
Follow-up study of immune defects in patients with dysmorphic disorders

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Background: In a previous study, we noted immunologic abnormalities in 46 (54.8%) of 84 individuals with dysmorphic disorders.

Objective: To reevaluate patients with dysmorphic disorders and immunologic abnormalities 2 to 3 years after an initial study to determine any changes in those abnormalities.

Methods: Information was gathered regarding significant infections during the previous 12 months. Blood samples were drawn for the immunologic tests that were previously performed (IgG, IgA, and IgM level determinations; complete blood cell count; and lymphocyte subset enumeration) and for determination of IgG subclasses and T-cell activation by CD69 expression.

Results: In the 21 patients available, 26 (63.4%) of the previously noted 41 low immunologic values were still present. In 5 patients, all previously noted immunologic abnormalities resolved. Of the 17 low values noted in 6 patients with Down syndrome, 12 (70.6%) were still present. Also, the 2 patients with Turner syndrome continued to have low IgA and IgM levels. Two patients had a low IgG4 level. A history of significant clinical infections within the previous 12 months was noted in 10 (58.8%) of 17 patients; 8 (47%) had current immune defects. There was a significantly lower T-cell response to staphylococcal enterotoxin B than in healthy controls. The T-lymphocyte activation response was low in 8 (38.1%) of the 21 patients.

Conclusions: Our study revealed a high rate of immune defects in patients with dysmorphic disorders, both during the initial study and 2 to 3 years later, which may contribute to their increased susceptibility to infections. This association was most obvious in patients with Down syndrome and Turner syndrome. The findings should alert for early immunologic evaluation when such patients have infections.

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INTRODUCTION

Patients with dysmorphic disorders have an increased risk of infections.¹ In many such patients, however, there is an obvious underlying cause such as a neurologic defect, an anatomical abnormality, malnutrition, or other associated chronic medical conditions, particularly cardiopulmonary disease. Furthermore, in a variety of genetic disorders and recognizable syndromes, immune abnormalities have been reported; these may contribute to the increased susceptibility to infection.²

In a previous study, patients with dysmorphic disorders were screened for general abnormalities related to circulating blood cells (complete blood cell count), B cells (immunoglobulin levels), and T cells (lymphocyte subsets).³ The highest proportion of immune defects was noted in 23 (79.3%) of 29 patients with chromosomal disorders, followed by the

group with a single gene disorder (15 [55.6%] of 27 patients); the lowest proportion was in the group with an unclassified dysmorphic disorder (8 [28.6%] of 28 patients).

In the present study, we looked for changes in the previously noted immunologic abnormalities and measured the IgG subclasses and T-lymphocyte proliferative response. We are also including a review of the literature available on the subject.

METHODS

Study Participants

Of the 46 patients with abnormal immunologic findings discovered by the previous investigation 2 to 3 years earlier, only 21 were available for reevaluation because 1 died and 24 who were living at a facility for developmentally handicapped individuals (Pine Crest Developmental Center, Alexandria, LA) were moved to other facilities away from the area. Their ages ranged from 3 to 77 years (median, 50 years). Eighteen patients were still living at the original residential facility, and 3 were observed at the Genetics Clinic of Louisiana State University Health Sciences Center–Shreveport.

The study was approved by the institutional review board of Louisiana State University Health Sciences Center–Shreveport, and all patients and/or guardians provided written informed consent. In all patients, the dysmorphic disorder was diagnosed and classified by a board-certified clinical geneticist (H.C.). Of the 21 patients, 12 had chromosomal

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disorders (6 had Down syndrome, 2 had Turner syndrome, and 1 each had a chromosome 2q37.1 deletion, a chromosome 2q32.2 deletion, Wolf-Hirschhorn syndrome, and fragile X mosaic). There were 6 patients with a single gene disorder (3 with phenylketonuria, 2 with Seckel syndrome, and 1 with mucopolysaccharidosis III) and 3 with an unclassified dysmorphic disorder (psychomotor retardation). In the latter group, all 3 had psychomotor retardation: 1 had fetal alcohol syndrome and an intrauterine infection; 1 had spastic quadriplegia, hypertelorism, and a prominent supraorbital ridge; and 1 had short stature and a high arched palate.

An interval history was obtained regarding infections, other illnesses, and hospitalizations within the last 12 months. A complete physical examination was performed, with particular attention to the presence of infection.

Laboratory Evaluation

Venous blood samples were obtained for laboratory evaluation using the previous clinicopathological parameters: complete blood cell count with differential; serum IgG, IgA, and IgM levels, measured by nephelometry; and lymphocyte subset analysis by flow cytometry, as previously described.³ In addition, IgG subclasses were measured (Specialty Laboratories, Valencia, California). The results of the immunoglobulin levels and lymphocyte subset enumeration were compared with established references by age.^{4,5} As in our initial study, laboratory values below the fifth percentile for age were considered abnormally low.

T-Cell Activation Assay by CD69 Marker Through Flow Cytometric Analysis

Peripheral blood specimens were collected using sodium heparin anticoagulant and were evaluated within 24 hours. Fourteen healthy patients were recruited as controls for this part of the investigation. To study the effect of stimulation on CD69 expression, whole blood was cultured on either plain Chang culture medium or culture medium supplemented with either phytohemagglutinin (PHA), 4 $\mu\text{g/mL}$, or staphylococcal enterotoxin B (SEB), 4 $\mu\text{g/mL}$. For each patient, 100 μL of whole blood was placed in each of 3-microtiter wells containing 100 μL of Chang medium. Two wells had 10 μL of either SEB or PHA. The third well served as an unstimulated control. The plates were incubated for 18 hours at 37°C.

After mitogen stimulation, 100 μL of blood was drawn from the microtiter wells and placed in 12- $\times$ 75-mm tubes. Each tube contained the commercial monoclonal antibody cocktail: CD3/CD4/CD8 (Beckman Coulter, Miami, Florida) and CD69 (Becton Dickinson Immunocytometry Systems, San Jose, California). The monoclonal antibodies were directly conjugated with fluorescein isothiocyanate, phycoerythrin, phycoerythrin-cyanin 5, or energy-coupled dye (phycoerythrin and red fluorescent dye [Texas Red]). The tubes were incubated in the dark for 20 minutes, then each sample was lysed and fixed (Q-Prep; Beckman Coulter). Three-color fluorescence analyses were performed per protocol using FC500 instrumentation (Beckman Coulter) for data acquisition. The percentage of unstimulated cells that expressed the activation antigen CD69 and the increase in the percentage of cells expressing CD69 after PHA or SEB stimulation were determined using Summit software (Dako, Fort Collins, Colorado). Digital FCS files were analyzed for each patient and evaluated with the software using the Overton subtraction method.⁶ Data for a standardized number of 2,000 CD3-positive lymphocytes were collected. Subtraction plots were made in comparison to the unstimulated histogram data from each specimen.

Statistical Analysis

The Fisher exact test was used to compare the 3 groups of dysmorphic disorders regarding the persistence of immunologic abnormalities noted in the initial study. The *t* test was used to determine significant differences between the means of CD69 expression of the dysmorphic group and the controls. *P* < .05 was considered statistically significant.

RESULTS

Persistent Immunologic Abnormalities

The initial study,³ which was performed approximately 3 years earlier, revealed immunologic abnormalities in 46 (54.8%) of 84 patients with dysmorphic disorders. For this follow-up study, 21 patients with 41 low laboratory values were available. In 5 patients (23.8%), the initial laboratory abnormalities have resolved. Of the 41 initial low values, 26 (63.4%) were still present (Table 1). In addition, 7 new low laboratory values were detected in 5 patients. Analysis of data

Table 1. Prevalence of Immunologic Abnormalities in Patients With Dysmorphic Disorders, Initially and at the 2- to 3-Year Follow-up

Dysmorphic group	Total No. of initially observed abnormalities	Abnormalities present at follow-up, No. (%) ^a	Abnormalities resolved at follow-up, No. (%) ^a	Newly discovered abnormalities ^a
Chromosomal (12 patients)	28	18 (64.3)	10 (35.7)	5 (in 3 patients)
Single gene (6 patients)	9	6 (66.7)	3 (33.3)	1
Unclassified (3 patients)	4	2 (50.0)	2 (50.0)	1
Total (21 patients)	41	26 (63.4)	15 (36.6)	7 (in 5 patients)

^a The Fisher exact test showed no significant difference between the 3 groups regarding the persistence of laboratory abnormalities (*P* = .99) or the presence of newly discovered abnormalities (*P* = .99).

according to the type of dysmorphic disorder showed persistence of the immunologic abnormality in 64.3% of instances in the chromosomal disorder group, 66.7% in the single gene disorder group, and 50.0% in the unclassified dysmorphic disorder group. The Fisher exact test showed no significant difference between the 3 groups of patients regarding the persistence of laboratory abnormalities ($P > .99$) or the detection of new abnormalities ($P > .99$). Although persistence of the immunologic abnormalities seemed to be more common in patients with Down syndrome, Turner syndrome, and phenylketonuria, the numbers were not large enough to allow sound statistical comparisons. None of the study participants had a low IgG level, in either the initial or the follow-up study.

Immunologic abnormalities noted in each of the 21 patients, in either the initial or the follow-up evaluation, are presented in Table 2. There was persistence of 70.6% (12/17) low laboratory values in the 6 patients with Down syndrome. The 2 patients with Turner syndrome had persistently low IgA and IgM levels. There was persistence of 66.7% (4/6) low laboratory values in the 3 patients with phenylketonuria. On the other hand, the abnormalities reported in a patient with chromosome 2q37.1 and in another with chromosome 2q32.2 deletion resolved by follow-up. Other patients who

had their immune abnormality resolved include a patient each with Down syndrome, Seckel syndrome, and psychomotor retardation.

Of 17 patients with current low laboratory values (Table 2), 8 (47%) were among the 10 who had a significant history of infections during the 12 months preceding this study (Table 3). On the other hand, of the 5 patients whose initial immunologic abnormality resolved, 2 (40%) had a history of significant infections. The 2 groups did not significantly differ (Fisher exact test) regarding the occurrence of infection.

T-Cell Activation Assay Findings

The mean (SD) of CD69 expression in the total dysmorphic group was 70.8% (20.3%) by PHA stimulation and 10.0% (10.8%) by SEB stimulation compared with 77.2% (7.7%) and 23.5% (8.7%) in healthy controls, respectively. Only the difference in response to SEB was statistically significant ($P < .001$).

By considering CD69 expression values below the mean of the control group by more than 2 SDs as abnormal, 8 (38.1%) of the 21 patients had a low T-lymphocyte response. Of these 8 patients, 6 had a low stimulation response to either SEB (3 patients with Down syndrome) or PHA (2 patients with

Table 2. Immunologic Abnormalities Noted in Each Patient, Initially and at the 2- to 3-Year Follow-up

Patient No.	Diagnosis	Abnormalities noted in the initial evaluation	Abnormalities noted in the follow-up evaluation
Chromosomal Disorder			
1	Down syndrome	CD8, CD3, and CD19	CD8, CD19, and CD69 expression ^a
2	Down syndrome	IgM and CD19	IgM and CD19
3	Down syndrome	Lymphocytes, ^b CD4, CD8, CD3, and CD19	Lymphocytes, ^b CD4, CD3, and CD19
4	Down syndrome	IgM and CD56	Lymphocytes, ^b CD19, CD56, and CD69 expression ^a
5	Down syndrome	CD4, CD19, and reversed CD4:CD8 ratio	CD4, CD19, reversed CD4:CD8 ratio, and CD69 expression ^a
6	Down syndrome	Lymphocytes ^b and CD8	CD69 expression ^a
7	Chromosome 2q37.1 deletion	CD56	None
8	Chromosome 2q32.2 deletion	CD8 and CD56	None
9	Turner syndrome	IgA, IgM, and CD56	Lymphocytes, ^b IgA, IgM, CD56, and CD8
10	Turner syndrome	IgA and IgM	IgA, IgM, and CD69 expression ^a
11	Wolf-Hirschhorn syndrome	Lymphocytes ^b and CD19	CD19
12	Fragile X mosaic syndrome	Lymphocytes ^b	IgM
Single Gene Disorder			
13	Phenylketonuria	Reversed CD4:CD8 ratio	Reversed CD4:CD8 ratio and lymphocytes
14	Phenylketonuria	CD4, CD3, CD19, and reversed CD4:CD8 ratio	CD19 and reversed CD4:CD8 ratio
15	Phenylketonuria	Lymphocytes ^b	Lymphocytes ^b
16	Seckel syndrome	CD56	None
17	Seckel syndrome	CD56	CD56
18	Mucopolysaccharidosis III	CD19	CD19
Unclassified Disorder			
19	Psychomotor retardation	Lymphocytes ^b and CD19	Lymphocytes ^b
20	Psychomotor retardation	Lymphocytes ^b	Lymphocytes ^b and CD4
21	Psychomotor retardation	CD56	None

^a Patients 1, 4, 5, 6, and 10 had low (2 SDs below the mean) CD69 expression by staphylococcal enterotoxin B stimulation in the follow-up evaluation.

^b Lymphocyte abnormality refers to a low (less than the fifth percentile) total number or percentage.

Table 3. Current Immunologic Abnormalities in the 10 Patients With Significant Infections During the Previous 12 Months

Patient No.	Dysmorphology diagnosis	Clinical history (frequency)	Immunologic abnormalities
2	Down syndrome	Chronic periodontitis	IgM and CD19
5	Down syndrome	Pneumonia (2) (hospitalized)	CD4, CD19, reversed CD4:CD8 ratio, and CD69 expression
8	Chromosome 2q32.2 deletion	OM (1), diarrhea (3), and URI (3)	None
11	Wolf-Hirschhorn syndrome	Sinusitis (1), bronchitis (1), and UTI (1)	CD19
12	Fragile X mosaic syndrome	Frequent infections of fingers and toes	IgM
13	Phenylketonuria	OM (1), pneumonia (1), and UTI (2)	Lymphocytes, ^a reversed CD4:CD8 ratio
16	Seckel syndrome	Recurrent herpes labialis	None
17	Seckel syndrome	Pneumonia (2), OM (1), diarrhea (1), and URI (1)	CD56
18	Mucopolysaccharidosis III	Abscess (1), folliculitis (1), and URI (2)	CD19
19	Psychomotor retardation	Aspiration pneumonia (2)	Lymphocytes ^a

Abbreviations: OM indicates otitis media; URI, upper respiratory tract infection; UTI, urinary tract infection.

^a Lymphocyte abnormality refers to a low (less than the fifth percentile) total number or percentage.

Seckel syndrome and 1 with Wolf-Hirschhorn syndrome) and 2 had a low response to both (1 with Down syndrome and 1 with Turner syndrome). Of these 8 patients, 3 had clinically relevant respiratory tract infections (pneumonia, sinusitis, and otitis media) during the previous 12 months and 1 had recurrent herpes labialis.

IgG Subclasses

Normal levels of IgG1, IgG2, and IgG3 subclasses were noted in all patients. Two patients (9.5%) had an undetectable IgG4 level (<1 mg/dL): a 47-year-old man with Down syndrome who had persistently low lymphocyte, CD4, CD3, and CD19 levels and a 17-year-old patient with Turner syndrome who had persistently low IgA and IgM levels.

DISCUSSION

This study showed that most (almost two-thirds) of the immunologic abnormalities noted 2 to 3 years ago in this series of patients with dysmorphic disorders were still present, irrespective of the dysmorphic group classification. Such an observation suggests that there may be an association between dysmorphic syndromes and certain underlying immune defects. In 5 patients, their previous immunologic abnormalities completely resolved. However, almost one-fourth of the low laboratory values were new. This suggests that the immunologic status at any point of time may vary and is not rigidly controlled by genetic factors alone. A possible role for the increase in age in the resolution of some defects is probably minor, if any. Of the 5 patients whose immunologic defects have resolved, only 1 was a child (age, 3 years); the other 4 were 36 to 75 years of age. Therefore, the observed changes are very unlikely to be due to a 2- to 3-year increase in age.

Certain genetic syndromes seem to be more associated with immunologic abnormalities.² In our series, there was a high percentage of abnormalities in patients with Down syndrome. Although the abnormalities in this subgroup were not homogeneous, a low CD19 level was still present in 4 of the 6 patients. This finding was consistent with findings in other

studies that showed a significant decrease in the absolute number and percentage of B lymphocytes in patients with Down syndrome.^{7,8} In a recent study of 96 noninstitutionalized children with Down syndrome, the B-cell number was the most decreased, with 61% of values below the fifth percentile.⁹ Other abnormalities reported in patients with Down syndrome include a high IgG and a low IgM level,¹⁰ reduced numbers of circulating CD4 cells,¹¹ a reversed CD4:CD8 ratio,¹¹ and low antibody responses to tetanus toxoid and influenza virus.¹² In institutionalized young adult patients with Down syndrome, Nurmi et al¹³ reported a decreased antibody response to pneumococcal vaccine compared with age-matched institutionalized patients with mental retardation and a normal karyotype or healthy control patients.

Our 2 patients with Turner syndrome continued to have low IgA and IgM levels. A study of 29 patients with Turner syndrome (age, 2–18 years) reported a low IgG level in 48%, a low IgM level in 41%, and a low IgA level in 3%.¹⁴ Cacciari et al¹⁵ also noted a significantly decreased IgG level in patients with Turner syndrome compared with healthy male and female patients. Naspitz and Sole¹⁶ described 2 patients with Turner syndrome with IgA deficiency and recurrent parotiditis. Another report¹⁷ described 3 patients with Turner syndrome with gluten intolerance and selective IgA deficiency.

In vitro evaluation of lymphocyte function is usually done by quantifying the mitogenic or antigenic response of T lymphocytes using radionucleotide-labeled tritiated thymidine. However, this procedure requires an extended incubation period and exposure to radioactive material. We used a flow cytometric assay for CD69, which is an early activation marker that appears rapidly on the surface of activated T cells after in vitro stimulation. Maino et al¹⁸ reported that CD69 expression after 4 hours paralleled thymidine incorporation measured after 72 hours. Prince and Lape-Nixon¹⁹ investigated the clinical use of CD69 expression to predict the outcome of lymphocyte response to anti-CD3 in patients with human immunodeficiency virus type 1. Compared with the

thymidine incorporation method, the CD69 activation assay showed a sensitivity of 86.2% and a specificity of 88.9%, with a positive predictive value of 92.6%.

In our study, 4 of 6 patients with Down syndrome were reported to have a poor T-lymphocyte activation response to stimulation by at least 1 mitogen (1 of 4 had a low response to both SEB and PHA). Similar findings were reported in other studies. Cuadrado and Barrena²⁰ reported low proliferative T-cell responses to anti-CD3 (58% of patients) and to tetanus toxoid (46.7% of patients) in those with Down syndrome. In a study of 42 patients, Spina et al⁸ reported decreased T-lymphocyte proliferative response to PHA. Another study²¹ reported a lower T-cell response to PHA in adult patients compared with age- and sex-matched controls. In our study, only 1 of 4 patients with Down syndrome and low CD69 expression had significant infections (hospitalized twice for pneumonia) during the previous year. This patient's other immunologic abnormalities were low CD4 level, low CD19 level, and reversed CD4:CD8 ratio.

We noticed a low T-cell response in 2 siblings with Seckel syndrome, a rare autosomal recessive disorder characterized by "birdlike" facies, growth retardation, microcephaly, and mental retardation.²² To our knowledge, this finding has not been reported before. One of the patients with Seckel syndrome had recurrent herpes labialis, and the other had episodes of pneumonia (twice), otitis media, and diarrhea.

Another patient with a low stimulation response to PHA has Wolf-Hirschhorn syndrome (chromosome 4 deletion). A study by Hanley-Lopez et al²³ identified antibody deficiencies in 9 of 13 infection-prone children with Wolf-Hirschhorn syndrome, none of whom had a T-cell defect. Last, a 17-year-old patient with Turner syndrome, who did not have any significant infection in the past year, had low T-lymphocyte proliferation to both SEB and PHA. A decreased in vitro response to concanavalin A, PHA, and pokeweed mitogen was reported in 20 patients with Turner syndrome.¹⁵ However, Lorini et al¹⁴ reported normal T-cell responsiveness to PHA and concanavalin A in 29 patients.

The T-lymphocyte response to SEB was lower than to PHA in both the dysmorphic group and the controls, which is expected. Bacterial superantigens bind mainly to the V β CDR2 (complementarity-determining region) loop of the T-cell receptor and are specific for one or a few V β gene products. Therefore, SEB can stimulate only 2% to 20% of T cells.²⁴ Staphylococcal enterotoxin B, a superantigen, reacts with a defined repertoire of V β specificities on T cells and has been demonstrated to react with V β 6 but not with V β 8.²⁵ On the other hand, T-cell mitogens, such as PHA, nonspecifically cross-link many T-cell surface structures, including those responsible for activation.

A significantly lower percentage of SEB-stimulated CD69 expression was observed in the dysmorphic group compared with the control group. Watson and Lee²⁶ reported that SEB stimulates only naïve T cells to proliferate, whereas peptide antigens stimulate both naïve T cells and memory T cells. They reported that signal transduction through the T-cell

receptor is impaired (ie, impaired activation of ZAP-70) in memory cells exposed to SEB. It may be postulated that the lower percentage of CD69 activation by SEB in our dysmorphic patients is secondary to a lower percentage of naïve T cells. A significant decrease of CD4+CD45RA+ cells (naïve T cells) was reported in both children and adults with Down syndrome compared with age-matched controls.²⁷ Further investigation is warranted in this area.

Last, none of our patients had low IgG1, IgG2, or IgG3 levels. Only 2 patients had low IgG4 levels: a 47-year-old man with Down syndrome and a 17-year-old girl with Turner syndrome. Low levels of IgG4 were reported in patients with Down syndrome, both children²⁸ and adults.²⁹ Stenberg et al³⁰ reported a low IgG4 level in 2 (13%) of 15 patients with Turner syndrome. Low levels of the IgG2 subclass were reported in some patients with Down syndrome²⁹ or Turner syndrome.³⁰ The immunologic abnormalities revealed by the additional tests in this follow-up study may have been present before rather than being newly developed.

A limitation of our study was the loss to follow-up of a good number (25 [54.3%] of 46) of our initial patients, which would not be remedied by adding new patients. Nevertheless, to our knowledge, it is the largest number being reported in a follow-up study. Unlike studies on prevalence or studies involving comparisons between interventions, the effect of numbers of patients is much less on our observational study that looked for interval changes. The findings are worthwhile in the management of patients with dysmorphic disorders and should stimulate further studies, both cross-sectional and longitudinal. Such studies may reveal some genetic associations between certain immune defects and specific dysmorphic disorders.

In summary, we found that almost two-thirds of the immunologic abnormalities in patients with dysmorphic disorders were still present 2 to 3 years later, particularly in patients with Down syndrome and Turner syndrome. This suggests a possible association between dysmorphic disorders and immune defects that may be contributing to their increased susceptibility to infection. The findings may serve as an alert for early evaluation for immune defects in such patients when they are affected by unusual or frequent infections.

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