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 gammapathy of undetermined significance (first IgG, which subsequently changed to IgM). The main clinical features and pathological findings are discussed.

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Keywords: biclonal gammapathy; 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 Bernischke[1]). 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 allogeneic 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 gammapathies 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-calciferol was initiated.

At 1 month of age she was hospitalised with generalised seborrhoeic dermatitis, diarrhoea, suppurative otitis, 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 leucocytosis caused by 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 4×10^9/l; red blood cell (RBC) count 2.47×10^{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.

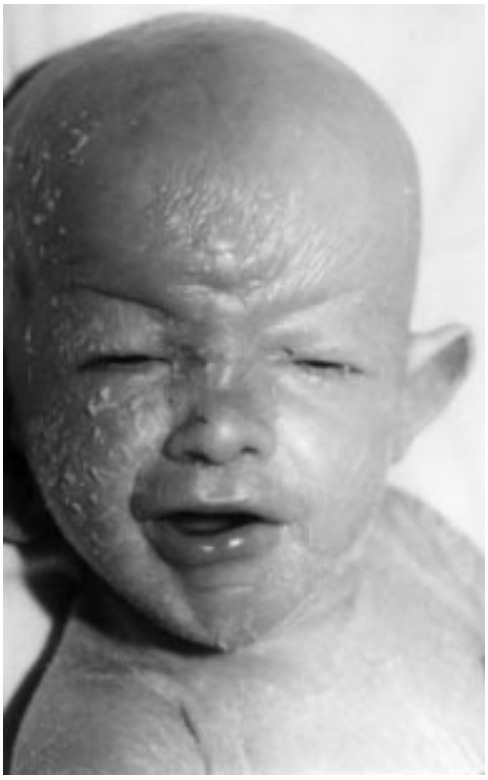

Figure 1 Clinical photograph of the patient.

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Approximately six months later, serum protein analysis and immunofixation electrophoresis revealed a monoclonal gammapathy (IgG1, λ 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) (titres ⩾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, λ 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 (II-18, fig 2). Echographic studies during pregnancy showed several placental infarcts. The grandmother (I-2, fig 2) had three spontaneous abortions (II-4, II-7, and II-11, fig 2), another malformed female fetus who died in utero shortly before delivery (II-12), one child who died at 17 days of age as a result of abnormal development (II-1), and another who died at 5 months of unknown causes (II-10). One of the patient's aunts was micrognathic and died of septicaemia at the age of 33 (II-9). The mother of the proband has three living healthy brothers (II-2, II-5, and II-15). The patient's grandmother (I-2), one healthy aunt (II-14), and one brother (born after her death) (III-13) also have micrognathia. A certain degree of consanguinity exists in this family: the girl's maternal greatgrandmothers were first cousins (data not shown).

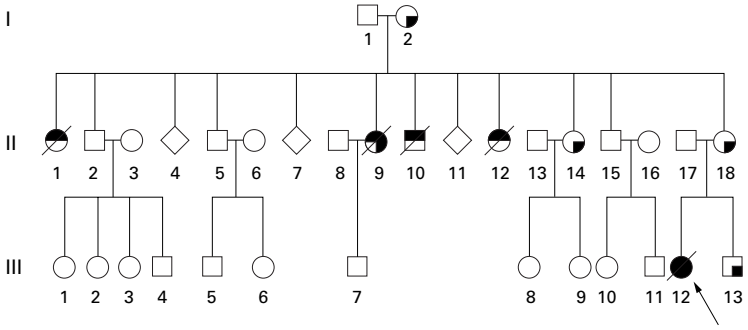

Figure 2 Family pedigree. Arrow indicates propositus. Unaffected individuals are represented by open symbols (squares, circles); open diamonds indicate spontaneous abortions. Individuals with evident facial anomalies (for example, micrognathia) and/or development disorders are represented by a black lower right segment, and those who died of infectious processes are marked with a black upper segment.

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.[3] *RAG1* and *RAG2* genes were screened for mutations as described previously.[4]

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 region of ZNF74 gene, located at 22q11.2,[5] was also tested in the patient and her parents. In order to investigate hemizygosity for chromosome 22q11, three highly informative microsatellite DNA markers, D22S264, D22S941 (CA 443), and D22S944 (CA N60C11), which map within the commonly deleted region in DGA, were used for deletion detection. The PCR and electrophoretical analysis were performed exactly as described previously.[6] Rearrangement of chromosome 10p was also studied as previously described.[7]

Results and discussion

CLINICAL AND GENETIC FEATURES OF DGA

DGA has now been redefined as a developmental disorder affecting structures which

originate embryologically from the third and fourth pharyngeal pouches. There is considerable variability in the defects and in the spectrum of associated anomalies.[8 9] The diversity of clinical features (partial versus complete forms) makes precise diagnostic criteria difficult to define.[10] About 90% of patients with DGA present hemizygosity for a region of chromosome 22q11 which is commonly deleted.[5 8 9] The acronym CATCH22 syndrome (cardiac defects, abnormal facies, thymic hypoplasia, cleft palate, hypocalcaemia) has been suggested to describe this clinical spectrum of conditions, now very broad, associated with 22q11 deletions.[8–10]

Although the clinical and pathological studies seemed clearly to indicate that our patient fulfils all the criteria for a diagnosis of DGA,[9] we sought additional evidence at a genetic level. Cytogenetic studies showed a normal 46XX karyotype with no deletions or translocations. No microdeletions were found at chromosome 22q11.2 by using D22S75 and Cos40 probes in FISH studies. In addition, the proband's parents did not show the 22q11 microdelection using M51 and sc4.1 cosmid probes (analysis kindly performed by P A In't Veld, Rotterdam). Furthermore, polymorphic markers for chromosome 22 did not reveal hemizygosity at this level (data not shown). The 10p deletion syndrome presented in cases with partial DGA exhibits specific phenotype characteristics, particularly sensorineural hearing loss.[7 11] However, hearing was apparently normal and no 10p deletions were found in our patient.

Although a molecular defect responsible for this anomaly was not detected it is possible that the presence of a chimerism (see below) could render the interpretation of studies with highly polymorphic markers more difficult. Maternal origin of the defect was strongly suspected because of the family history: multiple spontaneous abortions and neonatal deaths favour the hypothesis of a cryptic molecular defect and together with the other mildly affected family members (micrognathia) point to a dominantly inherited problem which was undetected.

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 ($CD3^+$, $TCR\alpha\beta^+$) 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 $CD3^+HLA\text{-}DR^+$, 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 $V\beta8^+$ 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 expresssing surface IgM ($CD19^+$, 63% and $sIgM^+$, 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 change in the monoclonal immunoglobulin, 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 $sIgM^+$ 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

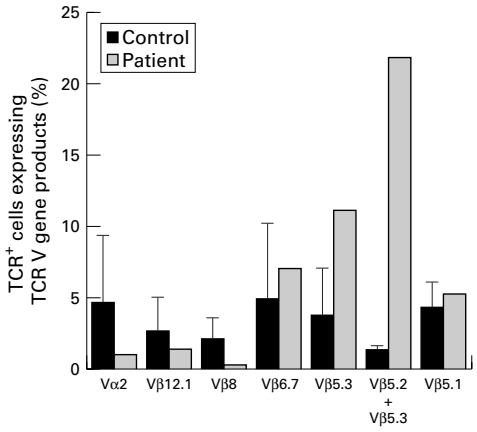

Figure 3 TCR diversity analysis of patient's T cells. Patient's peripheral mononuclear cells were analysed by flow cytometry using the indicated anti-TCR V monoclonal antibodies. Results are expressed as % of TCR expressing cells.

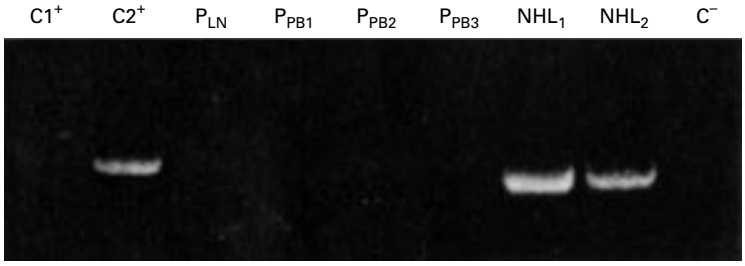

Figure 4 Clonal heavy chain gene rearrangements (lanes C1⁺ and C2⁺ are DNA extracted from the Namalwa B cell line and DNA from the erythroleukaemia K562 cell line, respectively. The P_{LN} lane is postmortem extracted DNA from the DGA patient's lymph nodes while P_{PB1-3} represent DNA extracted from peripheral blood at three different points during the clinical course. NHL_1 and NHL_2 indicate DNA from two unrelated patients with non-Hodgkin's B cell lymphomas. C^- represents a negative control with no DNA added.

polysaccharide vaccine and tetanus toxoid. No response was obtained against these antigens. Similarly, specific production of antibodies against viral antigens was also negative. Although routine serological studies did not detect antibodies to EBV, CMV, and HIV antigens, a postmortem search for DNA sequences in different tissues revealed the presence of EBV DNA sequences in spleen and lymph nodes as well as in a sample of frozen PBMC (not shown) but not in several other tissues tested (jejunal tissue, lung, skin). CMV and HIV were negative by PCR studies. This has been recently reported in two patients with DGA who died of an associated B cell lymphoma.[12 13] In both cases malignant cells expressed EBV. The high number of oligoclonal B cells observed in our patient could be related to an EBV driven expansion of these cells. However, we could not show histopathological evidence of malignancy on postmortem examination.

DEMONSTRATION OF MATERNO-FETAL CHIMERISM

Despite the patient's clinical and pathological findings indicating DGA, many other abnormalities seen in our patient, such as jejunal villous atrophy, exfoliative erythroderma, Evans' syndrome, monoclonal gammapathy, B cell proliferation, and antinuclear antibodies could be better explained by a chronic semiallogeneic interaction between maternal lymphocytes and recipient cells. In addition, the immunological alterations found (high IgE, restricted heterogeneity of TCR diversity, low proliferative response of PBMC to mitogens) could be also explained by GVHD. This could be caused by transplacental passage of maternal lymphocytes as shown by the presence of maternal DNA in the patient's tissues by HLA typing of the family (table 1). These results clearly indicated a maternally derived cell engraftment in the patient.

Table 1 Demonstration of materno-fetal chimerism by HLA typing

	*HLA loci**				
	A	*B*	*DRB1*	*DRB4*	*DRB5*
Father (II-17)	1/11	60/51	15/4	53	51
Mother (II-18)	1/31	18/39	1/4	53	51
Patient (III-12)	1/31/1	18/39/51	1/15/4	53	51/51

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

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 seen both in Omenn's syndrome and materno-fetal GVHD.[14] 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,[15] but this has not been shown by other authors.[16 17] There are reports of children with features of DGA with or without 22q11 deletion[18 19] 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)[15–17 23–25] 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.[26] This case report is the first, to our knowledge, which presents the simultaneous occurrence of severe GVHD, including Evans' syndrome, two monoclonal gammopathies of undetermined significance,[27 28] and other immunological dysfunctions in DGA. The early ocurrence 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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