Novel primary thymic defect with T lymphocytes expressing $\gamma\delta$ T cell receptor

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SUMMARY Flow cytometric analysis of the peripheral blood mononuclear cells in a six year old girl with a primary cellular immune deficiency showed a normal fraction of CD3 positive T cells. Most (70%) of the CD3 positive cells, however, expressed the $\gamma\delta$ and not the $\alpha\beta$ T cell receptor. Immunoprecipitation and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that most of the $\gamma\delta$ T cell receptors existed as disulphide-linked heterodimers. Proliferative responses to mitogens were severely reduced, but specific antibody responses after vaccination could be detected. A thymic biopsy specimen showed severe abnormalities of both the thymic lymphoid and epithelial component with abortive medullary differentiation and almost an entire lack of Hassall's corpuscles.

This patient represents a case of primary immune deficiency syndrome not previously described. Thymic deficiency associated with a high proportion of T cells expressing the $\gamma\delta$ T cell receptor has been described in nude mice, and it is suggested that the immune deficiency of this patient may represent a human analogue.

The thymus provides the microenvironment required both for inducing precursor cells to differentiate into T lymphocytes and for selecting among developing T cells those with an appropriate recognition specificity for antigen and major histocompatibility complex (MHC).^{1,2} Two separate lineages of T cells may be defined by the type of T cell receptor (TCR) expressed.³ Most T cells in the peripheral blood express the $\alpha\beta$ TCR, but a small fraction (0-10%) express the recently described $\gamma\delta$ TCR.⁴⁻⁶ Both the $\alpha\beta$ and the $\gamma\delta$ TCR are non-covalently associated at the cell surface with the monomorphic CD3 complex which comprises at least three different proteins (CD3- γ , δ , and ϵ).⁷ In the $\alpha\beta$ TCR/CD3 complex the $\alpha\beta$ TCR is involved in recognition, determining T cell specificity for antigen plus MHC structures, while the CD3 proteins have an important role in signal transduction during T cell activation.⁷ During intrathymic T cell differentiation, rearrangement and expression of the genes encoding the δ -, γ -, β - and the α -chains take place in an ordered sequence.^{8,9} The genes encoding the δ - and the γ -chain may also rearrange, and their products may be

expressed during an extrathymic T cell differentiation pathway,^{9,10} but this still remains to be elucidated.

Material and methods

The patient was born in 1982 as the third child of two healthy non-related parents. One brother born in 1972 is healthy showing no signs of immune deficiency. The other brother born in 1975 died aged 5 years from diffuse pneumonitis. Necropsy showed absent thymus and generalised lymphocytic depletion of lymphoid organs. Immediately after birth our patient was examined for immune deficiency. Moderate lymphopenia was found with normal distribution of T and B cells in the blood. Proliferative responses to mitogens were moderately reduced (70-80% of those of controls). She had an episode of lobar pneumonia when she was 4 years old, and had prolonged periods of monosymptomatic cough. Between the ages of 4 and 5 years, cytomegalovirus (CMV) was cultured on several occasions from the urine. Computed tomograms of the lungs yielded normal results.

At the most recent follow up she still had moderate lymphopenia ($1-2 \times 10^9/l$), but was not anaemic or

thrombocytopenic. Proliferative responses to mitogens were now severely reduced (10–20% of those of controls). Red cell adenosine deaminase and purine nucleoside phosphorylase activities were normal. The serum concentrations of IgG₁, IgG₂, IgG₃, IgG₄ and IgM were normal, but the IgA concentration was decreased. The patient's blood group is O and blood group A and B isohaemagglutinins were detected. Furthermore, antibodies against tetanus, diphtheria, and polio have also been detected, indicating that humoral immunity was not severely affected. The girl has developed normally and at the time of writing was otherwise in complete health, having had a physical examination showing no abnormalities.

Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation.¹¹

Monoclonal antibodies to leucocyte antigens were as follows: Anti-TCR- δ 1, which recognises the TCR δ -chain, was a kind gift from Dr MB Brenner (Dana-Faber Cancer Institute, Boston, USA). Ti γ A recognises the TCR γ -chain, and was a kind gift from Dr TH Hercend (Villejuif, France). Anti-Leu-1 (CD5), anti-Leu-2 (CD8), anti-Leu-3a (CD4), anti-Leu-4 (CD3), anti-Leu-5 (CD2), anti-Leu-11 (CD16), anti-Leu-7 which recognise a subset of T cells and natural killer cells, and WT-31, which recognises a framework determinant of the $\alpha\beta$ TCR were obtained unlabelled or phycoerythrin labelled from Becton Dickinson (Mountain View, California, USA). BMA-031, which recognises a framework determinant of the $\alpha\beta$ TCR was obtained from Behring Division (Roedovre, Denmark). β F1, which recognises the TCR β -chain, and δ -TCS-1 which recognises the TCR δ -chain, were obtained from T Cell Sciences Inc (Cambridge, USA). F25.29 (CD45), F103.12 (CD10), F110.08 (CD2), F101.01 (reactive with a conformational epitope of the TCR/CD3 complex,¹⁴ and F110.22 (CD11a) were produced by T Plesner (Copenhagen); Tü71 (CD5), Tü14 (CD7), Tü68 (CD8), Tü69 (CD25) were gifts from A Ziegler (Tübingen); Ber-H2 (CD30) and Ki-67 were supplied by H Stein (Berlin); UCHT1 (CD3) and VIT14 (CD27) were gifts from P Beverley (London) and W Knapp (Vienna), respectively; DAKO-T6 (CD1) and DAKO-HLA-ABC were obtained from Dakopatts (Copenhagen).

Monoclonal antibodies to epithelial antigens were as follows: AE1 (Hybritech, San Diego), LP34 (EB Lane, London) and K92 (DY Mason, Oxford) reactive with cytokeratins; BG3C8 reactive with an antigen of basal cells of stratified epithelia and subcapsular and medullary thymic epithelium¹²; and T2/30 and MR7 reactive with cortical epithelial cells obtained from the 3rd International Workshop on Human Leucocyte Differentiation Antigens, Oxford 1986.¹³

Single and two colour flow cytometric analysis was performed on a FACS IV (Becton Dickinson), as

described previously.¹⁴

The thymus biopsy specimen (0.5 × 0.5 × 0.5 cm) was snap-frozen in liquid nitrogen and stored at –70°C. Sections were cut at 7 μ m in a cryostat, fixed in acetone, and stained with monoclonal antibodies using a three step immunoperoxidase technique or an APAAP method described as previously.^{15 16}

Peripheral blood mononuclear cells were surface iodinated using the lactoperoxidase/glucose oxidase method followed by solubilisation and immunoprecipitation as described previously.^{14 17}

Results

FLOW CYTOMETRIC ANALYSIS

A normal prevalence of T cells in peripheral blood mononuclear cells, as judged by CD2 positive cells (75–85%), was found. One and two colour FACS analysis of T cell subsets, however, showed profound abnormalities. Only 24% of the peripheral blood mononuclear cells carried the $\alpha\beta$ TCR as determined by monoclonal antibodies WT-31 and BMA-031 (table). These cells expressed either CD4 (16%) or CD8 antigen (8%) and they all expressed normal titres of CD5 antigen. Fifty six percent of the peripheral blood mononuclear cells expressed the $\gamma\delta$ TCR as determined by TCR- δ 1 (normally below 10%). These cells were all CD4 negative; their expression of CD8 and CD5 antigen varied from negative to normal. Staining with WT-31 and TCR- δ 1 divided the CD3 positive T cells into two complementary groups (30% CD3⁺, WT-31⁺, TCR- δ 1[–] and 70% CD3⁺, TCR[–], δ 1⁺, WT-31[–]) (fig 1). A normal concentration of CD16 cells (8%) was found corresponding to the finding of normal NK cell activity (data not shown). An extraordinarily high number of Leu-7 cells (62%) was found.

Table Expression of cell surface molecules on lymphocytes isolated from peripheral blood

Surface molecule (antibody)	Case No	Normal range
CD2 (Anti-Leu-5)	85	73–90
CD3 (Anti-Leu-4)	80	64–89
$\alpha\beta$ TCR (WT-31)	24	50–81
$\alpha\beta$ TCR (BMA-031)	24	54–80
$\gamma\delta$ TCR (TCR- δ 1)	56	1–10
CD4 (Anti-Leu-3)	16	45–65
CD8 (Anti-Leu-2)	36	21–35
CD5 (Anti-Leu-1)	57	61–84
CD16 (Anti-Leu-11)	8	3–15
— (Anti-Leu-7)	62	0–20

*Values are the percentages of cells with fluorescence intensity above the upper limit of the negative control.

Fig 1 Two colour flowcytometric analysis of peripheral blood mononuclear cells from the patient. The designation of the staining antibody is given on the relevant axis. Anti-Leu-4 was applied as PE conjugated antibody. WT-31 was applied either as FITC conjugated antibody or as biotinylated antibody with PE-streptavidin as the second reagent. TCR- $\delta 1$ was applied with FITC-rabbit anti-mouse Ig as the second reagent.

IMMUNOPRECIPITATION AND SODIUM DODECYL SULPHATE—POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

SDS-PAGE showed that when lysate from ^{125}I -labelled peripheral blood mononuclear cells was precipitated with βF1 the $\alpha\beta$ TCR could be precipitated and that it existed as a disulphide-linked heterodimer with the same molecular weight as the $\alpha\beta$ TCR from the control

(non-reduced 90 kilodaltons, reduced 45 kilodaltons α -chain and 40 kilodaltons β -chain) (fig 2). Precipitation with TCR- $\delta 1$ showed that most of the $\gamma\delta$ TCR existed as a disulphide-linked dimer with a molecular weight of 80 kilodaltons in the non-reduced sample and 40–42 kilodaltons in the reduced sample. Furthermore, material with molecular weights of 55 kilodaltons and 40 kilodaltons were precipitated with TCR- $\delta 1$ in the non-reduced as well as reduced samples.

THYMIC MORPHOLOGY

The lobules looked mostly undifferentiated with no formation of Hassall's corpuscles or medullary differentiation, but a single corpuscle was recognised in one lobule and in others there was slight corticomedullary demarcation with central groups of small mature looking lymphocytes (fig 3). In sections stained with haematoxylin and eosin most lobules appeared almost lymphocyte depleted, consisting merely of epithelial cells.

THYMIC IMMUNOHISTOLOGY

Staining with pan-epithelial (AE1, LP34) and pan-leucocyte (CD45) monoclonal antibodies disclosed that about 50% of the cell population comprised epithelial cells, while the remainder were mostly lymphoid cells. The latter were large and about the size of the epithelial cells and therefore their numbers were underestimated by conventional microscopy. All lymphoid and epithelial cells stained intensely with monoclonal antibodies to MHC class I antigens.

Phenotype of the epithelial cells: The subcapsular epithelial cells seemed to be normally developed, as shown by labelling with BG3C8, but the medullary epithelial cells were scarce and entirely absent in most lobules. Cortical epithelial cells, as defined by labelling with monoclonal antibodies T2/30 and MR7, predominated in all parts of the lobules. Staining for epidermal keratin with K92 confirmed the presence of a single Hassall's corpuscle in one lobule.

Phenotype of the lymphoid cells: Small lymphocytes were rare and confined to occasional abortive medullary areas. They showed a normal late T cell phenotype (CD6^+ , CD27^+ , HLA-DR^- , $\text{CD4}^+/\text{CD8}^-$, or $\text{CD4}^-/\text{CD8}^+$). The predominant population of rather large CD45 positive cells that intermingled with cortical epithelial cells showed a normal cortical thymocyte phenotype for some differentiation antigens like CD3, CD7, and $\alpha\beta$ TCR. These cells, however, lacked or expressed only low amounts of CD1, CD2, CD4, CD5 and CD8 antigens. Double immunoenzymatic labelling for CD4 and CD8 antigens showed that these were never simultaneously expressed on the same cells as is the case in the normal thymic cortex. Only occasional small lymphocytes

Fig 2 SDS-PAGE of immunoprecipitates from ^{125}I -labelled peripheral blood mononuclear cells from the patient (lanes 1–6), control one (lanes 7–12), and control two (lanes 13–18). Precipitations were performed with normal mouse serum as negative control (lanes 1, 2, 7, 8, 13, 14), monoclonal antibody βF1 (lanes 3, 4, 9, 10, 15, 16), and monoclonal antibody TCR- $\delta 1$ (lanes 5, 6, 11, 12, 17, 18). Samples were run under non-reducing (uneven numbered lanes) or reducing (even numbered lanes) conditions.

stained with monoclonal antibodies to TCR γ - or δ -chains. Abnormal expression of CD10 antigen (weak) and HLA-DR (strong, also on epithelial cells) was

noted on all thymocytes. There was no expression of activation-associated antigens (CD25 and CD30); all lymphoid cells expressed normal amounts of CD11a.

Fig 3 Frozen section of thymic biopsy specimen showing lobules that seem to be almost lymphocyte depleted. There is only minimal medullary differentiation. A single Hassall's corpuscle is recognised in a lobule with some corticomedullary demarcation and the presence of small medullary lymphocytes. (Haematoxylin and eosin.)

Fig 4 Frozen sections of thymic biopsy specimen. (a) Stained for CD45 antigen with F25-29 showing about half of the cells to be of haematolymphoid origin; (b) stained for thymic cortical epithelial antigen with T2/30 showing predominance of epithelial cells with cortical phenotype; (c) normal infant thymus stained with BG3C8 showing intense labelling of subcapsular and medullary epithelial cells but no staining of cortical cells; (d) thymic biopsy specimen of the patient stained with BG3C8 showing the presence of subcapsular epithelium but absence of medullary phenotype epithelium. (a–g: immunoperoxidase (brown) and alkaline phosphatase anti-alkaline phosphatase (red) technique); h: immunoperoxidase and alkaline phosphatase technique).

The number of Leu-7 positive cells was not increased. Staining for proliferation associated nuclear antigen (Ki-67) showed almost all large lymphoid cells to be in a proliferating stage. CD1 positive dendritic cells were few in number. Fig 4 shows the immunohistological staining patterns.

Discussion

The patient described in this study may represent a primary immune deficiency not previously described. The survival to the age of 6 years without severe symptoms, the finding of an almost normal humoral immunity, and the relatively high concentration of CD3 positive lymphocytes argue against a severe combined immune deficiency.^{18–20} The possibility of DiGeorge's syndrome can be excluded because the patient lacked the characteristic clinical features associated with this disorder.¹⁸ The condition might be

analogous to that seen in some patients with Nezelof's syndrome (defective cellular immunity without severe impairment of humoral immunity).^{18,21} Because detailed studies of lymphocyte differentiation markers in cases of Nezelof's syndrome have not been reported, it cannot be excluded that our case resembles this syndrome. The thymic histology resembled the pattern of "simple dysplasia" described in various conditions—for example in patients with severe combined immune deficiency.²²

The finding of a thymic defect associated with a very high concentration of peripheral blood $\gamma\delta$ TCR positive cells could probably be due either to a primary, congenital thymic epithelial deficiency, or to a primary defect of the T lymphocyte progenitor cells. FACS analysis showed the presence, although in low numbers, of phenotypically normal $\alpha\beta$ TCR positive T cells expressing either CD4 or CD8 as well as normal titres of CD5. We suggest that these results may argue

Fig 4 Frozen sections of thymic biopsy specimen. (e) staining with UCHL1 for CD3 antigen; (f) staining with F101-01 against a conformational epitope of the TCR/CD3 complex; (g) staining of TCR δ -chain with TCR- δ 1; (h) double staining for CD4 and CD8 antigen with anti-Leu-3a (red) and Tü68 (blue), respectively.

against a primary precursor T lymphocyte defect and for a primary thymic epithelial abnormality.

A congenital thymic epithelial deficiency associated with a low number of T cells but potentially functional precursor T cells exists in nude athymic mice.²³ A high proportion of T cells isolated from nude mice express the $\gamma\delta$ TCR.^{10,24} Nude mice were initially thought to represent an animal model of DiGeorge's syndrome,²⁵ but no hypoparathyroidism, anomalies of great vessels, nor other diseases of the fourth pharyngeal pouch were found. We suggest that our patient may represent an isolated defect in thymic epithelial development which may be a human analogue to that of the nude mouse.

The existence of a thymus independent pathway for T cell differentiation has been described in mice.^{10,24} The immunohistological studies of the patient's thymus showed normal expression of CD3 antigen which compared well with the staining for the TCR β -chain. Staining for the TCR γ - and δ -chain showed only occasional positive lymphoid cells as in the normal thymus of childhood (personal observations).

This finding suggests that at least some of the $\gamma\delta$ TCR positive cells may have been differentiated in a thymic-independent pathway.

The expression of a disulphide-linked and a non-disulphide-linked form of $\gamma\delta$ TCR correlates with the use of C γ 1 and C γ 2 constant region gene segments, respectively.²⁶ Thus biochemical studies showed that most patients with $\gamma\delta$ TCR positive cells used the C γ 1 constant region gene segment encoding for the TCR γ -chain of 40 kilodalton disulphide-linked with the TCR δ -chain of 40 kilodaltons. Furthermore, a small fraction of $\gamma\delta$ TCR positive cells using the C γ 2 constant region gene segment encoding for the TCR γ -chain of 55 kilodaltons non-disulphide-linked with the TCR δ -chain of 40 kilodaltons were also shown. The relation, if any, between the use of C γ 1 or C γ 2 constant region gene segment and thymic-independent or thymic-dependent T cell differentiation still remains to be elucidated.

Recently it has been reported that WT-31 may react with $\gamma\delta$ TCR positive cells, depending on the state of glycosylation of the $\gamma\delta$ TCR.²⁷ Biochemical studies,

however, have shown the presence of both $\alpha\beta$ and $\gamma\delta$ TCR positive cells in the peripheral blood of our patient.

This case shows that monoclonal antibodies against subpopulations of thymic epithelial cells and T cells may further studies of the relation between thymic and peripheral T cell maturation. The studies of patients with immune deficiencies using immunohistological, flow cytometric, and biochemical methods might contribute to a better understanding of the normal development of the immune system and to a more precise classification of immune disorders.

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