An unusual concurrence of graft versus host disease caused by engraftment of maternal lymphocytes with DiGeorge anomaly

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Abstract
We describe a girl with DiGeorge anomaly and normal cytogenetic and molecular studies, whose clinical course was complicated by graft versus host disease caused by intrauterine materno-fetal transfusion, and several immunohaematological alterations including a monoclonal gammopathy of undetermined significance (first IgG, which subsequently changed to IgM). The main clinical features and pathological findings are discussed. (Arch Dis Child 2000;83:165–169)

Keywords: biclonal gammopathy; materno-fetal chimerism; graft versus host disease; DiGeorge anomaly; Omenn’s syndrome, Evans’ syndrome

Several studies have suggested that maternal blood cells can cross the placenta and migrate into the fetal circulation where they may be present in substantial numbers and for extended periods of time (reviewed in Bernischke1). Primary immunodeficiencies, mainly severe combined immunodeficiency, are sometimes associated with the persistence of maternal lymphocytes causing variable degrees of graft versus host disease (GVHD).2 In a congenitally immunodeficient host, maternal cells which gain entry into the fetus may provoke systemic alloimmune disease, worsening the prognosis.

We describe an unusual occurrence of chimerism caused by engraftment of maternal lymphocytes in a girl with DiGeorge anomaly (DGA). GVHD in this patient was unusual because two monoclonal gammopathies of undetermined significance were detected during the clinical course.

Case report
The propositus was born at 37 weeks gestation of normal weight. She was dysmorphic, with low set, asymmetrical, and posteriorly rotated ears, lateral displacement of inner canthi, antimongoloid palpebral fissures, slight facial asymmetry, a wide nasal root, micrognathia, a high arched palate, a small mouth, and short philtrum (fig 1). Hearing loss was not evident. No thymic shadow was present on chest x ray. Biochemical investigations showed low serum calcium (1.34 mmol/l) and parathyroid hormone (7 ng/l) concentrations with a normal magnesium concentration (1.06 mmol/l). Treatment with oral calcium supplementation and α-1-calciiferol was initiated.

At 1 month of age she was hospitalised with generalised seborrhoeic dermatitis, diarrhoea, suppurrative oitis, and cutaneous candidiasis. From the skin and ear exudates, Staphylococcus aureus and Pseudomonas aeruginosa were isolated. At 4–6 months of age she developed a desquamative erythroderma associated with eosinophilia (26%) and high concentrations of IgE (up to 7060 IU/ml), enlarged lymph nodes, and bilateral bronchopneumonia. A lymph node biopsy revealed significant T cell depletion with small follicles lacking germinal centres. Serological screening for human immunodeficiency virus (HIV), Epstein–Barr virus (EBV), and cytomegalovirus (CMV) was negative. Subsequently (age 8–10 months), she was diagnosed as having Evans’ syndrome (platelet count $4 \times 10^9$/l; red blood cell (RBC) count $2.47 \times 10^{12}$/l; a positive direct Coombs test; and presence of antiplatelet antibodies). Following corticosteroid therapy, her platelet and RBC counts returned to normal. The infectious and cutaneous picture persisted despite antibiotic treatment.

Figure 1  Clinical photograph of the patient.
Approximately six months later, serum protein analysis and immunofixation electrophoresis revealed a monoclonal gammopathy (IgG1, \(\lambda\) chain) without Bence–Jones proteinuria or skeletal lesions, and with normal bone marrow cytology. Because of the persistence of chronic diarrhoea, a jejunal biopsy was performed which showed evidence of villous atrophy. Antigliadin antibodies were undetectable.

Over the next several months she had several other infectious episodes (recurrent diarrhoea as well as otitis and sinusitis) of both bacterial and viral aetiology, persistent failure to thrive, and nasal speech (attributed to velopharyngeal insufficiency). Laboratory studies showed the presence of antinuclear antibodies (ANA) (titre >1/1280, with a homogeneous pattern). By this time (24–26 months of age), the ossification centre of the hyoid bone was not yet evident. A new episode of generalised exfoliative erythroderma with eosinophilia appeared at 30–32 months of age. A histopathological skin study was performed and the findings were compatible with GVHD.

Eight months later, her general status deteriorated with recurrent episodes of diarrhoea, bronchopneumonia, and cutaneous candidiasis. Serum electrophoresis revealed a new monoclonal band (IgM, \(\lambda\) chain). Again, no lytic lesions were observed in radiological studies and her bone marrow contained normal numbers of plasma cells. She died shortly afterwards at the age of 42 months from multiorgan failure, terminal respiratory insufficiency, and disseminated infections. Postmortem pathological findings confirmed the thymic absence and parathyroid gland hypoplasia as well as an aberrant right subclavian artery and mildly immature kidneys without genitourinary malformations. Generalised lymphadenopathy with severe T cell depletion was also shown.

The mother was mildly dysmorphic, also having a short philtrum and micrognathia. A certain degree of consanguinity exists in this family: the girl’s maternal greatgrandmothers were first cousins (data not shown).

### Methods

#### IMMUNOLOGICAL STUDIES

Serum concentrations of immunoglobulins, immunoelectrophoresis, and immunofixation as well as proliferative responses of peripheral blood mononuclear cells (PBMC) to optimal amounts of phytohaemagglutinin (PHA), concanavalin A (ConA), pokeweed mitogen (PWM), phorbol myristate acetate (PMA), and monoclonal antibody against CD3, were performed using standard methods. PBMC populations were enumerated by fluorescence activated flow cytometry with the use of monoclonal antibodies to cell surface determinants by routine procedures. HLA-A, -B, and -DR types were determined using polymerase chain reaction (PCR) with sequence specific primers (PCR-SSP) for both the girl and her parents. Finally, consensus primers to the joining segments and the framework region III of the variable segments of the IgH gene were used in a seminested PCR for clonality analysis of B lymphoid proliferation as previously reported.

#### CYTOGENETIC STUDIES, FISH, AND ANALYSIS OF POLYMORPHIC MARKERS IN 22q11

Chromosome preparations, obtained by standard techniques, of sufficiently high resolution (800–850 bands per haploid set) were G banded with trypsin and Leishman stain. Fluorescence in situ hybridisation (FISH) was performed with lymphocytes from the proband and the parents using a digoxigenin labelled cosmid DNA specific for the locus D22S75 (N25) (as control a cosmid for the locus D22S39 (pH17) in 22q13.3 was used). Cos 40 (provided by M Aubry, Montreal), a cosmid probe containing sequences of the zinc finger gene, located at 22q11.21 on April 12, 2022 by guest. Protected by copyright.http://adc.bmj.com/ Arch Dis Child: first published as 10.1136/adc.83.2.165 on 1 August 2000. Downloaded from http://adc.bmj.com/ on April 12, 2022 by guest. Protected by copyright.
DiGeorge anomaly and graft versus host disease

Despite the low number of T cells, almost all these cells (>80%) were CD45R/RO+ (normal range 29–44%). T cells also showed an “activated” phenotype (CD3+CD25+, 40% and CD3+HLA-DR+, 70%) (age matched normal ranges are 3–7% and 6–9%, respectively). Functional analysis revealed low T cell proliferative responses triggered by PHA as well as ConA (not shown). Proliferation to CD3/TCR or to PMA was also depressed with normal responses to PWM throughout the patient’s life (not shown). Using a panel of mAb against TCR V region gene encoded epitopes, we showed that T cells displayed a greatly reduced TCR diversity, characterised by a significantly diminished proportion of the TCR Vβ8 subset and an important expansion of the TCR Vβ5.2 and Vβ5.3 subsets (fig 3).

In contrast, a progressive increase in CD19+ cells was evident. The last phenotypic analysis performed when the IgM monoclonal paraprotein was detected, showed a high proportion of B cells mainly expressing surface IgM (CD19+, 63% and slgM+, 69.8%; normal range is 7–17%). The patient was routinely monitored for immunoglobulin concentrations. During the first six months of her life, serum IgG and IgM concentrations were within normal limits for her age. However, IgE was notably raised from birth and remained high for approximately one and a half years. Coinciding with the falling IgE concentration, IgG increased progressively and concentrations reached 2.5 g/l. A monoclonal IgG λ chain was detected on electrophoretic analysis by immunofixation. IgA concentrations were within normal range—or just below normal—on all occasions. IgM concentrations were also normal or slightly above normal, except shortly before death when a second monoclonal gammapathy (IgM λ) appeared. The significance of this finding in our patient lies in the isotype switch from IgG to IgM; such class switching is extremely rare. Next, we evaluated the IgH gene rearrangement by a seminested PCR method but were unable to detect clonal lymphoid proliferation of B cells shortly after the immunofixation detection of the IgG monoclonal band. However, PCR analysis of IgH rearrangements showed one band clearly detectable in the gel in the 80–120 bp range which allowed us to classify it as monoclonal. This coincided with the appearance of the IgM monoclonal band and the existence of a high proportion of IgM+ B cells (fig 4). We did not study whether maternal cells were functional in vitro, but proliferative responses to mitogens in this patient were probably decreased as a result of the low number of T lymphocytes. Antibody production was tested in vivo by immunisation with both pneumococcal

IMMUNOLOGICAL FOLLOW UP STUDIES

Analysis of peripheral blood lymphocytes on admission, showed CD3+, CD4+, and CD8+ subsets to be within normal ranges. Subsequently, the T cell numbers (CD3+, TCRαβ) gradually decreased, mainly as a result of a slow but progressive loss of CD4+ lymphocytes. Despite the low number of T cells, almost all these cells (>80%) were CD45R/RO+ (normal range 29–44%). T cells also showed an “activated” phenotype (CD3+CD25+, 40% and CD3+HLA-DR+, 70%) (age matched normal ranges are 3–7% and 6–9%, respectively). Functional analysis revealed low T cell proliferative responses triggered by PHA as well as ConA (not shown). Proliferation to CD3/TCR or to PMA was also depressed with normal responses to PWM throughout the patient’s life (not shown). Using a panel of mAb against TCR V region gene encoded epitopes, we showed that T cells displayed a greatly reduced
lymphocytes (both the patient and her parents); HLA typing was performed using PCR-SSP.

*High molecular weight DNA was isolated from lymph node cells (patient) and peripheral blood lymphocytes (both patient and both parents), HLA typing was performed using PCR-SSP.

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Table 1 Demonstration of materno-fetal chimerism by HLA typing

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OTHER DIAGNOSIS RELATED CONSIDERATIONS

Skin conditions, lymphadenopathy, hepatosplenomegaly, failure to thrive, chronic diarrhoea, recurrent infections, eosinophilia, increased IgE, and activated T cells with low proliferation are all features observed in both Omenn’s syndrome and materno-fetal GVHD.13 Some authors have reported clinicopathological findings resembling those observed in GVHD which argue in favour of the hypothesis that a lymphocytic chimerism caused by intrauterine transfusion is responsible for Omenn’s syndrome,13 but this has not been shown by other authors.13 17 There are reports of children with features of DGA with or without 22q11 deletion16 18 who developed Omenn’s syndrome but in whom no maternal cells were present and patients with eczematous skin lesions occurring simultaneously with the appearance of a restricted Vβ TCR and without materno-fetal chimerism.20 21 Thymic defects, including those of DGA, may have a role in the mechanism contributing to the restricted T cell repertoire seen in Omenn’s syndrome.22 Consequently, we also considered the possibility of Omenn’s syndrome because of the clinical features (diarrhoea, failure to thrive, several episodes of generalised erythroderma, eosinophilia, liver, spleen and lymph node enlargement, together with repeated infections)14 15 18 22 and certain immunological features (activated T cells with low proliferative response and restricted heterogeneity of TCR diversity) present in our patient. However, to our knowledge, Omenn’s syndrome could be ruled out in this case by because: (1) RAG1 and RAG2 mutations were not detected in our patient (not shown); (2) increased percentages of B cells were present throughout the life of our patient; and (3) as stated, the presence of maternal cells in the patient was unequivocally shown, a fact that could explain the Omenn’s syndrome like features.

CONCLUDING REMARKS

DGA is still an enigma.23 This case report is the first, to our knowledge, which presents the simultaneous occurrence of severe GVHD, including Evans’ syndrome, two monoclonal gammapathies of undetermined significance,27 28 and other immunological dysfunctions in DGA. The early occurrence of GVHD probably resulted in chronic allogeneic stimulation. This, together with the activation of residual T cell clones and lack of physiological programmed cell death in the thymus are linked processes which could trigger autoimmune manifestations (Evans’ syndrome, ANA). The immunoregulatory imbalance characteristic of DGA together with the evidence of transplacental passage of maternal T cells could explain this varied clinical picture. In addition, recurrent infections including EBV could have contributed to her grave clinical evolution. This girl could be included in the small group of patients in which neither FISH nor PCR analysis of polymorphic markers detect chromosome deletions. This does not rule out the existence of undetected...
smaller deletions or point mutations within DGA critical region genes.

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