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Monosomy 1p36 uncovers a role for OX40 in survival of activated CD4+ T cells

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Abstract

Monosomy 1p36 is a subtelomeric deletion syndrome associated with congenital anomalies presumably due to haploinsufficiency of multiple genes. Although immunodeficiency has not been reported, genes encoding costimulatory molecules of the TNF receptor superfamily (TNFRSF) are within 1p36 and may be affected. In one patient with monosomy 1p36, comparative genome hybridization and fluorescence *in-situ* hybridization confirmed that TNFRSF member OX40 was included within the subtelomeric deletion. T cells from this patient had decreased OX40 expression after stimulation. Specific, *ex vivo* T cell activation through OX40 revealed enhanced proliferation, and reduced viability of patient CD4⁺ T cells, providing evidence for the association of monosomy 1p36 with reduced OX40 expression, and decreased OX40-induced T cell survival. These results support a role for OX40 in human immunity, and calls attention to the potential for haploinsufficiency deletions of TNFRSF co-stimulatory molecules in monosomy 1p36.

Keywords

OX40; 4-1BB; TNFRSF superfamily; 1p36; T cell memory; primary immunodeficiency; subtelomeric deletion

Introduction

Monosomy 1p36 is a relatively common subtelomeric deletion syndrome presumably caused by haploinsufficiency of a number of different genes. The deletions are variable in size and location within chromosome 1p36 and may consist of terminal deletions, interstitial deletions, derivative chromosomes or complex rearrangements [1]. The syndrome occurs in 1:5000 births and is characterized by a phenotype including deep-set eyes, midfacial hypoplasia, asymmetric

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ears and pointed chin. Other clinical associations may include seizures, hypothyroidism, cardiomyopathy, developmental delay, hearing impairment and mental retardation [2,3,4,5]. To date, immunodeficiency has not been reported in these patients, however genes for various immunologically important members of the TNFR superfamily are located in or near the region of 1p36.

At least five genes encoding tumor necrosis factor receptor (TNFR) superfamily (SF) members co-localize to chromosome 1p36, including OX40 (CD134, TNFRSF4), glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR, TNFRSF18, AITR), herpesvirus entry mediator (HVEM, TNFRSF14), 4-1BB (CD137, TNFRSF9), and CD30 (Ki-1, TNFRSF8) [1,6,7]. The TNFRSF family is comprised of a diverse array of genes that provide costimulatory signals to achieve optimal T cell activation, proliferation, survival and longevity [6,7]. Of particular interest to this study is OX40; a TNFRSF family member which is proposed to be a key costimulatory molecule involved in the generation of memory CD4 T cells [8,9]. OX40 is induced after ligation and activates NF- κ B to promote expression of the anti-apoptotic proteins survivin, Bcl-2 and Bcl-x_L, thus prolonging effector T cell survival and increasing the memory T cell pool [10–15]. OX40 also functions in T cell-dependent B cell proliferation and differentiation, implicating its role in generating optimal IgG memory recall responses to foreign antigens. As an inducible costimulatory molecule, OX40 is suggested to be more important in regulating the secondary immune response when compared to constitutively expressed molecules like CD28 that are crucial for inducing primary responses. OX40 is preferentially expressed on activated CD4 T cells, and its absence or blockade results in decreased CD4 T cell viability, defective maintenance of CD4⁺ memory and an inability to generate an appropriate immune response to foreign antigen [16,11,17,18,19,12,20]. Furthermore, OX40 deficient mice exhibit reduced CD4⁺ responses to LCMV, VSV, contact hypersensitivity and common protein antigens. This is presumably due to decreased antigen specific T cells, generated late in the primary response as well as five weeks later when the memory response is formed [9,11–17,21–24]. In addition, reverse signaling through OX40L induces B cell proliferation, differentiation and immunoglobulin secretion [25,26].

In this report we describe a 3-year-old female with an abnormal karyotype and subtelomeric deletion in chromosome 1p36. This patients' particular deletion included OX40 and was associated with a specific decrease in CD4⁺ effector cell viability and an accelerated proliferation profile as measured by carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution. This is the first study showing functional differences in T cell proliferation and viability in a human haploinsufficient for OX40.

Materials and Methods

Patient evaluation

The patient and was evaluated at the Children's Hospital of Philadelphia and permission to obtain peripheral blood as well as report data was approved by the hospital's Institutional Review Board for the protection of human subjects. Consent for participation in this report was provided by the patient's parents or by the appropriate individual in the case of control donors. Clinical laboratory measurements were performed using standard techniques in a CLIA certified laboratory.

Array CGH (Comparative Genome Hybridization)

Microarray analysis was performed by Signature Genomic Laboratories (Spokane, Washington), using the Signature Chip, (www.signaturegenomics.com), which included assessment of 622 loci using 1887 BAC clones and DNA extracted from peripheral blood

mononuclear cells from the patient. The analysis was performed using previously published methods [27].

Cytogenetic and Fluorescence In Situ Hybridization (FISH) Analyses

Metaphase chromosomes were obtained from phytohemagglutinin (PHA) - stimulated peripheral blood lymphocytes and G-banding was performed by standard methods. Fluorescence in situ hybridization (FISH) studies were carried out to define the extent of the 1p deletion. Commercially available probes for the subtelomeric regions of chromosome 1 (TelVysion 1p, CEB108/T7 and TelVysion 1q) were used on metaphase chromosomes according to the manufacturer's instructions (Vysis, Inc. Downer's Grove, Ill). BAC clone (RP5-902P8,) was selected from the public databases to confirm deletion of the OX40 gene. The map position of each BAC clone was determined according to the UCSC Human Genome Project (May 2004 assembly) (<http://genome.cse.ucsc.edu>). All BAC clones were obtained through CHORI BACPAC Resources (Oakland, CA). BAC DNA was isolated (Puregene, Gentra System) and labeled by nick translation (Nick Translation Reagent Kit, Vysis, Inc.) in the presence of Spectrum Red (Vysis, Inc.). FISH probes were hybridized to metaphase preparations overnight; slides were then washed and counterstained with DAPI using standard protocols [28].

In vitro polyclonal stimulation

Peripheral blood mononuclear cells (PBMCs) from patient or adult control donors were processed from whole blood by Ficoll-hypaque density gradient centrifugation (Amersham, Uppsala, Sweden) and were used immediately in experiments, or cryopreserved in 10% DMSO with 90% FCS or Human AB serum for future studies. PBMCs from normal donor controls were obtained from the Human Immunology Core at the University of Pennsylvania. For evaluations of TNFRSF expression, PBMC were studied directly after isolation or cells were stimulated for 18 hours in RPMI containing 10% heat inactivated FCS and 2µg/ml PHA. After stimulation, the cells were washed three times in PBS with 1% BSA, and stained for flow cytometry analysis using monoclonal antibodies to CD3, CD4, OX40 (CD134), GITR and CD154 (CD40L). For proliferation and viability studies, PBMCs were thawed and labeled with fluorescent dye carboxyfluorescein diacetate succinimidyl ester (CFSE) in a final concentration of 5µM (Molecular Probes/Invitrogen). K562-based artificial antigen presenting cells (CD32 aAPCs) engineered to express the Fc receptor CD32 and 4-1BBL or OX40L were prepared and loaded with anti-CD3 (OKT3, Orthoclone) and co-cultured with PBMCs as previously described [29–31]. Cells were enumerated on a Coulter Multisizer 3 (Beckman Coulter). Proliferation and cell viability were assessed by CFSE dilution and Viaprobe (7-AAD) staining respectively, using flow cytometry. All antibodies used were obtained as direct fluorophore conjugates from BD Biosciences and staining procedures were followed according to the manufacturer. Cells were acquired using a FACScalibur (Becton Dickinson) and data was analyzed using FlowJo software (Treestar, Inc).

Relative CFSE Preservation index

Dilution of CFSE on day 6 of stimulation was calculated for the CFSE-labeled CD4+ T cells activated with anti-CD3 loaded K562 aAPCs expressing costimulatory molecules OX40L or 4-1BBL. Mean Fluorescent Intensity (MFI) of CFSE expression was measured by using FlowJo software (Treestar, Inc.) and relative CFSE preservation of expression was calculated for the patient and each normal donor from the following equation: $MFI \text{ of CFSE anti-CD3} + \text{costimulatory molecule} \div MFI \text{ of CFSE anti-CD3 alone}$. Averages of the relative values were calculated for conditions receiving 4-1BBL costimulation or OX40L costimulation from both the normal donor controls and patient.

Statistics

A two-tailed, student t-test was performed to compare values obtained from the patient and the normal donors. p values < 0.05 (*) and/or < 0.001 (**) were considered statistically significant as indicated in the appropriate figure legends.

Results and Discussion

Clinical history

Our patient was a 3 year old female with abnormal karyotype and subtelomeric deletion syndrome consistent with monosomy 1p36. Her clinical course included hyperinsulinism with resulting hypoglycemia, hearing loss, apnea requiring tracheostomy, multiple infections, and episodes of septicemia (Table 1). Her immunologic evaluation demonstrated low post-immunization titers against pneumococcal conjugate vaccination and decreased tetanus toxoid-specific antibody titer despite making an initial response. In addition, she demonstrated low tetanus- and absent Candida-induced lymphocyte proliferation (Table 2). Other immune parameters evaluated were generally within normal ranges including: quantitative immunoglobulins, mitogen-induced lymphocyte proliferation, quantitative lymphocyte subsets and leukocyte nitroblue tetrazolium reduction. Together these results could suggest imperfect maintenance of immunological responses, however her recurrent episodes of bacteremia might also have contributed. She was treated with IVIG for her poor specific antibody for several months, but died from septicemia.

Characterization of terminal 1p36 deletion

On G-banding analysis a small, subtelomeric deletion of the short arm of chromosome 1 was identified in 17/20 cells studied. Three cells had an apparently normal female karyotype. FISH using subtelomeric probes for 1p and 1q confirmed the deletion to be mosaic, present in 17/20 cells (Figure 1b, Left). Mosaicism has been previously described in 1p36, and these patients have been shown to manifest the clinical features of the 1p36 deletion syndrome [1]. The patient's clinical history of recurrent infections and immunodeficiency characterized by impaired memory recall responses led us to inquire whether the deletions included genes responsible for the immunodeficiency. The telomeric region of 1p36 contains several immunologically important members of the TNFRSF that likely arose from genetic duplication and have distinct, but related roles in the co-stimulation of T cells [6,32]. The relative locations of these genes are shown in Figure 1a. GITR and OX40 are found approximately one megabase from the telomere, while the loci for other TNFRSF members HVEM, 4-1BB and CD30 are located between 2–12 megabases from the telomere. A probe including the OX40 locus, BAC RP5-902P8, was used in FISH studies and was present on only one copy of chromosome 1, demonstrating haploinsufficiency for the region containing this gene (Figure 1b, Middle). Given that our patient was haploinsufficient for OX40, and OX40 has been previously determined to participate in immunological memory in murine studies, we sought to investigate the effects of this haploinsufficiency on T cell proliferation and viability. Hybridization of this BAC to both chromosomes in two other 1p36 deletion syndrome patients (data not shown) suggested that the deletion of this region is not a uniform feature of the disease. Array CGH was used to further map the breakpoint of the deletion using a targeted BAC array containing multiple clones representing this chromosomal interval. The array detected a single copy loss of 13 BAC clones at the subtelomeric region of chromosome 1p36. The apparent mosaicism resulted in detection slightly below the standard confidence since presumably the presence of cells containing 2 normal copies of chromosome 1p within the DNA led to decreased detection (Figure 1c). Despite the contribution of normal cells, the proximal boundary of this deletion was mapped on the array between clones RP5-907A6 (deleted) and RP11-33E3 (not deleted) indicating an approximately 2.9-3 Mb terminal deletion of 1p36 which includes the OX40 gene. Parental chromosomes were analyzed, and neither parent was found to carry a 1p deletion.

This *de novo* deletion is mosaic, and not present in all cells tested, it is presumed to have arisen mitotically, early in development.

Expression of OX40 on PBMC and T cells

To evaluate whether the deletion of the 1p36 region resulted in decreased expression of TNFRSF members, including OX40, freshly isolated PBMC from the patient and normal controls were stimulated overnight with PHA. CD40L (CD154), encoded by a gene located on the X chromosome and not affected by the 1p36 deletion, was induced at similar levels in CD4⁺ T cells from both the patient and a normal donor by flow cytometry and was used as control (Figure 2, left). Expression of GITR, also included in the deletion, was slightly decreased relative to control (Figure 2, middle). The most striking difference, however, was the reduced expression of OX40 on the patient's CD4⁺ T cells compared to normal control expression (Figure 2, right).

CD4⁺ T cell proliferation and viability

OX40 is known to play an important role in the costimulation of CD4 T cells resulting in increased survival and improved memory responses. To determine whether the decreased expression of OX40 observed in the patient had an effect on the survival or proliferation kinetics of CD4⁺ T cells, K562-based artificial antigen presenting cells (aAPCs) expressing OX40L or 4-1BBL were used to provide costimulation to patient and donor control cells. The aAPC system employed has been previously shown to support anti-CD3 mediated polyclonal T cell expansion and survival *in vitro* [29,33]. Using this system, we observed enhanced proliferation of the patient's CD4⁺ T cells when compared to normal controls (Figure 3A). Stimulation by anti-CD3 alone resulted in similar kinetics, with fully diluted CFSE for both patient and control, suggesting proliferation of the entire population within 6 days of stimulation. Proliferation of normal control cells stimulated with additional costimulatory signals OX40L or 4-1BBL appeared to occur more slowly than in patient cells, quantified in Figure 3B. The difference in CFSE dilution in the patient compared to the normal donor control CD4⁺ T cells stimulated with aAPCs expressing OX40L, but not 4-1BBL, was statistically significant ($n < 0.05$). This suggests that the patient cells did not respond normally to costimulation with these molecules, and instead fully diluted CFSE as in the anti-CD3 control. These findings are consistent with similar observations reported in 4-1BB-deficient CD4 T cells [34]. Upregulation of costimulatory molecules such as OX40 and 4-1BB normally occur after stimulation through the TCR, and may provide check point signals as well as upregulate survival signals to prevent activation-induced cell death.

Since the patient did not have an obvious lymphoproliferative syndrome, we hypothesized that the increased proliferation would be associated with a consequently increased amount of cell death. To evaluate this we analyzed the cell viability using VIAPROBE or 7-AAD on day 6 in response to costimulation (Figure 4). 7-amino-actinomycin D (7-AAD) is a nucleic acid dye, similar to propidium iodide, which is used as a viability probe. Dying cells with compromised membrane integrity will take up the VIAPROBE (7-AAD) dye, and can be detected using flow cytometry [35]. PBMCs from both patient and normal controls cocultured with aAPCs in the absence of anti-CD3 antibody resulted in cell death. While normal donor CD4⁺ T cells costimulated via OX40L maintained their viability, the viability of patient CD4⁺ T cells stimulated was reduced. Several experiments using multiple normal donors revealed that this result was statistically significant ($n < 0.05$). Stimulation with aAPC and anti-CD3 without costimulation showed increased survival in control cells, but not in the patient's, suggesting signaling through inducible endogenous costimulatory molecules was intact in the control cells. Costimulation with aAPCs expressing 4-1BBL rescued patient cells and the differences compared to the normal donor controls were not statistically significant, suggesting that signaling through 4-1BB was intact in the patient consistent with the genomic presence of two

copies of this gene. Several costimulatory signals are likely required for achievement of functional T cell responses, and different signals may be required at different times. As OX40 and 4-1BB are inducible signals and play a role to sustain ongoing immunologic responses, a coordinated and sequential process is suggested in mouse models and signaling through 4-1BB may follow OX40 and hypothetically explain the increased proliferation in 4-1BBL [6]. Notably, stimulation with aAPCs expressing OX40L resulted in decreased viability of expanded CD4 T cells from the patient relative to control. The increased activation induced cell death in patient cells suggests a lack of upregulation of anti-apoptotic genes that are normally induced by OX40 (Figure 4). There was no distinct difference in proliferation or survival of patient CD8 T cells compared to normal donor controls (data not shown), supporting the differential role of OX40 in CD4 and CD8 T cells. The anti-apoptotic genes normally upregulated by costimulatory survival signals may affect the proliferation kinetics of the normal donor cells in culture, but their lack of signaling in the patient's cells resulted in similar kinetics as stimulation with anti-CD3 alone. These results demonstrate a relatively specific insufficiency of OX40 function in the patient's CD4 T cells. This may have contributed to the patient's susceptibility to recurrent infections by impairing the survival of new effector cells generated during primary responses, and leading also to lack of efficient T cell help.

We described decreased OX40 expression in a patient diagnosed with monosomy 1p36, associated with the inefficient generation of immune responses possibly due to lack of proper T cell help. Haploinsufficiency in the majority of the patient cells likely resulted in the reduced expression of OX40. Immune evaluation revealed an inability to generate recall response to routine vaccine antigens. In addition, our patient had a history of recurrent infections and therefore was treated with IVIG.

Our patient's presentation parallels mouse models of OX40 deficiency. While studies of OX40 deficient mice have helped to elucidate the role of OX40, heterozygous mice have not been specifically characterized. Many studies using murine models provide evidence that OX40 plays a more important role in generating a secondary response to viral and protein antigens by inducing anti-apoptotic proteins such as Bcl-xl and promoting survival of CD4 T cells upon ligation [12,11,36,24,13,15,37,22,23,38]. Through these signals, OX40 is thought to increase the number of effector cells that can then enter the memory cell pool [11,6,39]. Thus, it is possible that OX40 haploinsufficiency in our patient led to inadequate survival of key memory T cells, consistent with the patient's inability to respond to recall antigens *in vitro*.

The expression of other TNFRSF molecules was also reduced in the patient, including 4-1BB and CD30, compared to normal controls following activation and it is unclear at this time why this was observed as she was not haploinsufficient for 4-1BB. TNFRSF expression was only studied for 6 days, thus it is also possible that the induction of the other molecules occurred earlier in the patient's cells, correlating with the enhanced proliferation kinetics. Despite lower 4-1BB surface expression at day 6, CD4 T cell proliferation was enhanced when patient cells were stimulated through 4-1BB, similar to what has been reported in 4-1BB deficient mice [34]. Increased cell death was only seen after OX40 signaling, thus demonstrating that despite increased 4-1BB induced proliferation, this effect was likely suppressed phenotypically in the patient. Unfortunately, limited patient samples due to her untimely death from sepsis prevented further studies from being conducted.

Many patients with deletions of 1p36 are haploinsufficient for the region containing OX40 [1]. Other patients with deletions in this region may have had unrecognized immunological deficits. Our results suggest that our patient's immune system was affected by her haploinsufficiency for OX40. Alternatively, her monosomy may have uncovered a novel unidentified mutation in her remaining 1p36 region, leading to an immunologic defect. However, due to the presence of reduced levels of OX40 by FACS in the patient's cells compared to normal controls, our

results demonstrate specific functions for OX40 *in vitro*. Other patients who have a deletion including this gene may have milder forms of immunologic abnormalities and present an important focus for further evaluation of this syndrome. Because many TNFRSF family members are located in the same region, it may be important to screen other monosomy 1p36 patients for terminal deletions that include these genes and compare their immune status. The close chromosomal location of many TNFRSF family members implies that they may be derived from duplication [32], but studies indicate that they have distinct roles in the immune system and one molecule may not compensate for the loss of another [6,40,41,42]. Deletions can be detected in these patients that vary from 1Mb to greater than 10.5 Mb [42], further supporting the need to investigate whether varying degrees of immunodeficiency occur in patients with this syndrome. Identification of patients with monosomy 1p36 who also have homozygous or heterozygous mutations in these TNFRSFs not only would increase our current understanding of T cell costimulation, but also prompt interventions such as prophylactic antibiotics, immunoglobulin replacement, or perhaps even agonistic antibody treatments if necessary resulting in generally improved clinical outcomes for these patients.

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Figure 1. FISH analysis and microarray profile define the 1p deletion

a. Map of chromosome 1p36 demonstrating the location of TNFRSF members and BAC probes used to define patient deletion. The deletion breakpoint is marked. **b.** FISH studies on metaphase spreads from the patient. (Left) Subtelomeric probes for 1p - CDB108/T7 labeled in green, and 1q - 1qtell9 labeled in red, reveal a deletion of the 1p probe. (Middle) BAC RP5-902P8 includes the OX40 locus and is present in only one copy of chromosome 1, demonstrating deletion of this locus. (Right) BAC RP4-758J18, which maps proximal to the OX40 locus, within 1p36 is present on both copies of 1p. **c.** Microarray profile for a mosaic, terminal deletion of 1p36 characterized by array CGH. Each clone represented on the array is arranged along the x-axis according to its location on the chromosome with the most distal/telomeric p-arm clones on the left and the most distal/telomeric q-arm clones on the right. The blue line plots represent the ratios from the first slide for each patient (control Cy5/patient Cy3) and the pink plots represent the ratios obtained from the second slide for each patient in which the dyes have been reversed (patient Cy5/control Cy3). Typical deletions show deviations

greater than 0.3 and -0.3. **(top)** Plot for a normal chromosome 1 showing a ratio of 0 on a log2 scale for all clones. **(bottom)** Plot showing single-copy loss of 13 clones at 1p36.3 indicating a terminal deletion of 2.9Mb. Note that for most of the clones the deviations do not reach the expected 0.3 and -0.3 because of the presence of mosaicism.

Figure 2. Decreased expression of OX40 on patient PBMC compared to healthy control
PBMC from patient or normal donor control were stimulated with PHA and IL-2 overnight and surface expression was measured for CD40L, GITR and OX40. Histograms represent expression of these costimulatory receptors on patient CD4⁺ T cells (black) compared to isotype (gray) and normal donor control (heavy black).

Figure 3. Polyclonal stimulation of patient CD4 results in enhanced proliferation

Cryopreserved PBMCs from patient or normal donor control were thawed, labeled with CFSE and co-cultured with CD32 aAPCs alone (no stimulation) or loaded with anti-CD3 that were expressing costimulatory ligands 4-1BBL or OX40L. Proliferation was assessed by flow cytometry and data analyzed using FlowJo software. A. Overlay of histograms representing CFSE dilutions of CD4⁺ cells on day 6 in the culture. Experiment was conducted in duplicate, and gated on CD4⁺ T cells. B. Relative CFSE preservation index. MFI of CFSE for each of the cultures on day 6 of stimulation was used to calculate the relative preservation of CFSE expression as described in the materials and methods. Mean values obtained for patient CD4⁺ T cells (black) stimulated with anti-CD3 loaded K562 aAPCs expressing OX40L or 4-1BBL compared to 8 normal donors (gray). Error bars represent $\pm$ standard deviation. A two-tailed

student t-test was performed and p values (* <0.05) were considered statistically significant. Plots are representative of three independent experiments using patient PBMCs and normal donor controls.

Figure 4. Polyclonal stimulation of patient CD4 results in decreased viability

Cryopreserved PBMCs from patient or normal donor control were thawed and co-cultured with CD32 aAPCs alone (no stimulation) or loaded with anti-CD3 that were expressing costimulatory ligands 4-1BBL or OX40L. Decrease in viability of CD4⁺ cells from patient compared to healthy donor. Cell viability was measured on day 6 in culture using VIAPROBE (7-AAD). Experiment was conducted in duplicate. Viability was assessed using flow cytometry and analyzed using Flowjo software. Left: Histograms representing VIAPROBE (7-AAD) +/

– expression in CD4⁺ T cells from normal donor control (top) and patient (bottom). Right: Mean percentages of VIAPROBE⁺ CD4⁺ T cells for patient (black) were calculated and compared to 8 normal donor controls (gray). Error bars represent $\pm$ standard deviation. A two-tailed student t-test was performed, and those p values (* $p < 0.05$, ** $p < 0.001$) were considered statistically significant. Representative of at least three independent experiments.

Table 1

Documented Clinical Infections*.

Age		Infections
10.5mo-15mo	•	Parainfluenza type 3
	•	Rotavirus
35–38 months	•	Multiple episodes of bacteremia/sepsis (<i>P. aeruginosa</i> , <i>P. putida</i> , <i>Alcaligenes</i> , <i>Xanthomonas</i> , <i>S. aureus</i> , <i>Klebsiella</i>)
	•	Pneumonia (<i>Klebsiella</i>)
	•	Recurrent pulmonary infiltrates and right sided consolidation
	•	Groin abscess (<i>Klebsiella</i> , <i>α-Strep</i>)
	•	Tracheitis (<i>P. aeruginosa</i> and <i>putida</i>)

* Confirmed by viral culture, cultures of blood, sputum, or wound. Patient had central line at 18months, tracheostomy at 34months complicating assessment of immunodeficiency due to inherent increased risk of infection with implanted medical devices.

Table 2

Immunologic evaluations.

Immune Parameter	38 – 40 months	Reference Range
Total Leukocytes, (cells/ μ L)	15,200	5000–15000
Neutrophils	%	cells/ μ l 9728 >1000
Lymphocytes	30	1700–6900

HUMORAL IMMUNITY		
<i>Quantitative Immunoglobulins</i>		
IgG, (mg/dL)	458 ^I	908 ⁴ 477 – 1334 22–159 47–200 0.19–16.9
IgA, (mg/dL)	40 ^I	
IgM, (mg/dL)	79 ^I	
IgE, (IU/mL)	4.5 ^I	
<i>Qualitative IgG</i>		
Tetanus titer (IU/mL)	1.27 ^I	> 0.1 > 0.1 > 1.0 μ g/mL ³
Diphtheria titer (IU/mL)		
Pneumococcal serotypes, (0/14 serotypes tested),	0.37 ^{I,2} 0.47 ^I , 0/7 serotypes in conjugate vaccine, (0/14 serotypes tested),	
(μ g/mL)	0.16 ⁵ ,	
Varicella Ab		

CELLULAR IMMUNITY		
<i>Lymphocyte Subset Analysis</i>		
CD3+	cells/ μ l	cells/ μ l
CD3+/CD4+	1719	3192
CD3+/CD8+	470	2371
CD19+		775
CD3–/CD16+/CD56+		1231
CD4+/CD45RA+		137
CD4+/CD45RO+		2006
<i>Lymphocyte Proliferation</i>		
Candida (cpm)	CPM	CPM
Tetanus (cpm)	421	Cntrl 20387
PHA (cpm)	3674	Cntrl 65782
Con A (cpm)	311215	Cntrl 377817
PWM (cpm)	179255	Cntrl 254756
	136473	Cntrl 166838

INNATE IMMUNITY		
CH50		
NBT test	208units Normal	101– 300 units

^I Pre Immunoglobulin Replacement

² Protective titer waned in 2 months time

³ In light of prevnar immunization, would expect 7 serotypes to be >1.0 μ g/mL

⁴ Post Immunoglobulin Replacement

⁵ No clinical varicella and no vaccine