Patients with XLP Type 1 have variable numbers of NKT cells

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Abstract

X-linked lymphoproliferative disease (XLP1) is a rare primary immunodeficiency that usually presents in early childhood. Patients with XLP1 have been reported to have absent NKT cells, and it has been suggested that this can be diagnostic for the disorder. Whilst NKT frequency in adults is variable, little is known about their frequency in children. Therefore, we established a paediatric reference range for these cells. In contrast to previous reports, in our cohort of XLP1 patients NKT cell numbers were found to be variable, and we would advise against using the finding of NKT cells to exclude a diagnosis of XLP1.

Background

NKT cells are a sublineage of T cells with unique properties, that include expression of an invariant T cell receptor ($V\alpha_{24}V\beta_{11}$ in humans) which binds glycospingolipids presented by the MHC class I-like molecule, CD1d, and they have the ability to rapidly produce many cytokines after stimulation[1]. NKT cells develop in the thymus and are positively selected by CD1d expressing bone marrow cells, rather than by cortical epithelial cells like conventional T cells. NKT cells are most prevalent in the thymus, spleen, liver and bone morrow and are much less abundant in lymph nodes. NKT cells make up only approximately 0.1% of human peripheral blood T cells. There is a high degree of variability in the frequency of NKT cells in the peripheral blood of normal individuals. In a study of 70 normal controls, NKT cell numbers varied from 10 – 30,000 per mL [2]. Although SLAM-associated protein (SAP) is not required for the development of the majority of lymphocytes, it plays a crucial role in

the development of NKT cells. $SAP^{-/-}$ mice have greatly reduced numbers of NKT cells in secondary lymphoid organs [3,4].

X-linked lymphoproliferative disease (XLP1) is a rare primary immunodeficiency disorder in which the gene (*SH2D1A)* which encodes the protein SAP is defective. XLP1 patients have been reported to have greatly reduced numbers of NKT cells in peripheral blood[4,5]. In one study[4] XLP1 patients (n = 17) had 0 – 58 NKT cells/mL peripheral blood which was greatly reduced compared to the numbers in normal individuals: 10 controls with 120 – 1596 NKT cells/mL. These studies used small numbers of control individuals (8 age matched controls (1 to 27 years old)[5], 10 healthy individuals (unspecified ages)[4]). As the number of NKT cells in peripheral blood is known to be extremely variable in adults[2] and the average age of onset of XLP1 is 2.5 years[6], reference ranges for peripheral blood NKT cells in children need to be established using larger numbers of controls as a preliminary step to determining whether NKT cell enumeration may aid in the diagnosis of XLP1.

Aim

The aim of this study was to develop a paediatric reference range for NKT cells in peripheral blood and compare NKT cell numbers in children and in adults. We then aimed to enumerate NKT cells in known XLP1 patients and to determine the usefulness of NKT cell enumeration in the diagnosis of XLP1.

Methods and materials

The method for NKT cell enumeration was based on a published method[2]. Peripheral blood was stained with directly conjugated fluorescent monoclonal

antibodies to CD3 and Vα24/Vβ11 subunits of the NKT cell invariant TCR. Following lysis of erythrocytes, cells were washed and re-suspended in fixative and analysed by flow cytometry. Absolute cell counts were calculated based on the proportion of beads acquired compared to cells acquired, **and also by using the lymphocyte count obtained from the full blood count**. Samples from 28 pediatric controls, 15 adult controls, and 5 XLP1 patients were analysed.

Results

NKT cells were identified by flow cytometry as CD3+ T lymphocytes expressing the invariant TCR, Vα24Vβ11 (**Figure 1**, R1 and R2 and R4). NKT cell counts were very variable and showed a skewed distribution, tending towards lower NKT cell numbers. (**Figure S1, and Table S2**).

The range for paediatric samples was 24-8766 NKT cells/mL, with a median of 658 NKT cells/mL The adult range was determined as 27-7572 NKT cells/mL with a median value of 1142 NKT cells/mL There was no significant difference between paediatric NKT cell counts and adult NKT cell counts (p=0.168 using 2-tailed Mann-Whitney U test)

Peripheral blood NKT cells were enumerated in 5 known XLP1 patients. NKT cell counts ranged from 12 to 115 cells/mL Two patients (Patient 1 and Patient 2) had an NKT cell count within the paediatric reference range **(Figure 2)**. Patients with very low NKT cell numbers had several different mutations, while the two patients with an NKT cell count within the paediatric reference range had a deletion of exon 2 and 4 in SH2D1A **(Table S1)**.

Discussion

Peripheral blood NKT cell numbers are variable in children and adults, and showed a skewed distribution. There was no significant difference between paediatric and adult NKT cell counts in this study. NKT cell numbers in 2 known XLP1 patients (Patient 1 and Patient 2) were low but within the normal range, whilst NKT cell numbers were low and below the reference range in 3 patients. This is in contrast to previous reports[4,5] which found that NKT cells were absent in XLP1 patients. Patient 1 and 2 are noted to be siblings and share the same mutation; it is unclear if and how this exon deletion affects NKT cell development. **Further work to establish NKT numbers and any correlation of genotype and phenotype in a larger cohort of XLP1 patients through a multi-centre study would be beneficial, although the rarity of the condition and the fact that most of the patient have been treated with HSCT makes this difficult.**

In summary, as NKT cell numbers are variable and are not completely absent in XLP1 patients, ‘normal’ numbers of NKT cells should not exclude a diagnosis of XLP1. We recommend that measurement of SAP protein by flow cytometry, followed by confirmatory genetic analysis should be undertaken where a diagnosis of XLP1 is suspected.

Acknowledments

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Authorship

ER, JH and KG designed the research study, ER and JH performed the research and analysed the data, CB contributed patient data, ER wrote the manuscript, all authors critically revised the manuscript and approved the final version.

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Figures:

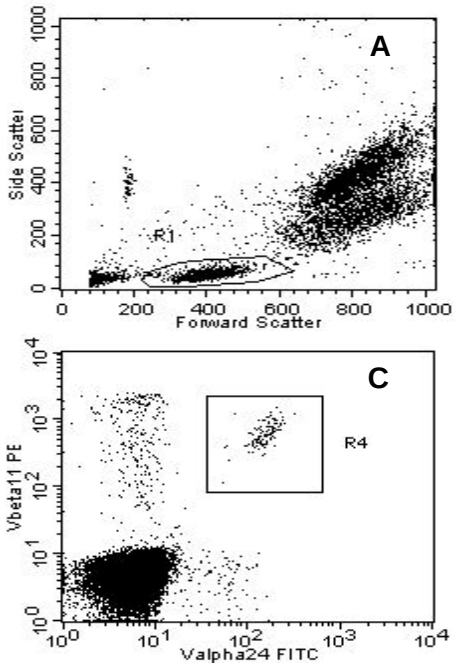

Figure 1 NKT cell immunophenotyping strategy
Lymphocytes were gated based on forward and side scatter (**A**, R1). Within the lymphocyte gate, CD3+ T lymphocytes were identified (**B**, R2). NKT cells were identified as CD3+V24+V11+ lymphocytes (**C**, R1+R2+R4).
This is representative of both adult and paediatric controls and XLP1 patients

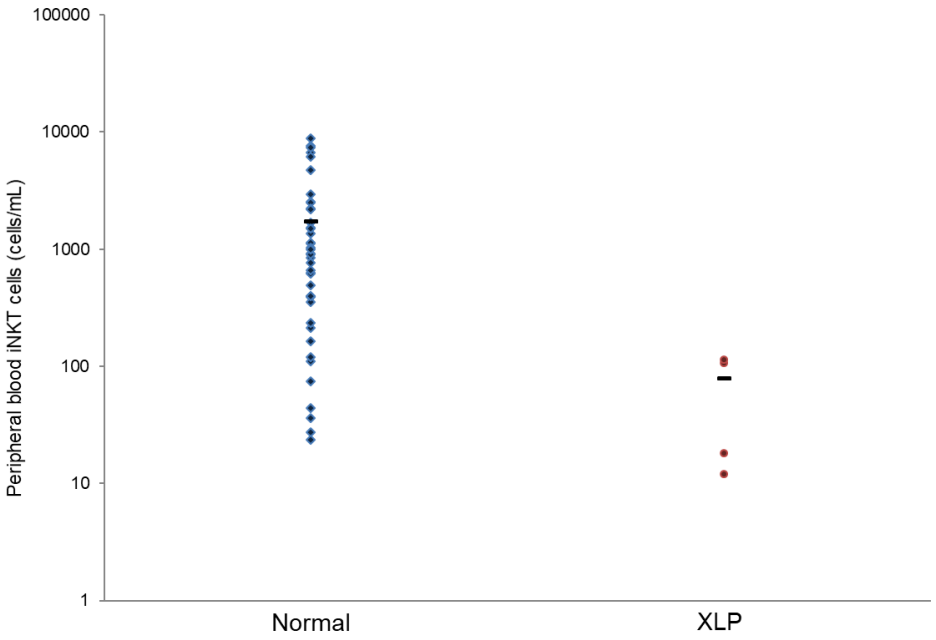

Figure 2
Paediatric peripheral blood NKT cells in normal controls and known XLP1 patients
NKT cells were enumerated in 28 normal controls (diamonds, line represents 50th percentile) and 5 known XLP1 patients (circles, line represents 50th percentile).

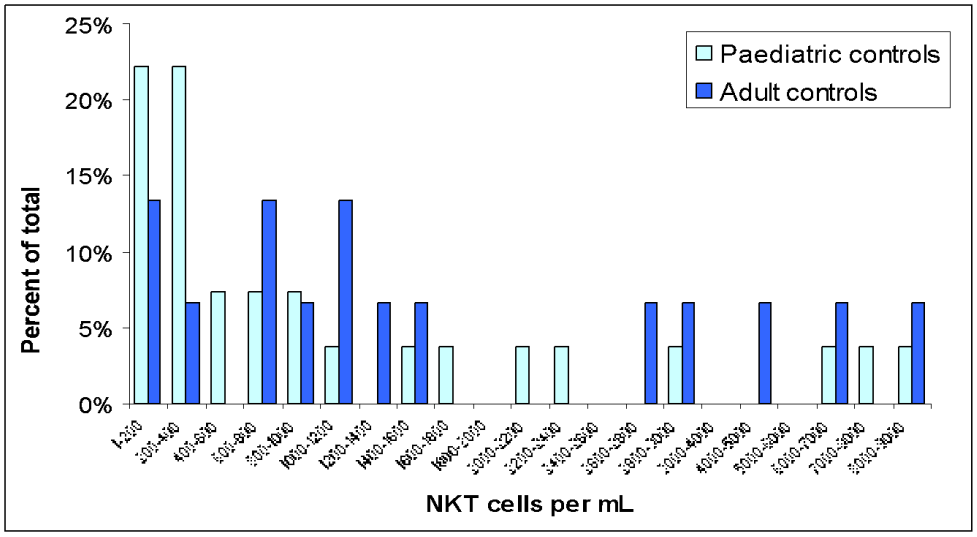

Figure S1
Distribution of NKT cell counts in adults and children
NKT cells were enumerated in duplicate in 28 children (light blue bars) and 15 adults (dark blue bars).

Patient	Mutation in *SH2D1A*	NKT cell count (/ml)
1	Deletion Exon 2 and 4	107
2	Deletion Exon 2 and 4	115
3	C245DUP	18
4	Deletion Exon 2	12
5	C163c>t	18

Table S1
Comparison of mutation in *SH2D1A* with absolute NKT cell count

Paediatric control	NKT cell count (/ml) (calculated using lymphocyte count)	Paediatric control	NKT cell count (/ml) (calculated using lymphocyte count)
1	374	**15**	1869
2	633	**16**	196
3	2478	**17**	32
4	383	**18**	2021
5	265	**19**	1670
6	861	**20**	2091
7	925	**21**	Lymphocyte count not available
8	290	**22**	115
9	637	**23**	49
10	113	**24**	1157
11	332	**25**	23
12	165	**26**	6028
13	178	**27**	8363
14	610		

Table S2
Paediatric peripheral blood NKT cells in normal controls, calculated using lymphocyte count.
NKT cells were enumerated in 28 normal controls