycocyanin-labeled mAbs to CD4 and IgM; fluorescein isothiocyanate-labeled mAbs to CD3, CD27, CD45RA, and TCR- $\alpha\beta$; phycoerythrin-labeled mAbs to CD27, CD31, HLA-DR, IgD, and T-cell receptor (TCR)- $\gamma\delta$; and peridinin chlorophyll protein-labeled mAbs to CD3, CD19, and CD45RA (BD Biosciences, Oxford,

UK). RTEs were identified in the CD3⁺CD4⁺ population by expression of CD45RA and CD31 and naive CD4⁺ T cells identified by expression of CD45RA and CD27 (see this article's Fig E1, A and B, in the Online Repository at www.jacionline.org). Intracellular FoxP3 expression (Fig E1, C) was analyzed by using a commercially available kit (eBiosciences, San Diego, Calif) as previously described.²¹ Data acquisition was performed by using Cellquest software (BD Biosciences). MultiSet (BD Biosciences) and FlowJo 7.2 (Tree Star, Ashland, Ore) software was used for data analysis.

Statistical analysis

To correct for age-related changes, comparison between groups was performed by using an analysis of covariance model with lymphocyte populations and controls or patients as main effects and age as the covariate. Correlation analysis was performed by calculating the 2-tailed Pearson correlation coefficient. A *P* value less than .05 was considered significant. Linear regression analysis was used to assess the relationship between T-cell populations and age. Statistical analysis was performed by using XLStat-Pro (Addinsoft, Paris, France).

RESULTS

Surface markers of peripheral lymphocytes were analyzed for 54 controls and 27 patients. Demographic data and mean values for lymphocyte populations for each age group for controls (see this article's Table E1) and patients (see this article's Table E2) are available in the Online Repository at www.jacionline.org.

T cells

The CD3⁺, CD3⁺CD4⁺, and CD3⁺CD8⁺ T-cell counts decreased significantly with age in controls and patients (see this article's Fig E2 in the Online Repository at www.jacionline.org). Adjusting for age, the CD3⁺, CD3⁺CD4⁺, and CD3⁺CD8⁺ T-cell counts were significantly lower in patients (*P* < .001, *P* = .023, and *P* < .001, respectively). Although patients started with lower counts, the decline in T-cell counts with age occurred at a slower rate such that the age-related decrease was blunted. Using linear regression, T cells declined at a rate of 153 cells/ μ L/y in controls, compared with 79 cells/ μ L/y in patients. CD4⁺ T cells in patients declined at a rate of 55 cells/ μ L/y, compared with 117 cells/ μ L/y for controls. CD8⁺ T cells in patients declined at a rate of 21 cells/ μ L/y compared with 35 cells/ μ L/y for controls. There was no statistical difference in the CD4/CD8 ratios between patients and controls. HLA-DR expression did not show any significant change with age for both patients and controls. However, adjusting for age, there was an increase in HLA-DR expression in patients (*P* = .019). There was no age-related change in the proportions of TCR- $\alpha\beta$ or TCR- $\gamma\delta$ expression of T cells in controls or patients. Consistent with the decreased CD3⁺ T cells in the patient group, there was a significant decrease (*P* < .001) in TCR- $\gamma\delta$ CD3⁺ T-cell counts in patients compared with controls. Patients also had significantly lower proportions of TCR- $\gamma\delta$ T cells compared with controls (*P* = .009).

Naive T cells and RTEs

To examine the kinetics of change with age in T-cell populations, the naive and RTE populations within the T-cell pool were analyzed. The naive CD4⁺ T-cell population was identified by expression of CD45RA and CD27, which has been shown to correlate with naive T cells.²² Absolute counts and proportion of naive cells in the CD4⁺ T-cell compartment significantly decreased

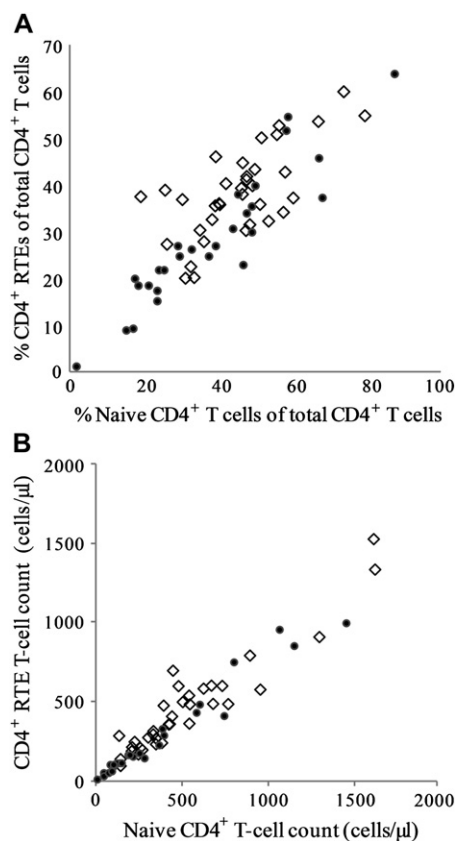


FIG 1. Naive CD4⁺ T cells vs CD4⁺ RTE counts in patients and controls. The proportions in the CD4⁺ T-cell population (**A**) and absolute counts of naive (CD45RA⁺CD27⁺) vs RTE (CD45RA⁺CD31⁺; **B**) from controls (◇) and patients (●) are shown. Correlation coefficients for patients: $r = 0.929$; controls: $r = 0.636$ (**A**); patients: $r = 0.979$; controls: $r = 0.948$ (**B**).

with age in each group ($P < .001$). Although patients showed a trend toward lower counts and proportion of CD4⁺ naive cells compared with controls, this did not reach statistical significance ($P = .062$ and $P = .108$, respectively). CD4⁺ RTEs were identified by expression of CD31 and CD45RA, which has previously been shown to correlate with T-cell receptor excision circle analysis.^{23,24} In both patients and controls, the numbers and proportion of RTEs declined with age ($P < .001$). However, patients had significantly lower numbers and proportions of RTEs compared with controls ($P = .001$ and $P = .041$, respectively). CD3⁺ T-cell counts correlated strongly ($P < .001$) with RTE counts (patients, $r = 0.927$; controls, $r = 0.926$, respectively). This strong correlation with RTEs was also seen with CD4⁺ T-cell counts (patients, $r = 0.976$; controls, $r = 0.962$, respectively). Importantly, there was a significant linear correlation for both absolute counts and proportion of CD4⁺ T cells between the CD4⁺ naive T-cell and CD4⁺ RTEs for both groups (all P values $< .001$; Fig 1).

Regulatory T cells

To assess the effect of thymic hypoplasia on nTreg cells, we analyzed the CD4⁺FoxP3⁺ nTreg populations for patients and controls. The proportion of CD4⁺ T cells expressing FoxP3 increased with age in controls ($r = 0.557$; $P < .001$) but not patients ($r = 0.147$; $P = .473$), such that in patients older than 2

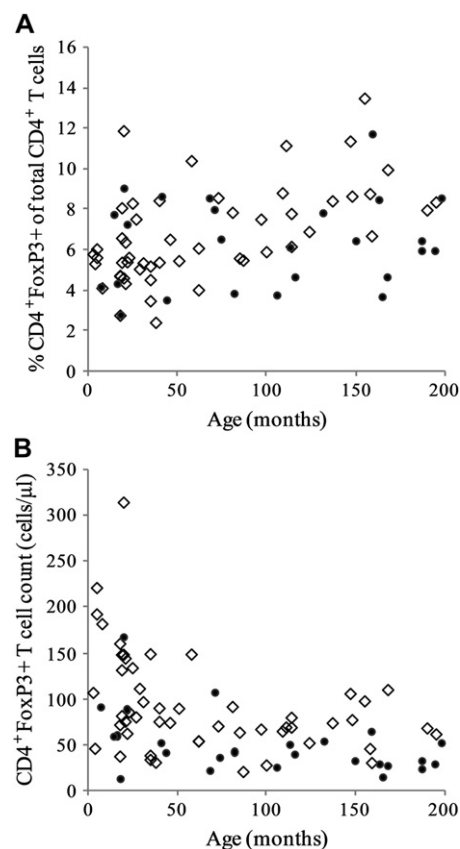


FIG 2. Absolute count and proportion of CD4⁺FoxP3⁺ nTreg cells with age in patients and controls. The proportion of nTreg cells in the CD4⁺ population (**A**) and absolute counts (cells/μL; **B**) of nTreg cells from controls (◇) and patients (●) are displayed according to age in months.

years, there was a significant decrease in the proportion of FoxP3⁺ cells within the CD4⁺ T-cell population compared with controls ($P = .030$; Fig 2, **A**). CD4⁺FoxP3⁺ nTreg cell counts decreased with age in both patients and controls ($r = -0.642$, $P = .011$; and $r = -0.609$, $P = .010$, respectively). However, the CD4⁺FoxP3⁺ T-cell counts were significantly lower in patients ($P = .008$; Fig 2, **B**). In patients and controls, the nTreg cell counts significantly correlated with thymic output as measured by RTEs (both $P < .001$; Fig 3).

Natural killer cells

Similar to CD3⁺ T cells, natural killer (NK) cell counts significantly decrease with age in patients and controls ($P = .009$ and $P < .001$, respectively). Although there was an age-related increase in the relative percentage of NK cells of lymphocytes in the control group, there was no difference in the patient group ($P = .005$ and $P = .150$, respectively). There were no significant differences in NK cell counts between patients and controls (Fig E2).

B cells

CD19⁺ B cells were stained for surface expression of CD27, IgD, and IgM. CD27 expression has been shown to correlate with memory B cells, and CD27⁺ B cells can further be divided on the basis of surface IgD and IgM expression into nonclass-switched (CD27⁺IgM⁺IgD⁺) and class-switched (CD27⁺IgM⁺IgD⁺)

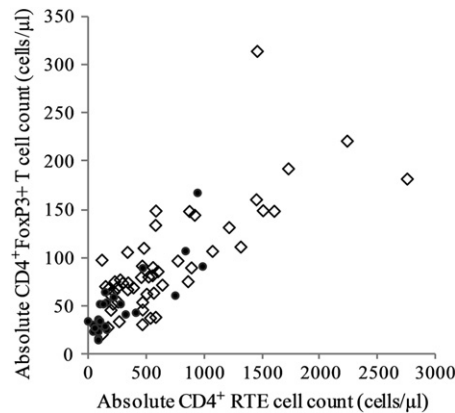


FIG 3. Correlation between nTreg cells and RTEs in patients and controls. The absolute counts of nTreg cells and CD4⁺ RTEs (CD45RA⁺CD31⁺) from controls (◇) and patients (●) are shown. Correlation coefficient for patients: $r = 0.829$; controls: $r = 0.779$.

memory B cells.^{25,26} There was no difference in B-cell numbers between patients and controls ($P = .310$; Fig E2). In both patients and controls, there was a significant increase in CD27⁺ memory B cells with age ($P = .003$ and $P < .001$, respectively; Fig 4). In controls, the proportion of both class-switched and nonclass-switched memory B cells significantly increased with age ($P < .001$ and $P = .006$, respectively). In patients, the proportion of class-switched and nonclass-switched memory B cells increased with age ($P = .004$ and $P = .061$, respectively). Comparison of patients and controls showed a significantly decreased proportion of CD27⁺ memory B cells in patients ($P < .001$), seen in both the nonclass-switched and class-switched (both P values $< .001$) memory B-cell populations.

Immunoglobulins

Serum immunoglobulin levels were measured for 26 patients (data not shown). One patient on intravenous immunoglobulin (IVIG) replacement for specific antibody deficiency was excluded. No patient had complete IgA deficiency. There were minor immunoglobulin deficiencies in 15 of 26 patients; 1 had low IgA and low IgM, 2 had low IgA only, and 12 had low IgM only. There was no correlation between IgM and B-cell counts or proportion of memory B cells. The level of IgA correlated with B-cell counts ($P = .047$). IgA levels significantly correlated with the proportion of class-switched but not nonclass-switched memory B cells ($P < .001$ and $P = .742$, respectively). In contrast, total IgG levels correlated with the proportion of nonclass-switched but not class-switched memory B cells ($P = .007$ and $P = .119$, respectively). There was no correlation between total IgG levels and B-cell counts. IgG subclasses levels were available for 23 patients; no patients had evidence of IgG subclass deficiency.

DISCUSSION

22q11.2 Deletion syndrome is associated with a highly variable immunodeficiency ranging from profound life-threatening immune deficiency to normal immunity. Early studies revealed decreased T-cell counts but an unpredictable clinical course, and the T-cell numbers may not be predictive of infection in patients with mild to moderate T-cell lymphopenia.⁵ Previous studies have

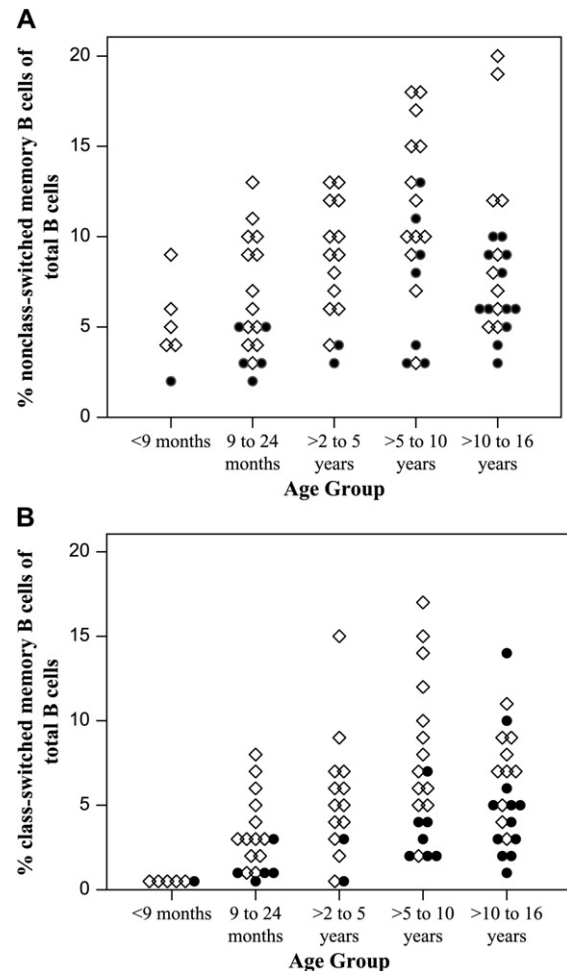


FIG 4. Memory B cells in the B-cell population in patients and controls. Proportion of nonclass-switched memory B cells (CD27⁺IgM⁺IgD⁺; A) and class-switched "memory" B cells (CD27⁺IgM⁺IgD⁺; B) in the total B-cell population of controls (◇) and patients (●) is displayed according to age group.

shown the cellular immunocompromise is age-dependent, with the greatest deficit seen in the neonatal period, and in patients there is a slower decrease in the normal age-related decrease in T cells such that relatively there is an improvement in T-cell counts with age. This study confirms the slower rate of decline in CD3⁺ T cells in patients that is dominant in CD4⁺ T cells, as suggested in recent studies.^{12,27} Although specific subsets of TCR- $\gamma\delta$ T cells have been shown in mice to develop extrathymically,²⁸ the decreased numbers seen in this cohort suggest the thymus plays a predominant role in the generation of this population in human beings. Analysis of RTEs showed significantly lower counts in patients compared with controls for all ages, consistent with decreased thymic output in these patients.²⁴ The numbers of RTEs correlated strongly with CD3⁺ and CD3⁺CD4⁺ T-cell counts, consistent with impaired thymic output playing the major role in the T-cell lymphopenia in these patients. Importantly, there was a significant linear correlation between the naive CD4⁺ T cells and CD4⁺ RTE populations. This is in contrast with the findings of Piliero et al,²⁹ who found a nonlinear correlation with age in patients, with evidence of increased replication in the naive T-cell pool by telomere length spectratyping. They

suggested the correction in the degree of T-cell lymphopenia may be a result of “homeostatic proliferation” within the naive T-cell pool. In contrast with their data in older children and adults, our data in children show a linear correlation between the naive T-cell pool and RTEs, suggesting that thymic output, not proliferation, is predominantly responsible for maintaining the naive T-cell pool in both patients and controls. This is consistent with previous studies which, using T-cell receptor excision circle analysis, have suggested that the bulk of the naive T-cell pool is sustained primarily from thymic output for individuals younger than 20 years of age, whereas proliferation within the naive phenotype is dominant for older individuals.^{30,31} Although it is clear that lymphopenia-induced proliferation occurs in the naive T-cell pool in lymphopenic states, this proliferation is typically associated with the conversion of naive T cells to a memory/effector phenotype.³² Memory T cells show enhanced homeostatic proliferation compared with naive T cells and impair their expansion and phenotypic conversion in an IL-7 receptor–dependent manner.³³ Consistent with this, there was a significant increase in HLA-DR expression in our patients that significantly correlated with lower proportions of naive T cells ($P < .001$; $r = -0.585$). HLA-DR expression has been shown to be increased in effector and memory T cells compared with naive T cells.³⁴ Taken together, these data suggest that the improvement with age in the degree of T-cell lymphopenia in children with 22q11.2 deletion is not a result of “homeostatic proliferation” of the naive T-cell pool but rather an increase in the effector/memory T-cell populations.

For the first time, this study analyzed the FoxP3⁺ nTreg compartment in patients with 22q11.2 deletion syndrome. The absolute counts of nTreg cells in both patients and controls correlated strongly with thymic output as determined by RTEs. For all ages, patients showed significantly lower nTreg cell counts compared with healthy controls. Although there is strong evidence in mice for the thymic origin of nTreg cells, there is relatively little evidence in human beings.^{35,36} Some have suggested the thymus in human beings plays a less significant role in the generation of nTreg cells. A model has been suggested of predominant peripheral nTreg cell generation occurring after activation, during which all activated T cells attain a FoxP3⁺ regulatory state with some cells persisting as nTreg cells.³⁶ Despite increased T-cell activation markers in patients compared with controls, there is no increase in the proportion of FoxP3⁺ nTreg cells seen in patients. Furthermore, the decreased counts and proportions of nTreg cells in patients with developmental thymic hypoplasia and the strong correlation of nTreg cells with RTEs suggest that the generation and maintenance of the FoxP3⁺ nTreg cell pool, at least in children, is directly related to thymic function rather than peripheral production. Relatively little is known about homeostatic control of the nTreg cell population in human beings, and it is possible that persistence of this population throughout adulthood involves peripheral induction or expansion.^{18,37} Decreased nTreg cells in these patients may contribute to the increased prevalence of autoimmune disease seen in patients with 22q11.2 deletions. Although the absolute count or proportion of nTreg cells in the patients with autoimmunity was decreased compared with controls, there was no difference compared with patients without autoimmune disease. This is consistent with a previous study showing decreased CD4⁺CD25⁺ T counts in patients, but no difference between patients with or without autoimmunity.²⁰ Other factors must also be involved in determining susceptibility to autoimmune disease in these patients.

Early studies of 22q11.2 deletion syndrome suggested the immune defect was a result of a T-cell defect. However, alterations in humoral immune responses have been reported in these patients. In one study of 13 children with 22q11.2 deletion syndrome, 43% of patients exhibited evidence of antibody deficiency (IgA deficiency, IgM deficiency, IgG subclass deficiency, or specific antibody deficiency), and there was a significant correlation between the presence of recurrent infections and humoral abnormalities.¹¹ Analysis of B-cell phenotype in these patients showed a decrease in CD27⁺ memory B cells, predominantly affecting CD27⁺IgM⁺ B cells. In another study of 26 patients, 50% had abnormal immunoglobulin levels, with 55% showing impaired antibody responses to pneumococcal polysaccharide antigen.⁹ In contrast, other studies have failed to demonstrate significant antibody deficiencies. In a study by Chinen et al¹² of 45 patients, in whom 36 had immunoglobulin levels measured, only 2 patients had decreased immunoglobulin levels (1 low IgA, 1 low IgM), but all patients had normal specific antibody responses. In another study of 30 patients with 22q11.2 deletions, none had significant immunoglobulin deficiency or specific antibody deficiency.¹³ Analysis of humoral immunity in our cohort showed evidence of immunoglobulin deficiency in 59% of patients. Low IgM levels were common, and selective IgM deficiency in 22q11.2 deletion syndrome has been described.^{38,39} Although there was no difference in the B-cell counts in patients, significant differences were seen in peripheral memory B-cell populations. Analysis of both nonclass-switched and class-switched memory B cells showed an age-related increase in the proportion of both subsets in patients and controls. The proportion of both class-switched and nonclass-switched memory B cells was significantly decreased in patients compared with controls. The decrease in class-switched memory B cells is perhaps not surprising in the context of impaired T-cell immunity. B-cell class-switch recombination is T-cell–dependent, mainly through the interaction of CD40 ligand expressed on activated T cells and CD40 expressed by B cells. The reason for the decrease in nonclass-switched memory B cells is less clear. These cells seem to function as innate cells that produce high-affinity IgM antibodies and represent first-line defense against encapsulated organisms.²⁶ The role of T cells in the development of these cells is not known, but impairment of so-called *T-independent antibody responses* is seen in other primary T-cell immunodeficiencies, including HIV infection. Defects in the microenvironment for B-cell development and impaired T-cell recruitment in later phases of the T-independent B-cell response may play a role.⁴⁰

Given the high rate of infections in these patients, it is possible that some of the immunologic findings may be a result of increased infection compared with controls. Within the population studied, there are a small number of patients who do not have a history of recurrent or severe infections. Comparison of the immunologic features showed no difference between those patients with and without infections, suggesting the immunologic abnormalities are not a result of infection. The reason certain patients do not have infections despite similar immunologic parameters is not clear. Whether patients with severe or recurrent infections show more subtle immunological deficits (such as impaired TCR repertoire diversity, lymphocyte proliferation responses, or specific antibody production) was not assessed in this study, and future studies should address this. There was no correlation between clinical phenotype (eg, cleft palate, cardiac defects, or feeding difficulties) and infection to suggest that

anatomical abnormalities were associated with increased infections in these patients (data not shown).

In summary, this study showed significant impairment of the T-cell compartment in patients with 22q11.2 deletion syndrome. Although this decrease was age-related, there was a strong correlation between CD4⁺ RTEs and CD4⁺ naive T cells, suggesting that the increase in T-cell counts with age in children is not related to "homeostatic expansion" of the naive T-cell pool but an increase in memory or effector cells. Crucially, this is the first study that shows a significant decrease in both numbers and proportion of CD4⁺FoxP3⁺ nTreg cells in patients. Whether this defect plays a role in increasing susceptibility to autoimmune disease is not clear, and other factors may be involved. In addition to T-cell abnormalities, defects in humoral immunity were common in our cohort, with a high incidence of mild immunoglobulin deficiencies and decreases in class-switched and nonclass-switched memory B-cell compartments. Investigation for humoral immune defects should be considered in patients with 22q11.2 deletion syndrome.

Clinical implications: Decreased nTreg cells in patients may play a role in the increased incidence of autoimmunity seen in 22q11.2 deletion syndrome. Investigation of humoral immunity should also be considered in these patients.

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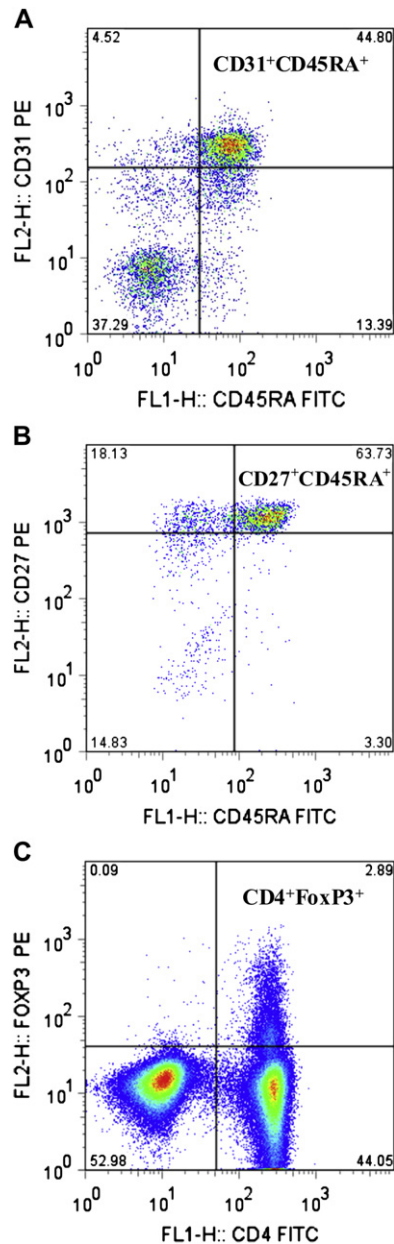


FIG E1. Phenotypic characterization of CD4⁺ RTEs, CD4⁺ naive T cells, and CD4⁺FoxP3⁺ regulatory T cells. **A**, The *dot plot* shows surface expression of CD31 and CD45RA RTEs. **B**, The *dot plot* shows surface expression of CD27 and CD45RA naive cells. **C**, The *dot plot* shows surface expression of CD4 and FoxP3 nTreg cells. The cells in *A* and *B* are gated on scatter and CD3⁺CD4⁺ T cells, and cells in *C* are gated on lymphocytes by scatter. FITC, Fluorescein isothiocyanate; PE, phycoerythrin; FL, fluorescent channel.

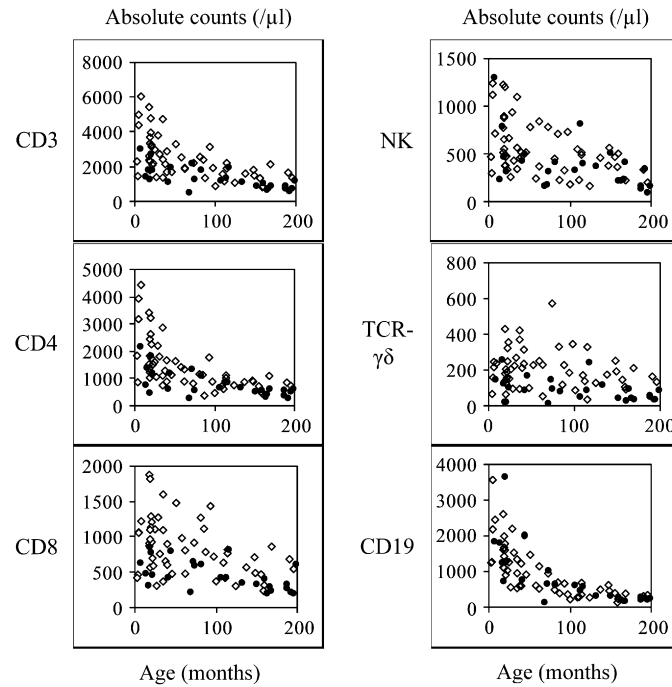


FIG E2. Absolute counts of peripheral blood lymphocyte populations in patients and controls. Data shown for 54 healthy controls ($\diamond$) and 27 patients ($\bullet$).

TABLE E1. Demographic data and lymphocyte populations according to age group in normal controls

	Age groups				
	3-9 mo (n = 5)	9-24 mo (n = 13)	2-5 y (n = 13)	5-10 y (n = 13)	10-16 y (n = 10)
Sex	4 M + 1 F	12 M + 1 F	9 M + 4 F	7 M + 6 F	3 M + 7 F
Mean age	5 mo	20 mo	3.1 y	7.6 y	13.2 y
Total lymphocytes (CD45 ⁺)	6689 (3078-10149)	5288 (2211-9502)	4293 (2269-7289)	3042 (1337-4269)	2369 (1603-2885)
T cells (CD3 ⁺)	3834 (1469-6024)	3160 (1813-5423)	2598 (1385-4732)	1944 (899-3143)	1559 (834-2149)
B cells (CD19 ⁺)	2150 (1261-3565)	1504 (919-2612)	1075 (553-2208)	558 (239-1160)	445 (154-637)
T _H cells (CD3 ⁺ CD4 ⁺)	2867 (867-4459)	1962 (1035-3426)	1504 (751-2887)	889 (298-1602)	843 (376-1782)
T cytotoxic cells (CD3 ⁺ CD8 ⁺)	841 (412-1220)	1071 (560-1881)	953 (376-1782)	806 (362-1437)	593 (226-858)
NK cells (CD16 ⁺ CD56 ⁺)	768 (299-1238)	671 (336-1224)	559 (243-1095)	492 (182-841)	398 (454-1105)
% Activated T cells (HLA-DR)	7 (3-15)	11 (5-28)	11 (5-17)	11 (6-24)	11 (6-24)

M, Male; F, female.

Mean values for lymphocyte counts (cells/ μ L) are displayed with minimum and maximum values for the group in parentheses.

TABLE E2. Demographic data and lymphocyte populations according to age group in patients with 22q11.2 deletion syndrome

	Age groups				
	3-9 mo (n = 1)	9-24 mo (n = 5)	2-5 y (n = 2)	5-10 y (n = 7)	10-16 y (n = 12)
Sex	1 M	5 M	2 M	4 M + 3 F	7 M + 5 F
Mean age	7 mo	18 mo	3.5 y	7.5 y	14.6 y
Total lymphocytes (CD45 ⁺)	6104	4048 (2574-6900)	3426 (2370-4482)	2327 (835-2989)	1746 (1116-3474)
T cells (CD3 ⁺)	3010	1784 (1259-2682)	1533 (1133-1932)	1452 (496-2191)	838 (558-1207)
B cells (CD19 ⁺)	1872	1768 (774-3655)	1414 (803-2024)	625 (173-1063)	281 (193-363)
T _H cells (CD3 ⁺ CD4 ⁺)	1840	1135 (486-1840)	897 (598-1196)	803 (266-1329)	482 (272-682)
T cytotoxic cells (CD3 ⁺ CD8 ⁺)	637	577 (303-859)	613 (416-810)	528 (203-819)	307 (192-605)
NK cells (CD16 ⁺ CD56 ⁺)	306	463 (237-794)	461 (426-496)	378 (164-813)	267 (96-513)
% Activated T cells (HLA-DR)	11	16 (7-49)	17 (13-21)	15 (6-32)	23 (9-30)

M, Male; *F*, female.

Mean values for lymphocyte counts (cells/ μ L) are displayed with minimum and maximum values for the group in parentheses.