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Primary T lymphocyte immunodeficiency associated with a selective impairment of CD2, CD3, CD43 (but not CD28)-mediated signal transduction

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SUMMARY

A 2-year-old female with important signs of immune response failure against virus, bacteria, fungi and protozoa and no obvious humoral or lymphocyte phenotypical defect was studied. Both peripheral blood mononuclear cells and IL-2-dependent T cell lines derived from the patient showed a severe selective T cell activation impairment via CD2, CD3 and CD43; however, this defect was reversible with the addition of either IL-2, or phorbol myristate acetate (PMA) or anti-CD28 antibodies. Concordantly, the induction of IL-2 (and, in part, IL-3 and IL-4) messenger RNA was severely reduced in stimulated T cells, but that of other cytokines was either normal (IL-5) or only slightly diminished (interferon-gamma (IFN-γ)). It is concluded that an activation T cell defect exists previous to protein kinase C (PKC) and between membrane receptors and the activation pathway of certain response genes encoding for interleukins involved in proliferation (i.e. IL-2, IL-3 and IL-4), but not of others (i.e. IL-5). The use of T cell lines from human T lymphocyte activation deficiencies allows dissection of T cell pathology and the corresponding physiological pathways. In the present description, there is an evident independence of the CD28 T cell activation pathway from those induced through CD2 or CD3, and the differential gene regulation of the different interleukins.

Keywords T lymphocyte signal transduction proliferation immunodeficiency IL-2 deficiency

INTRODUCTION

T cell activation is a complex, finely tuned chain of biochemical events that requires the participation of many molecules, both at the cell surface and within the cell [1]. T cell activation is initiated by the binding of antigens (or mitogens) to the T cell receptor/CD3 complex. Other surface antigens, like CD2, CD43 and CD28, also participate in early or late stages of the process. After antigen binding, several signals are sent to the T cell nucleus, where different groups of genes are activated or inhibited [2]. Certain universal signal transduction mechanisms are known to exist in T cells: protein phosphorylation/dephosphorylation, G-protein-dependent hydrolysis of inositol phospholipids and, as a consequence, activation of protein kinase C (PKC) and an increase in cytosolic Ca^{2+}. As a final effect, an activated T cell exerts preprogrammed effector functions, measurable *in vitro* as proliferation, lymphokine secretion or cytotoxicity.

Although T cell activation is a central step of the immune response, specific T cell activation pathology is only beginning to be recognized, presumably as a result of a limited availability of appropriate laboratory tests. In the present study, we have used proliferation in both peripheral blood mononuclear cells (PBMC) and IL-2-dependent T cell lines as an indicator of T cell function in a 2-year-old infant with clinical signs of immunodeficiency, including repeated viral, bacterial, fungal and parasitic infections.

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PATIENT AND METHODS

Patient

The patient is a 2-year-old female born to healthy unrelated Spanish parents. She had a history of recurrent bacterial, fungal, parasitic and viral infections, including several episodes of urine infections by *Escherichia coli*. ADA and PNP deficiencies were excluded as causes of immunodeficiency. She was 1 week old when an episode of conjunctivitis was recorded. At 10 weeks of age she was admitted with a gastroenteritis episode and diarrhoea. Laboratory examination revealed *Giardia lamblia* cysts, which appeared again when the girl was 4 months old. Oral candidiasis developed the first of three recorded times when she was 3 months old, and the last when

Table 1. Peripheral blood mononuclear cell phenotype*

Molecule		Reference range
Lymphocytes		
Absolute number/μl	5500 ± 2066	1500–4000
CD2	77 ± 6	74–86
CD3	63 ± 8	54–71
CD3	67 ± 8	58–73
TCRγδ	7	2–12
CD4	44 ± 8	29–52
CD4CD45RA	28	15–35
CD4CD45RO	5	7–19
CD8	17 ± 3	17–31
CD7	63	58–72
CD28	51	35–55
CD25	2 ± 1	1–12
CD43	71	74–86
HLA-DR	20 ± 4	14–21
CD20	19	10–20
CD21	17 ± 7	10–18
CD57	4 ± 4	1–14
CD56	17 ± 10	8–33
CD16	7 ± 5	6–20
CD11b	5 ± 4	2–35
Monocytes		
Absolute number/μl	775 ± 310	400–1000
CD14	89 ± 8	80–100
CD4	28 ± 12	15–46
CD11b	84 ± 8	65–100
HLA-DR	72 ± 5	65–95
CD18	89	82–100

*Expressed as a percentage of positive cells. The results are shown as mean ±s.d., except when only one determination was available.

she was 20 months old. An unidentified viral infection was diagnosed at 4 months; also a colitis episode due to salmonellosis was observed at 13 months old. Levels of serum immunoglobulins (IgG 599 ± 299 mg/dl, IgA 22 ± 15 mg/dl and IgM 87 ± 29 mg/dl), IgG subclasses and isohaemagglutinins were found to be normal for her age. The total IgG and IgG1 subclass response following vaccination with tetanus toxoid and the total IgG response to polysaccharide from *Haemophilus* were also normal. However, the IgG2 subclass response to *Pneumococcus* polysaccharide was decreased, whereas the total IgG production was within normal values. Serial determinations of serum complement levels (C3, C4) and haemolytic complement activity (CH_{50}) were also found to be normal.

Isolation of lymphocytes

PBMC were obtained by density gradient centrifugation using Ficoll–Hypaque (Lymphoprep; Nyegaard, Oslo, Norway) as described [3].

Cytofluorographic analysis

Whole blood samples were stained by direct immunofluorescence as described [4] with the use of the following MoAbs: OKT3 (anti-CD3), OKT4 (anti-CD4), OKT8 (anti-CD8), OKM14 (anti-CD14) and OKB7 (anti-CD21) (Ortho Pharmaceuticals, Raritan, NJ); Leu-4 (anti-CD3), Leu-11c (anti-CD16), Leu-16 (anti-CD20), Leu-45RO (anti-CD45RO), Leu-7 (anti-CD57), and Leu-19 (anti-CD56) (Becton Dickinson, Sunnyvale, CA); T11 (anti-CD2), IL-2-R1 (anti-CD25), MO1 (anti-CD11b) and 2H4 (anti-CD45RA) (Coulter, Hialeah, FL); IOT18 (anti-CD18) (Immunotech, Marseille, France); anti-CD43 (Cymbus Bioscience, Southampton, UK); anti-CD7 (Sera-Lab, Crawley Down, UK); Kolt-2 (anti-CD28) (CLB, Amsterdam, The Netherlands); and TCRδ1 (anti-TCRγδ) (T Cell Diagnostics, Cambridge, MA). Stained cells were analysed by an Epics C cell sorter (Coulter). The results were recorded as the percentage of stained cells by each MoAb (those displaying fluorescence intensities above the upper limit of a negative control) within the lymphocyte population (electronically gated by size/complexity criteria). The expression level of the different molecules was simultaneously recorded in arbitrary units for comparative purposes only.

Cell lines

Cell lines were derived from PMBC and cultured with RPMI 1640 medium (Biochrom, Berlin, Germany) containing 10% fetal calf serum (FCS) (Flow Laboratories, UK), 1% phytohaemagglutinin (PHA; Difco Labs, Detroit, MI) and 50 U/ml of human recombinant IL-2 (kindly provided by Hoffmann-La Roche, Nutley, NJ) and some of them also with allogeneic feeder Epstein–Barr virus (EBV)-transformed cells. Cells from the patient were cultured in parallel with cells from different healthy individuals for 15–35 days, and the latter were used as controls in all experiments.

Functional assays

Proliferative assays. The proliferative assays of PBMC and IL-2-dependent T cell lines were carried out as previously described [4]. Eighty thousand cells were placed in round-bottomed microtitre wells (Nunc, Roskilde, Denmark) in 0·2 ml of final culture medium RPMI 1640 (Biochrom) supplemented with 10% FCS, 1% antibiotic (streptomycin, penicillin and fungizone) and 1% L-glutamine 20 mM (Whittaker, Walkersville, MD). The following stimuli or their combinations were: anti-CD3 (OKT3) (soluble, 12·5 ng/ml, or attached to plastic) [5]; anti-CD28 (Kolt-2, 50 ng/ml purchased from Janssen Biochimica, and Kolt-2 1:60 000 dilution of ascitic fluid from K. Sagawa, Fukuoka, Japan); anti-CD2 (D66 plus X11 at 1:2000, and 1:1600 dilution of ascites fluid, from A. Bernard, Villejuif, France); anti-CD43 (at 0·5 μg/ml purchased from Janssen Biochimica); concanavalin A (Con A) 10 μg/ml (Calbiochem, La Jolla, CA); PHA 1:100 final (Difco); pokeweed mitogen (PWM) 1% v/v (Difco); Enterotoxin A and Enterotoxin C1 1 ng/ml (Serva, Heidelberg, NY); phorbol myristate acetate (PMA; Sigma, St Louis, MO) 10 ng/ml; ionomycin (Calbiochem) 1 μM, and human recombinant IL-2 (rIL-2) (Genzyme, Cambridge, MA) 50 U/ml.

Cytokine gene induction. IL-2-dependent T cell lines were stimulated under identical conditions as described for proliferative assays. The induction of cytokine-specific transcripts (IL-2, IL-3, IL-4, IL-5 and interferon-gamma (IFN-γ)) was analysed using a commercial protein chain reaction (PCR) reagent kit (Mapping; Clontech, Palo Alto, CA). Appropriate age-matched controls were always tested in parallel.

IL-2Rα expression. PBMC were stimulated with PHA or PHA plus PMA under identical conditions as described for

Table 2. Peripheral blood mononuclear cell function*

Stimulus	Experiment 1		Experiment 2		Experiment 3	
	Patient 7 months	Normal control	Patient 9 months	Normal control	Patient 32 months	Normal control
Controls						
Medium	1	1	1	6	<1	<1
PMA	3	2	2	5	3	2
Interleukins						
IL-2	12	6	4	12	2	4
Antigens						
Enterotoxin A	16	38	19	25	–	–
Enterotoxin C1	***1***	8	–	–	–	–
Monoclonal antibodies						
Anti-CD3	***<1***	8	***3***	18	***5***	9
Anti-CD3 + IL-2	38	49	147	100	55	54
Anti-CD3 + PMA	57	137	50	238	79	114
Anti-CD3+anti-CD28	–	–	120	67	24	42
Anti-CD2	–	–	–	–	***<1***	43
Anti-CD2 + PMA	–	–	–	–	192	111
Anti-CD2 + anti-CD28	–	–	–	–	136	161
Anti-CD43	–	–	–	–	***<1***	12
Anti-CD43 + IL-2	–	–	–	–	41	52
Anti-CD43 + PMA	–	–	–	–	53	152
Anti-CD28 + PMA	–	–	209	256	–	–
Lectins						
PHA	***5***	79	***8***	168	***23***	233
PHA + IL-2	253	248	361	364	–	–
PHA + PMA	288	133	213	345	249	260
Con A	***2***	32	***11***	239	***41***	120
Con A + IL-2	167	121	280	321	–	–
Con A + PMA	212	86	–	–	–	–
PWM	15	8	45	23	–	–
PWM + PMA	77	35	10	123	–	–

*Expressed as ^{3}H-thymidine incorporation in ct/min $\times 10^{-3}$. Bold italics denote consistently low proliferative responses.
PMA, Phorbol myristate acetate; PHA, phytohaemagglutinin; Con A, concanavalin A; PWM, pokeweed mitogen.

proliferative assays, and analysed at 48 h by cytofluorography (Epics C) with the IL-2-R1 antibody.

RESULTS

PBMC phenotype (Table 1)

Patient PBMC showed normal numbers of T, B and natural killer (NK) cells. In particular, the CD4 and CD8 T cell subsets were within normal relative values, and the surface densities of the analysed molecules were comparable to those obtained in the controls (data not shown). However, a slightly reduced number of memory ($CD4^+CD4RO^+$) T cells was observed in one instance.

PBMC function

Isolated PBMC from the patient and healthy controls were challenged *in vitro* with different cell-surface as well as transmembrane stimulatory reagents or their combinations. A severe impairment of CD3-mediated proliferative response was immediately apparent and stable within the studied period of time; a similar impairment was observed in the proliferative response to PHA and Con A (Table 2). Certain other responses, such as CD2- and CD43-mediated proliferation, were also severely diminished. By contrast, other membrane stimuli (IL-2, enterotoxin A and PWM) elicited normal proliferative responses. These defects could not be due to a lack of CD2, CD3 or CD43 epitopes or to gross disbalances in T cell subsets (Table 1 and unpublished fluorescence intensity data). The defects were probably associated with a low induction of IL-2, because the addition of exogenous recombinant IL-2 effectively restored control proliferative responses to anti-CD3, anti-CD43, PHA and Con A (Table 2). These responses were also completely restored by addition of the transmembrane PKC activator PMA. Taken together, the results revealed the existence of a stable signalling defect between membrane receptors (notably CD3, CD2 and CD43) and certain responses genes involved in PBMC proliferation (like that of IL-2). The fact that submitogenic doses of PMA corrected the responses suggests that the defects may be membrane-proximal, previous to PKC in the biochemical T cell activation pathway. The impaired proliferative response following CD3 and CD2 triggering was also

Table 3. IL-2-dependent T cell line function*

Stimulus	Experiment 1		Experiment 2		Experiment 3	
	Patient 7 months	Normal control	Patient 7 months	Normal control	Patient 32 months	Normal control
Controls						
Medium	<1	2	<1	<1	<1	<1
PMA	8	13	–	–	<1	<1
Interleukins						
IL-2	45	58	27	120	12	24
Monoclonal antibodies						
Anti-CD3	***4***	146	***4***	309	***<1***	13
Anti-CD3 + IL-2	164	138	86	212	36	72
Anti-CD3 + PMA	127	101	–	–	–	–
Anti-CD2	–	–	–	–	***<1***	17
Anti-CD2 + PMA	–	–	–	–	82	22
Anti-CD2 + anti-CD28	–	–	–	–	79	20

*Expressed as ^{3}H-thymidine incorporation in ct/min $\times 10^{-3}$. Bold italics denote consistently low proliferative responses. The CD3 molecule was shown to be present in > 95% of T cells from patients and controls, and the CD4/CD8 subset ratio was comparable in all cell lines. Cell lines cultured with (experiments 2 and 3) or without (experiment 1) feeder cells were used (see Patient and Methods).

PMA, Phorbol myristate acetate.

restored when anti-CD28 monoclonals were added as costimulus. These results support that the CD28 activation pathway is different [4], and suggest that it may collaborate with the biochemical machinery engaged by the CD3 and CD2 pathways for the induction of the intermediate messengers involved in T cell activation (i.e. IL-2).

A further analysis of PBMC function was performed by studying the induction of IL-2α (CD25) as a response gene after PHA stimulation (CD3 stimulation could not be used to measure CD25 induction due to the low signal/background ratio in normal controls). The results indicated that the patient's PBMC had a significant defect in CD25 induction (Fig. 1). This defect was corrected by addition of PMA, suggesting again the existence of a pre-PKC primary defect in this individual.

T cell line function

In order to test whether the observed PBMC proliferation defects were due to primary T cell-specific deficiency, several IL-2-dependent T cell lines were derived from the patient and, along with control T cell lines from healthy individuals, analysed for CD3- and CD2-mediated proliferation and cytokine gene induction in response to CD3 engagement. The results of the proliferative assays (Table 3) clearly demonstrate a consistent severe T cell-specific impairment in CD3- and CD2-mediated proliferation. The decreased proliferation of patient's T cells in response to CD3 engagement was completely restored by addition of exogenous IL-2 or by direct PKC activation using PMA, as shown previously in PBMC. T cell lines also revealed a severely impaired proliferative response following CD2 triggering, which was restored when PMA or anti-CD28 monoclonals were added as costimulus.

Lastly, a direct assessment of cytokine gene induction after CD3 triggering was carried out (Fig. 2). The results demonstrated the existence of a profound defect in the induction of IL-2 mRNA and diminished (IL-3, IL-4) or slightly diminished levels (IFN-γ) of other cytokine messages. This defect was not due to a general impairment of cytokine message induction by the patient's lymphocytes, because other cytokine transcripts (IL-5) were normal. As expected from previous functional data, the IL-2 message induction in response to CD3-mediated stimulation was restored when PMA was added as costimulus (Fig. 2).

DISCUSSION

The primary genetic and biochemical defect giving rise to the observed activation impairments (proliferative response, IL-2

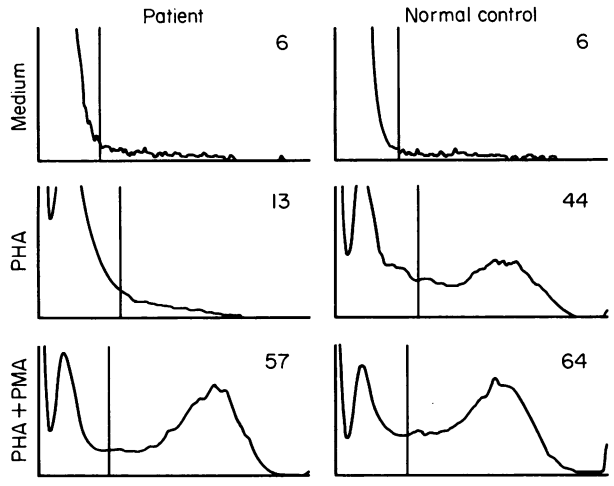

Fig. 1. Defective induction of IL-2Rα expression after activation by phytohaemagglutinin (PHA) of peripheral blood mononuclear cells from patient compared with a normal control. The percentage of positive cells appears in the top-right of each graph. The vertical lines within each graph denote the upper limit of the negative control staining. PMA, Phorbol myrisate acetate.

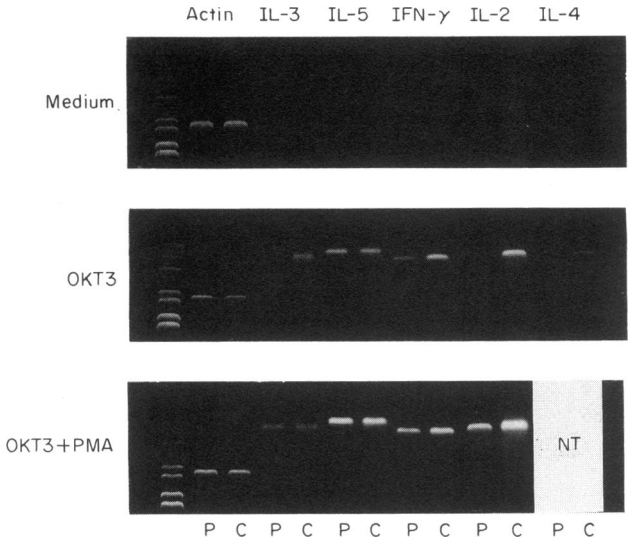

Fig. 2. Cytokine mRNA induction deficiency after CD3 triggering of an IL-2-dependent T cell line derived from patient's peripheral blood mononuclear cells (PBMC). Unstimulated and stimulated cells (patient and control) were harvested at 16 h and their cytokine message content assayed by specific PCR amplification (see Patient and Methods). RNA recovery efficiency (positive control) was measured using actin-specific oligonucleotides. Negative control lanes contained no oligonucleotides in the PCR mixture (data not shown). The amplified fragment sizes were: actin 1·2 kbp, IL-2 0·462 kbp, IL-3 0·459 kbp, IL-4 0·462 kbp, IL-5 0·414 kbp and INF-γ 0·5 kbp. NT, Not tested; P, patient; C, normal control; PMA, phorbol myristate acetate.

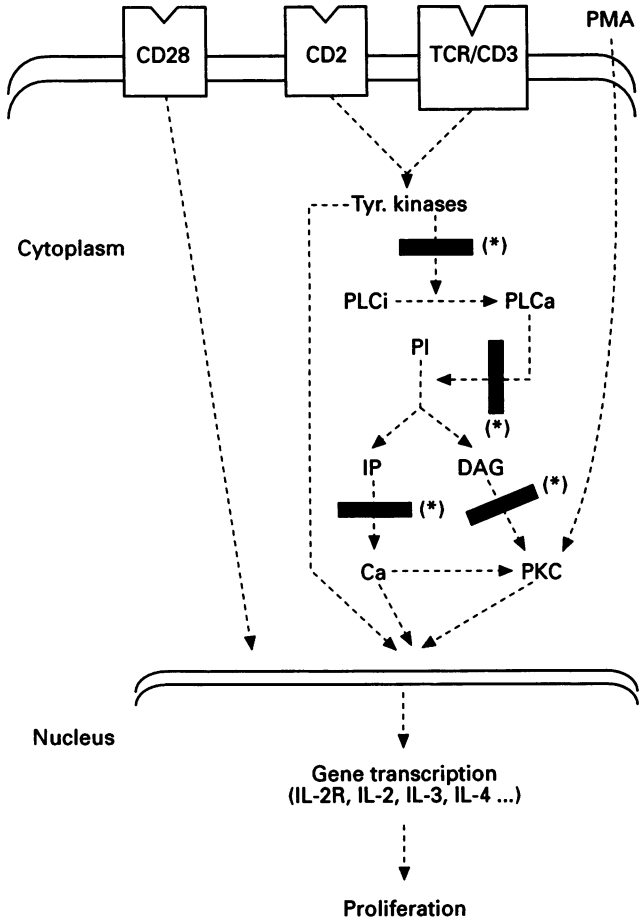

Fig. 3. Proposed defect in the patient's T cells. CD3- and CD2-mediated proliferation as well as CD3-mediated gene induction (IL-2) become normal when anti-CD28 MoAbs or phorbol myristate acetate (PMA) are added as costimulus, suggesting the existence of a membrane-proximal defect. The defect may reside between membrane receptors (CD2, CD3) and PKC activation (*). PLCi, Inactive phospholipase C; PLCa, activated phospholipase C; PI, phosphatidyl inositides; IP, inositides phosphate; DAG, diacylglicerol; PKC, protein kinase C; Tyr. kinases, tyrosine kinases.

production and IL-2 receptor α chain induction) in the studied individual remain undefined. However, the analysis of T cell lines permits a clear distinction between primary and secondary or acquired T cell activation defects. This may be of diagnostic as well as therapeutical use in the growing field of human T cell pathology [6].

In this patient, a primary T cell activation defect was ascertained (Fig. 3). Further studies are required, using the available T cell lines, to establish the biochemical basis of this novel type of immune defect. Interestingly, the proliferation impairment of PBMC in this individual was more severe using CD3-specific antibodies than enterotoxins as stimuli (Table 2). As both reagents engage the T cell receptor/CD3 complex, the results suggest that enterotoxins deliver a more complete, stronger or physiological signal to T cells or induce additional stimuli through alternative activation pathways initiated by the engagement of the TCR/CD3 receptor, overcoming in part the defective signal transduction observed via CD3 with anti-CD3 antibodies [7,8]. In this patient, the impaired response to CD3- and CD2-specific antibodies was restored when anti-CD28 antibodies were added as costimulus (Table 2), which may reflect the induction by CD28 of additional regulatory factors that are not induced by CD3 or CD2 alone in this individual [9]; this may be due to a pre-PKC defect that can be restored when the PKC activator PMA is added as costimulus (Tables 2 and 3, Fig. 3). The fact that when PMA is added as costimulus the responses (proliferation, IL-2 production, IL-2R expression) are restored, may suggest that an additional pathway of T cell activation, different to the PKC pathway (e.g. tyrosine kinase activity) is functional in the patient's T cells (Fig. 3).

On the other hand, inadequate signalling via the T cell antigen receptor may induce a state of unresponsiveness of T cells, which is referred to as anergy [10,11]. In fact, anergic $CD4^+$ T cells have a greatly reduced ability to proliferate and to produce IL-2 after antigenic restimulation. This can be reversed *in vitro* by IL-2 stimulation [12] or CD28 triggering [13]. Also, activation-induced cell death by apoptosis *in vitro* can be prevented by CD28 engagement [14] or PKC activation [15]. Since apoptosis may be induced in mature T cells as a consequence of activation through the TCR/CD3 complex or the CD2 molecule [14–17], the observed restoration of the patient's T cell proliferative responses to CD3 and CD2 triggering, when IL-2, PMA or anti-CD28 antibodies are added as costimulus, may result from preventing anergy and/or activation-induced cell death. Indeed, the slightly reduced numbers of memory ($CD45RO^+$) $CD4^+$ T cells in the patient's PBMC (Table 1) may be due to their depletion by apoptosis, or may be secondary to the inadequate activation of these cells.

The antibody response (IgG and IgG1) to T cell-dependent

antigens (tetanus toxoid) was normal in this patient, while IgG2 levels in response to polysaccharides from *Pneumococcus* (which was thought not to be dependent on T cells) were reduced, although the total IgG response was within normal values. These results strengthen the possibility that the specific IgG2 response to polysaccharide antigens may in certain cases be partially dependent on T cell collaboration, as has been previously observed in other primary T cell immunodeficiencies [18,19]. It is known that certain immunoglobulin switching is dependent on T cell–B cell interaction (i.e. CD40–CD40L for switching to IgG, IgE and to IgA [20]). Moreover, IgG4 and IgE secretion are dependent on IL-4 production by T cell subsets [21], and transforming growth factor-beta (TGF-β) and IL-10 cooperate to induce IgA secretion [22]. A related defect (either B cell–T cell interaction, or a T cell-secreted factor) might be responsible for the lack of IgG2 response to polysaccharide antigens in this patient.

This presently described immunodeficiency may be classified as a primary signal transduction defect, together with other deficiencies which have previously been published [23–30].

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