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ORIGINAL RESEARCH

Post-Transplantation B Cell Function in Different Molecular Types of SCID

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Abstract
Purpose Severe combined immunodeficiency (SCID) is a syndrome of diverse genetic cause characterized by profound deficiencies of T, B and sometimes NK cell function. Non-ablative HLA-identical or rigorously T cell-depleted haploidentical parental bone marrow transplantation (BMT) results in thymus-dependent genetically donor T cell development in the recipients, leading to a high rate of long-term survival. However, the development of B cell function has been more problematic. We report here results of analyses of B cell function in 125 SCID recipients prior to and long-term after non-ablative BMT, according to their molecular type.
Methods Studies included blood immunoglobulin measurements; antibody titers to standard vaccines, blood group antigens and bacteriophage Φ X 174; flow cytometry to examine for markers of immaturity, memory, switched memory B cells and BAFF receptor expression; B cell chimerism; B cell spectratyping; and B cell proliferation.
Results The results showed that B cell chimerism was not required for normal B cell function in IL7Rα-Def, ADA-Def and CD3-Def SCIDs. In X-linked-SCID, Jak3-Def SCID and those with V-D-J recombination defects, donor B cell chimerism was necessary for B cell function to develop.
Conclusion The most important factor determining whether B cell function develops in SCID T cell chimeras is the underlying molecular defect. In some types, host B cells function normally. In those molecular types where host B cell function did not develop, donor B cell chimerism was necessary to achieve B cell function. 236 words

Keywords B cell function · B cell chimerism · bone marrow transplantation · severe combined immunodeficiency · molecular type · memory B cells

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Introduction

Severe combined immunodeficiency (SCID) is a syndrome of diverse genetic cause characterized by profound deficiencies of T and B cell function and, in some types, also of NK cells [1]. This condition is uniformly fatal in the first 2 years of life unless immune reconstitution can be accomplished [1–3]. SCID is currently known to be caused by mutations in at least 13 different genes. X-linked SCID is caused by defects in the common gamma chain (γc) [4, 5]. Mutations in the genes encoding adenosine deaminase (ADA) [6], Janus kinase 3 (Jak3) [7], the α chain of the IL-7 receptor (IL7Rα) [8], recombinase activating genes 1 or 2 (RAG1 or RAG2) [9], CD45 [10], the Artemis gene [11], ligase IV [12], DNA protein kinase catalytic subunit (DNA-PKcs) [13], CD3δ [14], CD3ε [15] or CD3ζ [16] also result in SCID and are inherited as autosomal recessive traits.

Since 1968, the standard treatment for all forms of SCID has been allogeneic bone marrow transplantation from HLA-identical or haploidentical related donors or, in some instances, from unrelated donors. The principal causes of death in such infants have been fatal viral infections present at the time of referral.[1] Infants with all genetic types of SCID given T cell-depleted HLA-identical or haploidentical bone marrow stem cells without or with pre-transplant chemoablation or post-transplant GVHD prophylaxis develop phenotypically and functionally normal, genetically-donor T cells at between 90 and 120 days post-transplantation [1–3, 17–20].

While bone marrow transplantation has resulted in life-saving T cell reconstitution in most SCIDs, correction of B cell function has been more problematic. It has been suggested that the need for post-transplantation immunoglobulin (IG) replacement is due to a lack of donor B cell engraftment, attributed to a lack of pre-transplant chemoablative conditioning, although data to support the latter premise have been far from clear. Few studies have been published regarding longterm B cell function in patients with SCID who have received bone marrow transplants [2, 3, 19, 21–30]. A review of 19 reports from Europe and the United States published over the past two decades found that the percentage of survivors with B cell chimerism and/or function was higher and the percentage requiring IG replacement was lower at those Centers that used pre-transplant conditioning [31]. However there were substantial numbers of patients requiring IG replacement at all centers, so pre-transplant conditioning does not guarantee development of B cell function. More importantly, survival rates were higher at those centers that did not use pre-transplant conditioning or immunosuppressive drugs post-transplantation for graft-versus-host disease (GVHD) prophylaxis [31]. This paper reports on B cell function in 125 surviving SCIDs who received non-ablative bone marrow transplants without post-transplantation GVHD immunosuppressive drugs at a single center over a 28 year period.

Methods

Patients and Transplantations

We studied the 125 surviving SCID patients out of a total of 165 (76 %) transplanted at Duke University Medical Center from May 19,1982 to May 19, 2010 without pre-transplant chemotherapy or post-transplantation GVHD prophylactic drugs for the status of their B cell function. One hundred forty-eight patients received rigorously T-cell depleted haploidentical parental bone marrow stem cell transplants. The other 17 received either unfractionated bone marrow or T cell depleted bone marrow cells from an HLA-identical relative. Thirty (75 %) of the 40 patients who died succumbed to viral infections present at the time of transplantation, but none died of GVHD. At the time of this analysis, patients ranged from 8 months to 28 years post-transplantation, with 81 of them being more than 10 years post-transplantation. Fifty-eight of the surviving patients have γc-Def SCID [5], 8 have Jak3-Def SCID[7], 17 have IL7Rα-Def SCID [8], 18 have ADA-Def SCID, 6 have RAG-1 or RAG-2 Def SCID) [9]. 3 have mutations in the various chains of CD3 (CD3-Def) [32], one has CD45-Def SCID [10], 1 has Artemis-Def SCID [33], 1 has cartilage hair hypoplasia (CHH), 11 have autosomal recessive (AutoRec) SCID of unknown molecular type and there is 1 male SCID of unknown molecular type (Table I). All studies were performed with the approval of the Duke University Medical Center Institutional Review Board and with the written informed consents of the parents.

Donor bone marrow was depleted of T cells by agglutination with soybean lectin and two cycles of rosetting with sheep erythrocytes that had been treated with aminoethylisothiuronium bromide as previously described [17]. The

Table I B cell chimerism vs need for IG treatment

Group	# (%) with Donor B Cell Chimerism	# (%) On IG Treatment
X-linked *N*=58	21 (36)	38 (66)
Jak3 Def *N*=8	2 (25)	3 (38)
IL-7R Def *N*=17	1 (6)	1 (6)
ADA Def *N*=18	6 (33)	4 (22)
CD3 Chain *N*=3	0	0
RAG 1/2 *N*=6	1 (17)	5 (83)
AutoRec *N*=11	3 (27)	8 (73)
CD45Def *N*=1	0	1 (100)
Artemis *N*=1	1 (100)	0
CHH *N*=1	0	1 (100)
Unknown *N*=1	1 (100)	0
Totals *N*=125	36 (29)	61 (49)

method of T cell depletion was the same for all recipients over the 28 years of this study. Forty-three of these patients were treated with a non-ablative booster BMT in an effort to improve T cell function or, in two cases, to achieve B cell function and the outcomes of the booster transplants are described in a separate manuscript (submitted). Four of the patients underwent gene therapy elsewhere (2 with ADA-Def in Italy, 2 with γc-Def SCID at the NIH). Gene therapy was successful in the two ADA-Def but unsuccessful in the 2 γc-Def patients who subsequently each received a matched unrelated donor (MUD) transplant following reduced intensity conditioning. Two ADA-Def patients are currently receiving polyethylene glycol modified bovine adenosine-deaminase (PEG-ADA), and one received a MUD bone marrow transplant elsewhere. One Artemis-Def patient received a MUD bone marrow transplant elsewhere.

Serum Immunoglobulin and Antibody Measurements

Serum IgG, IgA, IgM and IgE were quantified by single radial diffusion or nephelometry [34]. Anti-diphtheria and anti-tetanus antibodies were determined by tanned red cell hemagglutination [35] or by an ELISA after standard vaccines had been administered, and isohemagglutinins were measured by a microtiter plate assay. Bacteriophage ΦXl74 responses were assessed after the administration of 0.02 ml/kg of bacteriophage intravenously by measuring antibody responses and isotypes of the antibodies following primary and secondary immunizations, as reported by Ochs et al. [36].

B Cell Chimerism

The relative proportion of donor B cells was assessed on EBV-transformed B cell lines established at varying times post-transplantation or on blood B cells using fluorescence in situ hybridization (FISH) [37], and more recently by restriction fragment length polymorphism (RFLP) or short tandem repeats (STR) in the cases where the donor and recipient were of the same sex.

Flow Cytometry

Multi-color flow cytometry was performed on blood B lymphocytes with the use of murine monoclonal antibodies to CD19, CD20, CD22, CD10, CD5, CD27, CD23, CD38 and IgD, purchased from Beckman Coulter (Miami, FL), Invitrogen (Carlsbad, CA) and Becton Dickinson (San Jose, CA). CD10, CD5 and CD38 are molecules present on immature B cells, whereas CD19, CD20 and CD23 are found on both immature and mature B cells. Switched memory B cells were detected as described, using monoclonal antibodies to CD22, CD27 and IgD [38]. The expression of BAFF-R on CD19+ B cells was detected by flow cytometry using biotinylated or fluorescently labeled mAbs (eBioscience, CA) following the manufacturer's recommendation. The receptor for BAFF (BAFF-R), a normal B-cell survival and differentiation factor, is expressed preferentially on B cells.

B Cell Isolation, Culture and Function

Venous blood from healthy controls and patients was collected into heparinized tubes. PBMC were isolated using Ficoll density gradient centrifugation. Fresh or cryopreserved PBMC were used for the isolation of B cells. Benzonase was added to cryopreserved cells upon thawing [39]. CD19+ B cells were positively selected using anti-CD19 magnetic beads (MACS; Miltenyi Biotec, Auburn, CA). The positively selected cell populations contained >86 % B cells, as detected by flow cytometric staining with an anti-CD20 mAb (data not shown). The cells were resuspended in RPMI 1640 supplemented with 10 % human serum (Gemini) and 1 % PSG (100U/mL penicillin, 100ug/mL streptomycin and 2 mmol/LL-glutamine) (Sigma-Aldrich). The flat-bottom 96-well plates were set up in triplicate with 10^5 CD19+−selected B cells per well in a total volume of 200 μL in the presence of CpG oligonucleotides, unmethylated single-stranded DNA motifs (a gift from Dr. James H. Robinson, Tulane University) in Threalose (1.25 μg/mL), IL4 (5 ng/mL) and anti CD40 (0.5 μg/mL), IL 21 (10 ng/mL) and anti CD40 (0.5 μg/mL), or media alone. Cell proliferation was assessed by incorporation of [^{3}H] thymidine for the last 18 h of culture. Data are expressed as proliferation index [mean counts per minute (cpm) in test wells/mean cpm in media control wells].

B Cell Receptor Spectratyping

RNA was prepared from 2 to 5×10^6 fresh or cryopreserved PBMC specimens using Trizol (Life Technology, Gaithersburg, MD) and reverse transcribed to single stranded cDNA with AMV reverse transcriptase using an oligo dT primer according to the manufacturer's protocol (Promega, Madison, WI). The newly synthesized cDNA was then used as a template for individual PCR reactions. The PCR reactions were carried out in 20 μl volumes by standard procedures using six VH forward primers (VH1: CCATGGACTGGACCTGG; VH2: ATGGACATACTTTGTTCCAC; VH3: CCATGGAGTTTGGGCTGAGC; VH4: ATGAAACACCTGTGGTTCTT; VH5: ATGGGGTCAACCGCCATCCT; VH6: ATGTCTGTCTCCTTCCTCAT) and a reverse JH consensus primer (JH-cons: AACTGCAGAGGAGACGGTGACC).[40] The samples were subjected to 41 cycles of denaturation (1' at 94 °C), annealing (30″ at 60 °C), and elongation (1′ at 72 °C).

After the last cycle, a final elongation step (5′ at 72 °C) was performed. The PCR products were visualized on a 2 % agarose gel by ethidium bromide staining before using 2 μl of the amplified products for a run-off elongation reaction with a fluorescent (G-FAM) JH nested primer (FAM-TATATTGT(G/C/T)CC(C/A/T)TGGCCCCAG). The elongation products were then run on a sequencing gel. Fluorescence-labeled size markers (Applied Biosystems, CA) were loaded with the run-off products. After analysis on an automated sequencer (Applied Biosystems, CA) size determination of the run-off products and the analysis of the CDR3 region products were performed using GeneScan software. Spectratype profiles showed a polyclonal Gaussian distribution of peaks (327–422 bp in size) in samples of normal control PBMC; an oligoclonal distribution, from two human lymphoma B cell lines Namalwa [41] and OCI-Ly80 [42] and from normal human EBV-transformed B cells; no amplified products from a T cell line (Jurkat).

Statistical Analysis

Individual comparisons of the mean numbers of B cells bearing the different markers prior to transplantation and at the latest transplant date between pairs of molecular types of SCIDs and with normal controls were performed using a two-sided Satterthwaite test [43]. To maintain an overall Type 1 error rate near 0.1 for each individual set of comparisons between the SCID types and normal controls a conservative Bonferroni [44] (0.1/k) alpha level was used, where k is the number of SCID type to normal control comparisons in any individual analysis set. In most analyses $k=7$ SCID type versus normal control comparisons are made and therefore comparisons with p-values less than 0.0143 (≈0.1/7) were considered statistically significant. However, in certain individual analyses $k=6$, 5, and 2 and therefore p-values less than 0.0167, 0.02, and 0.05 were considered significant.

Results

B Cell Chimerism and IG Replacement

As seen in Table I, B cell chimerism was found in only 36 (29 %) of the 125 patients at the latest time studied post-transplantation. The molecular defects with the highest percentages of donor B cell chimerism were X-linked SCIDs, of which 21 (36 %) had donor B cells, and ADA-Def SCIDs of which 6 (33 %) had donor B cell chimerism, with smaller percentages of donor B cell chimerism found among the other molecular types. We have done longitudinal studies of B cell chimerism and have found that this does not change significantly with time (data not shown). The two patients who received booster BMT with the sole purpose of achieving B cell chimerism failed to do so. Eighty-nine (71 %) of the patients do not have donor B cell chimerism. Nevertheless, only 61 (48.8 %) of the 125 survivors require immunoglobulin (IG) replacement therapy. Thus, 28 of the survivors without B cell chimerism do not require IG replacement. Sixty-two percent of those requiring IG replacement are X-linked SCIDs; 38 patients are currently receiving it and 37 of them do not have B cell chimerism. In the one remaining case with donor chimerism, it is too soon to know whether IG therapy can be discontinued. Other molecular types with a high percentage receiving IG replacement are RAG-Def SCIDs (83 %) and autosomal recessive SCIDs of unknown molecular type (73 %). By contrast, only 1 (6 %) of the patients with IL7Rα-Def SCID, 4 (22 %) of the ADA-Def SCIDs and none of the CD3-Def SCIDs require IG replacement. Only 1 of the IL7Rα-Def SCIDs and none of the CD3 chain deficient SCIDs have donor B cell chimerism. Thus, host B cells appear to be able to cooperate with donor T cells and function normally in these particular SCID defects.

Serum Immunoglobulins and Antibodies

Figure 1 shows the concentrations of serum IgA, IgM and IgE prior to (Fig. 1a) and at the latest time studied post-transplantation (Fig. 1b). These isotypes are not present in transplacentally-transferred immunoglobulin or in IG preparations. Many of the infants were neonates or receiving IG when referred, and 62 of the survivors are currently receiving IG replacement. Prior to transplantation (Fig. 1a), the $\log_{10}$ Ig levels for the following SCID groups were significantly smaller when compared to the values for the normal controls: IgA for X-Linked SCID ($p=0.0044$), IgM for ADA-Def, AutoRec and X-linked SCIDs ($p=0.0022$, 0.0141, <0.0001), and IgE levels for ADA-Def, RAG-Def and X-linked SCIDs ($p=0.0026$, 0.0136, 0.0003). All other comparisons were not different from normal. At the latest time post-transplantation (Fig. 1b), the $\log_{10}$ Ig levels for the following SCID groups were significantly smaller when compared to the values for the normal controls.: IgA for the ADA-Def, AutoRec and X-Linked SCIDs ($p=0.0122$, 0.0127, <0.0001), IgM for ADA-Def, RAG-Def and X-linked SCIDs ($p=0.0025$, 0.0007, <0.0001), and IgE levels for AutoRec, IL7Rα-Def, RAG-Def and X-Linked SCIDs ($p=0.0067$, <0.0001, 0.0031, <0.0001).

Measurement of antibody titers to standard vaccines is non-informative when patients are receiving IG replacement. Table II displays the antibody titers in the patients who are no longer receiving IVIG. As can be seen, most but not all of these patients have normal serum immunoglobulin levels and appropriate antibody titers to tetanus and diphtheria toxoids and to blood group antigens. In the cases of the IL7Rα-Def, ADA-Def and CD3-Def SCIDs, there was a

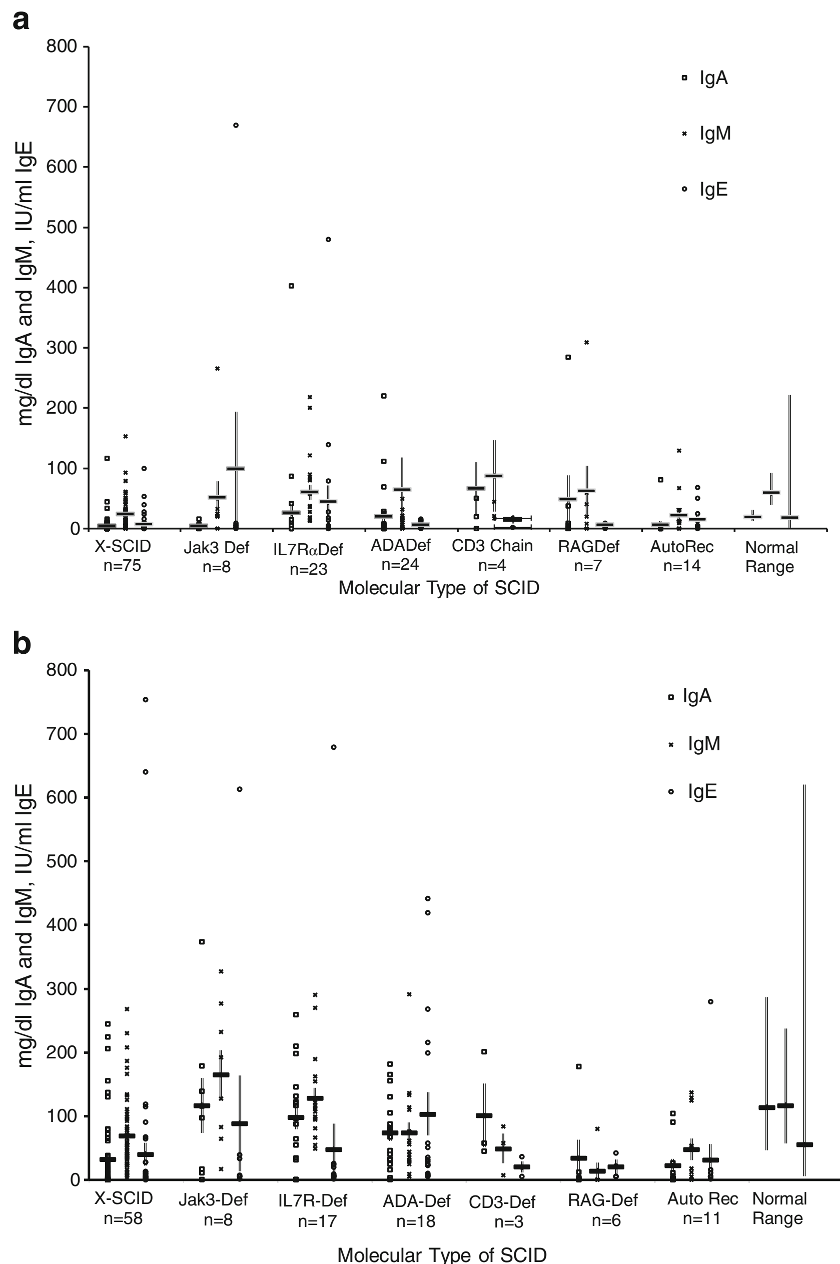

Fig. 1 **a** Serum concentrations of IgA □, IgM **X** and IgE ○: prior to (**a**) and at the latest time studied after (**b**) rigorously T cell-depleted nonablative related donor bone marrow transplantation. The *symbols* represent individual patients, the *bars* the means and the vertical lines 2 SD above and below the means. Normal control values are for age 6 months in (**a**) and for older children and adults in (**b**)

very low rate of B cell chimerism. Antibody production capability can be assessed in those receiving IG replacement by giving them the neoantigen bacteriophage Φ X174 and measuring antibody responses and isotypes of the antibodies following primary and secondary immunizations. Neither IVIG nor subcutaneous immunoglobulin (SCIG) contains antibodies to this neoantigen. In an effort to determine whether IG therapy could be discontinued, we have been able to administer bacteriophage Φ X174 to 61 of these patients, and the results we found in all 61 of the immunized patients are displayed in Fig. 2. All 61 patients have donor T cell chimerism and good T cell function. Only 20 of the 61 patients had B cell chimerism: 12 of the 33 X-linked, 5 of the 10 ADA-Def, and 1 each of the RAG-Def, IL7Ra-Def and Jak3-Def. After transplantation, the mean peak antibody response to bacteriophage for CD3-Def SCIDs was significantly smaller ($p<0.0001$) than that for the normal controls (Fig. 2a) while the mean peak antibody responses to bacteriophage for all other SCID types were not significantly different from normal controls. The mean percentages of IgG antibody made after the second immunization are shown in Fig. 2b according to the molecular type of SCID. These percentages were significantly smaller than that for normal controls for Jak3-Def ($p<0.0001$), X-Linked ($p<0.0001$) and ADA-Def ($p=0.0475$) SCIDs. The most striking differences were seen in the first two types, and those with Jak3-Def SCID had a lower mean percentage of IgG antibody those with X-linked SCID ($p=0.0156$). Despite the fact that both CD3-Def patients had

Table II Immunoglobulins[a], antibody titers[a] and B cell chimerism in patients off of immunoglobulin therapy45

Mol. Type	Patient #	IgG	IgA	IgM	IgE	Tetanus	Diphtheria	Anti-A	Anti-B	B Cell Chimerism
X-linked	1	1290	245	132	9	0.11	0.07	2	32	100 %
	2	934	224	59	12	243.00	ND[c]	0	64	100 %
	3	585	29	65	3	ND	ND	ND	ND	100 %
	4[b]	425	61	71	5	0.03	0.05	0	0	40 %
	5[b]	785	65	103	9	177,147	ND	0	8	81 %
	6	570	12	176	118	0.45	0.10	0	0	ND
	7	1170	137	268	64	0.27	0.08	256	128	0
	8	413	16	92	2	ND	ND	ND	ND	100 %
	9	923	32	50	115	0.50	0.09	256	64	100 %
	10	859	72	166	90	3.20	0.36	0	0	100 %
	11	627	14	39	ND	ND	ND	ND	ND	99 %
	12	518	4	21	1	ND	ND	ND	ND	37 %
	13	1060	80	71	4	6,561	2187	ND	ND	25 %
	14	938	79	125	641	0.14	0.03	0	0	15 %
	15	325	130	99	27	0.08	0.02	0	16	0
	16[b]	1080	206	95	13	0.06	0.04	4	4	30 %
	17	816	19	50	754	1.57	0.39	0	0	0
	18[b]	743	155	57	5	0.14	0.05	0	0	10 %
	19	650	18	78	13	>7.00	>3.00	2	0	100 %
	20[b]	604	6	41	5	81	ND	0	0	99 %
Jak3 Def	21	389	17	127	39	729	81	0	32	0
	22[b]	1,050	374	192	5	0.31	0.15	64	0	0
	23	1368	139	277	33	2,187	729	128	128	ND
	24	921	116	232	7	0.04	0.04	8	4	0
	25	869	97	64	5	0.92	0.19	0	0	100 %
IL7Rα Def	26	944	259	113	25	19,683	19,683	0	64	0
	27[b]	1117	122	81	5	531,441	531,441	64	8	0
	28	771	0	154	5	0.22	0.05	0	0	0
	29	1370	198	121	5	0.19	0.85	0	128	0
	30	845	210	111	1	81	81	32	0	0
	31	829	31	162	23	59,049	59,049	128	0	0
	32	421	96	96	5	0.47	0.74	32	0	0
	33	681	34	107	6	531,441	19,683	32	0	0
	34	1060	64	66	680	177,147	ND	128	64	0
	35	1310	117	270	9	1.68	0.38	0	0	1
	36	882	120	189	19	0.25	0.18	32	2	0
	37	807	54	99	7	19,683	ND	8	0	0
	38	1540	146	290	5	7.00	0.29	0	16	0
	39	864	0	54	5	1.22	0.18	0	0	0
	40	1230	0	116	5	0.91	0.26	2	2	0
	41	834	87	94	5	0.96	0.15	256	256	0
ADA Def	42	1180	165	136	7	2,187	81	0	16	0
	43	738	82	35	31	6,561	6,561	128	4	16 %
	44[b]	883	182	61	199	2.08	0.17	32	32	100 %
	45	1190	78	44	28	ND	ND	8	2	79 %
	46[b]	1030	130	74	419	7.00	3.00	32	34	0
	47	492	0	28	24	531,441	19,683	0	0	71 %
	48[b]	1410	155	110	35	19,683	6,561	ND	ND	99 %
	49[b]	991	105	114	5	7.00	1.51	0	32	0

Table II (continued)

Mol. Type	Patient #	IgG	IgA	IgM	IgE	Tetanus	Diphtheria	Anti-A	Anti-B	B Cell Chimerism
	50	501	67	30	57	243	243	128	0	0
	51	607	87	76	22	2,187	729	64	0	100 %
	52	586	46	75	268	59,049	19,683	0	2	0
	53	423	3	5	10	ND	ND	0	0	0
	54	1260	32	9	6	19,683	19,683	0	0	0
	55	1010	80	55	51	1.62	0.23	8	16	0
	56[b]	793	31	133	442	3.00	7.00	128	64	0
CD3 Chain Def	57	1,260	57	84	5	1.79	1.21	32	32	0
	58[b]	842	201	57	36	4.30	>3.00	0	16	0
	59	936	45	7	19	1.73	0.20	4	8	0
RAG 1	60[b]	751	178	80	21	243	81	32	16	100 %
AutoRec	61	624	26	129	5	0.30	0.05	0	0	0
	62[b]	690	90	124	280	6,561	2,187	16	0	4 %
	63	982	104	52	11	177,147	2,187	0	0	100 %
Unknown	64	1700	125	123	49	7.00	2.46	0	16	100 %

[a] Values are expressed as mg/dl (IgG, IgA, IgM) or IU/ml (IgE). Tetanus and diphtheria antibody titers are expressed as 1:X, omitting the 1, or as μg/dl. Isohemagglutinins are expressed as 1:X, omitting the 1.

[b] HLA Identical Transplants

[c] *ND* not done

quantitatively low antibody titers to bacteriophage Φ X174, they both had normal IgG isotype switching and made normal antibody titers to their standard vaccines after IG therapy was discontinued. Neither had donor B cell chimerism.

B cell Phenotypes

Prior to transplantation, the mean number of CD19+ B cells was significantly larger ($p=0.0005$, Fig. 3a) for the X-Linked SCIDs than that for the normal controls, while the mean number of CD19+ B cells was significantly smaller in ADA-Def SCIDs ($p<0.0001$) than that for normal controls. B cells were not detected in RAG-Def, CHH and Artemis-Def SCIDs (not shown in Fig. 3). The mean numbers of CD19+ B cells for all other SCID types were not significantly different from normal controls. The X-linked, Jak3-Def and IL7Rα-Def SCIDs also had markedly increased expression of three molecules present on immature cells: CD10, CD5 and CD38 (Fig.3a) ($p<0.0001$, 0.0035, <0.0001). The mean numbers of CD5+ B cells were significantly larger in X-linked and IL7Rα-Def SCIDs ($p<0.0001$ and $p<0.0001$) than in normal controls. The mean number of CD38+ B cells were significantly larger in X-linked SCID ($p<0.0001$) than in normal controls. Prior to transplantation, the mean number of CD23+ B cells was significantly smaller than normal ($p<0.0001$) in the ADAdef SCIDs and significantly larger than normal ($p<0.0001$) in the X-Linked SCIDs.

After successful T cell reconstitution by bone marrow stem cell transplantation, the RAG-Def, Artemis-Def, and CHH did not develop B cells. As shown in Fig. 3b, the mean numbers of CD19+ total B cells for ADA-Def and IL7Rα-Def SCIDs were significantly smaller ($p<0.0001$, $p=0.00015$) when compared to age-appropriate normal controls, whereas the mean numbers for all other SCID types were not significantly different from normal. The mean number of CD10+ B cells for X-Linked SCIDs was significantly larger ($p<0.0059$) when compared to normal controls, but the mean numbers for all other SCID types were not different from normal. The mean number of CD5+ B cells for the AutoRec SCIDs was significantly smaller ($p=0.012$) than that for normal controls, but the mean numbers for all other SCID types were not different from normal. The mean numbers of CD38+ B cells for ADA-Def, CD3-Def, and IL7Rα-Def SCID types were significantly smaller ($p=0.0025$, 0.0079, <0.0001) when compared to normal controls, but the mean numbers for all other SCID types were not different from normal. Therefore, SCID B cells do not retain an immature phenotype after transplantation. At the latest time tested post-transplantation, the mean numbers of CD23+ B cells were lower in the ADA-Def ($p=0.001$) and IL7Rα-Def ($p=0.0023$) SCIDs than in the normal controls, but were not significantly different from normal for all other SCID types.

We were also interested in knowing about the appearance of CD27+ (memory) B cells. Memory B cells are capable of producing diverse plasma cells making antibodies of

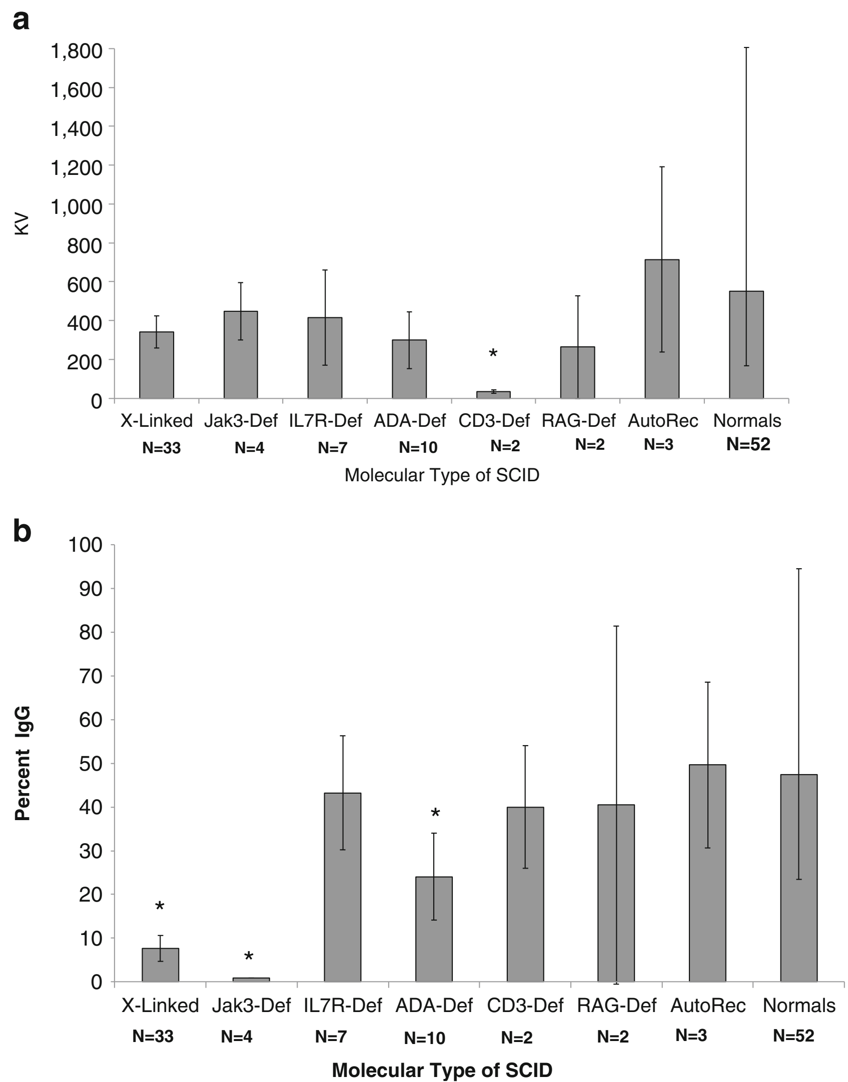

Fig. 2 Antibody responses to bacteriophage Φ X174 in all 61 SCID patients immunized with this vaccine post-transplantation. **a** The mean peak antibody response to bacteriophage for CD3-Def SCIDs was significantly smaller ($p<0.0001$) than the mean peak antibody response to bacteriophage for the normal controls, but those for all other SCID types were not significantly different from those of normal controls [36]. **b** The mean percentages of IgG antibody made after the second immunization were significantly lower than normal in Jak3Def ($p<0.0001$), X-Linked ($p<0.0001$) SCIDs and ADA-Def ($p=0.0475$) SCIDs. Those with Jak3Def SCID had an even lower mean percentage of IgG antibody those with X-linked SCID ($p=0.0156$). All other comparisons were not significantly different from normal controls [36]. All patients have donor T cells and good T cell function

different isotypes when antigen is encountered again, whereas naïve B cells would produce plasma cells producing only IgM antibodies. As seen in Fig. 4a, before transplantation the mean percentages of CD19+/CD27+ B cells for IL7Rα-Def, Jak3-Def, and X-Linked SCIDs were significantly smaller ($p<0.0003$, = 0.020, <0.0001) than that for age appropriate normal controls. Post-transplantation, the mean percentages of CD19+/CD27+ B cells for only the Jak3-Def and X-Linked SCIDs remained significantly smaller ($p<0.0001$, <0.0001) than normal controls (Fig. 4b). Patients were included in these studies regardless of whether they were receiving IG therapy. We were also able to assess the percentage of switched memory B cells in 55 of the patients (Table III). As can be seen, the molecular types of SCID with the lowest percentages of switched memory B cells are the X-SCIDs still receiving IG therapy ($p=<0001$), the autosomal recessive SCIDs of unknown molecular type ($p=<0001$), the ADA-Def SCIDs ($p=0.0017$) and the CD45-Def SCID. The percentages are normal for all of the other molecular types except the RAG-Def, Artemis-Def and CHH patients (not listed) who do not have any B cells.

B Cell Responses In Vitro

Interactions between CD40 on B cells and its ligand on activated CD4+ T cells are crucial in T cell-dependent B cell responses, and are vital to the generation of memory B cells and germinal centers [45]. IL-4 and IL-21, produced by T cells, promote the expansion of mature B cells that have been stimulated through CD40 [46]. While IL-4 stimulates B cells using the γc-containing IL-4 receptor and the non-γc-containing IL-13 receptor [46], IL-21 only utilizes the γc-containing IL-21 receptor [30, 47]. The goal of these studies was to test whether poor B cell function post-transplantation was attributed to the underlying SCID-causing molecular defect or whether it was a consequence of inadequate T cell help. We studied the function of CD19+ selected B cells from PBMC of five X-linked SCIDs (who

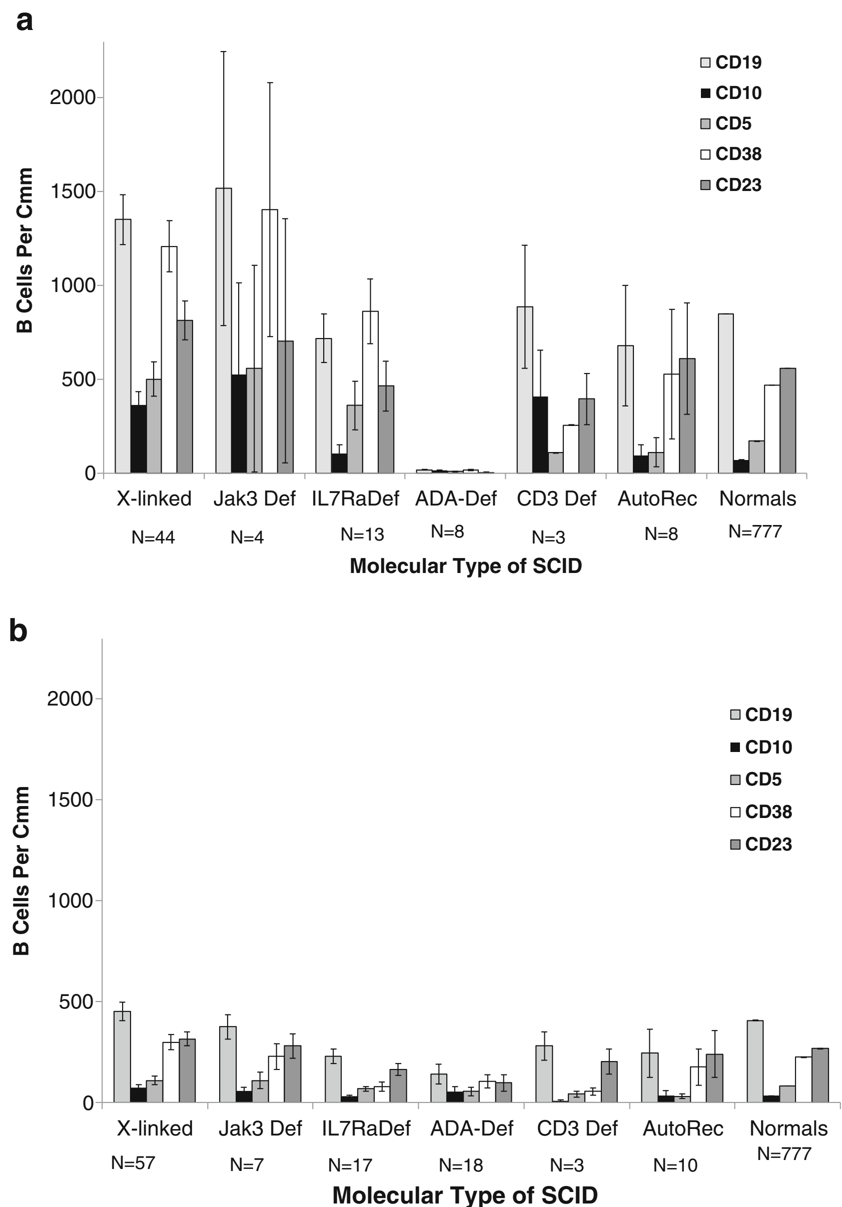

Fig. 3 **a** Prior to transplantation, the mean number of CD19+ B cells was significantly larger (p=0.0005) for the X-Linked SCIDs than that for the normal controls, while the mean number of CD19+ B cells was significantly smaller in ADA-Def SCIDs (p<0.0001) than that for normal 6 month old controls. B cells were not detected in RAG-Def, CHH and Artemis-Def SCIDs (not shown). X-linked, Jak3-Def and IL7Rα-Def SCIDs also had markedly increased expression of three molecules present on immature cells: CD10, CD5 and CD38 (Fig. 3a) (p<0.0001, 0.0035, <0.0001). **b** At the latest time studied post-transplantation, the mean number of CD10+ B cells for X-Linked SCIDs was significantly larger (p<0.0059) when compared to normal adult controls, but the mean numbers for all other SCID types were not different from normal

had no detectable donor-derived B cells and no B cell function but good T cell function), two IL7Rα-Def SCIDs (with no detectable donor-derived B cells but good B cell function), and five normal controls. CD19+ B cells were cultured with human IL-4 or human IL-21 and anti-CD40 mAb, or with the mitogen CpG, or with media. The results of stimulation with IL-21+ anti-CD40 size (Fig. 5a) showed substantially lower proliferation of all X-SCID patients' B cells tested, compared to B cells from controls and from IL7Rα-Def SCID B cells, although the difference was not statistically significant due to the small sample size (two sided p values 0.063 and 0.1516 in a Satterthwaite test). Conversely, both X-SCID and IL7Rα-Def B cells proliferated similarly to B cells from normal controls in response to stimulation with IL-4+ anti CD40 (Fig. 5b) and to CpG (Fig. 5c).

BAFF-R Expression

Normal B-cell survival and differentiation depends upon B-cell activation factor (BAFF). Defects in mouse genes encoding BAFF or BAFF-R abolish the generation of mature B cells. The receptor for BAFF (BAFF-R) is expressed preferentially on B cells. We thus tested whether the expression of BAFF-R on B cells from SCID patients after BM transplantation was different between molecular types that developed B cell function compared to those that didn't, and compared to controls. Flow cytometric studies were performed to test for BAFF-R expression on CD19+ B cells in 56 PBMC samples from 27 SCID patients after BM transplantation, and in 15 control PBMC samples (14 individuals of ≥20 years of age and one newborn). The results,

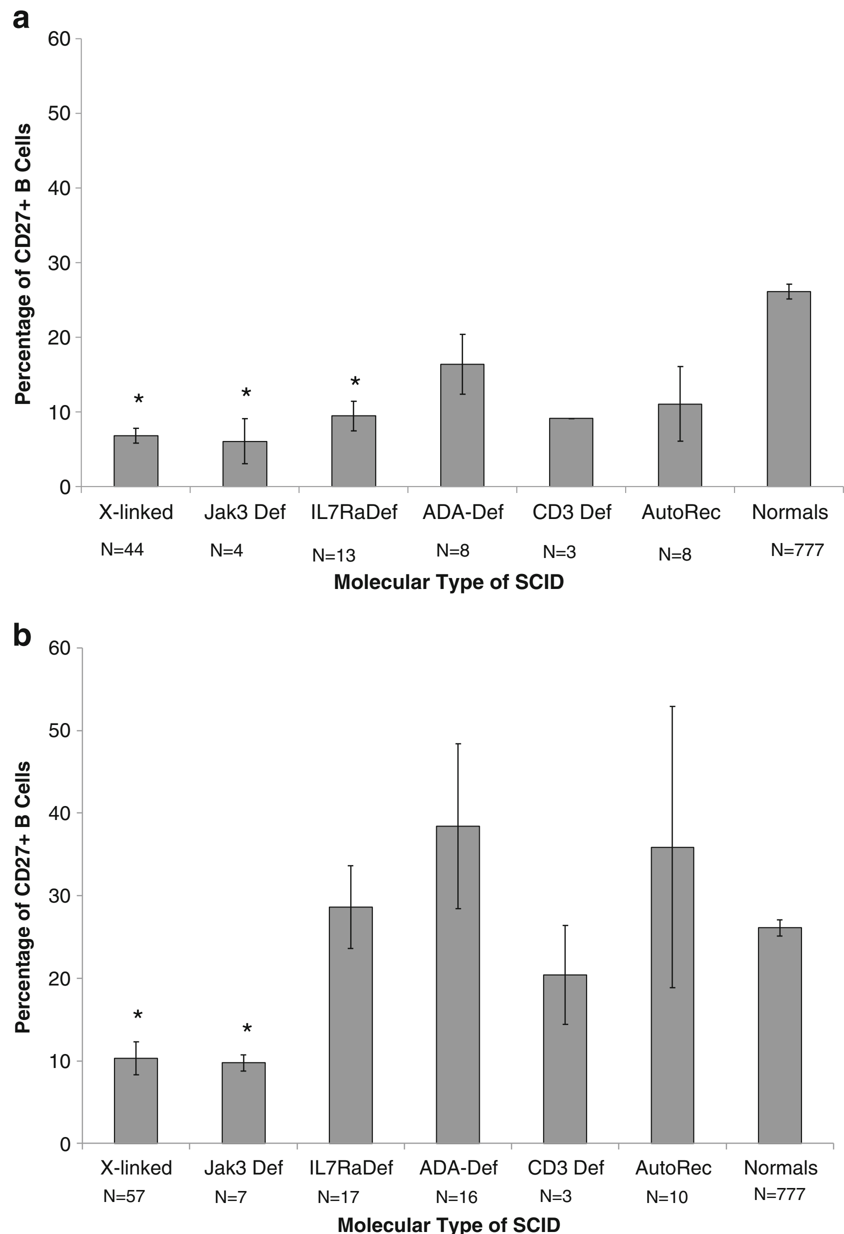

Fig. 4 CD27+ (memory) B cells: **a** Prior to transplantation the mean percentages of CD19+/CD27+ B cells for IL7Rα−-Def, Jak3-Def, and X-Linked SCIDs were significantly smaller ($p<0.0001$, 0.020, <0.0001) than that for normal controls. **b** Post-transplantation the mean percentages of CD19+/CD27+ B cells remained significantly smaller than normal only for the Jak3-Def and X-Linked SCIDs ($p<0.0001$, <0.0001). Patients shown included those who were or were not receiving IG therapy

shown in supplemental Figure 1, revealed that BAFF-R is constitutively expressed on host CD19+ B cells from all SCID types (X-linked, Jak3-Def, ADA-Def, CD3δ-Def and IL7Rα-Def SCID) after bone marrow transplantation. Only 7 % of SCID samples (3 IL7Rα-Def and one Jak3-Def) showed a lower percentage of CD19+ BAFF-R+ cells at early times after transplantation (<146 days), similar to that found in a normal newborn sample. Further out after transplantation and successful immune reconstitution by donor stem cell-derived T cells, the percentage of CD19+ BAFF-R+ normalized in all of the SCID samples despite the fact that the B cells remained of host origin in 22/27 patients tested. One of the five with donor B cell chimerism was the Jak3-Def patient who had a lower percentage of CD19+ BAFF-R+ cells early after transplantation.

BCR Spectratype Analysis

We next determined whether the B cell functional deficiency in SCID recipients of non-ablated T cell-depleted bone marrow stem cell transplants derives from restricted utilization of immunoglobulin (Ig) genes, resulting in lack of development of a diverse repertoire. This question was addressed by spectratyping the six major VH gene families in an area encompassing the Complementarity Determining Region 3 (CDR3). The selected patients had no evidence of maternal B cells in their PBMC samples, as determined by FISH or RFLP (data not shown). Compiled results from the spectratype analyses of nine BMT treated SCID patients are presented in Table IV. The number of size peaks from each VH family was determined in the patients‘ samples, and

Table III Percentages of switched memory B cells

Molecular Type SCID	N	Mean[a]	St. Dev.	S.E.M.
ADA-Deficient	6	27.67*	10.60	4.33
CD3 Chain Deficient	2	56.75	1.85	1.31
IL7Ralpha-Deficient	8	48.79	12.41	4.39
ARSCID	4	11.05**	3.69	1.85
CD45 Deficient	1	8.40	NA[b]	NA[b]
Jak3-Deficient	3	30.87	13.99	8.08
X-SCID on IG	21	11.41**	11.16	2.44
X- SCID not on IG	10	34.68	22.26	7.04

[a] Normal adult mean % and range: 51.4 (32.3–70.5)

[b] NA=not applicable for statistics as there is only 1 patient of this type.

*p=0.0017

**p<0.0001

compared with the range of peaks from normal controls. Only 2/9 patients studied (X-3 and X-6) did not express amplification of the VH1, VH5 and VH6 gene families, while all of the other patients revealed a complete representation of the six VH families. Examples of spectratype results from an X-linked (X-1), a Jak3-Def (J-2), an IL7Rα-Def (7–4) patient and a normal control are shown in supplemental Fig. 2, panels A, B, C and D, respectively). When comparing the spectratype results for the BCR repertoire analysis with that of the TCR repertoire analysis for each patient tested (see last column in Table IV) [48] the results were different and unpredictable. In fact, a complete polyclonal TCR repertoire, seen in patient X-3, was associated with a partially skewed BCR repertoire, while patient X-1 did not develop a diversified TCR repertoire (0 % polyclonality) but developed a normal BCR repertoire (Table IV).

Discussion

The studies presented here revealed that normal B cell function can develop in some SCIDs who become T cell-reconstituted following non-ablative rigorously T cell-depleted haploidentical (N=108) or HLA-identical (N=17) bone marrow transplants despite having only host B cells. The genetic types with the best B cell function (IL7Rα-Def, ADA-Def, CD3-Def and some autosomal recessive SCIDs of unknown molecular type) were also found to have the highest percentages of B cells bearing the CD27 memory B cell marker and the highest percentages of switched memory B cells. This demonstrates that host B cells in patients with IL7Rα-Def, ADA-Def, CD3-Def and some autosomal recessive SCIDs of unknown molecular type can function normally if T cell help is provided. The finding of normal B cell function in the IL7Rα-Def SCIDs with only host B cells is in contrast to the situation in IL7Rα murine knockouts where

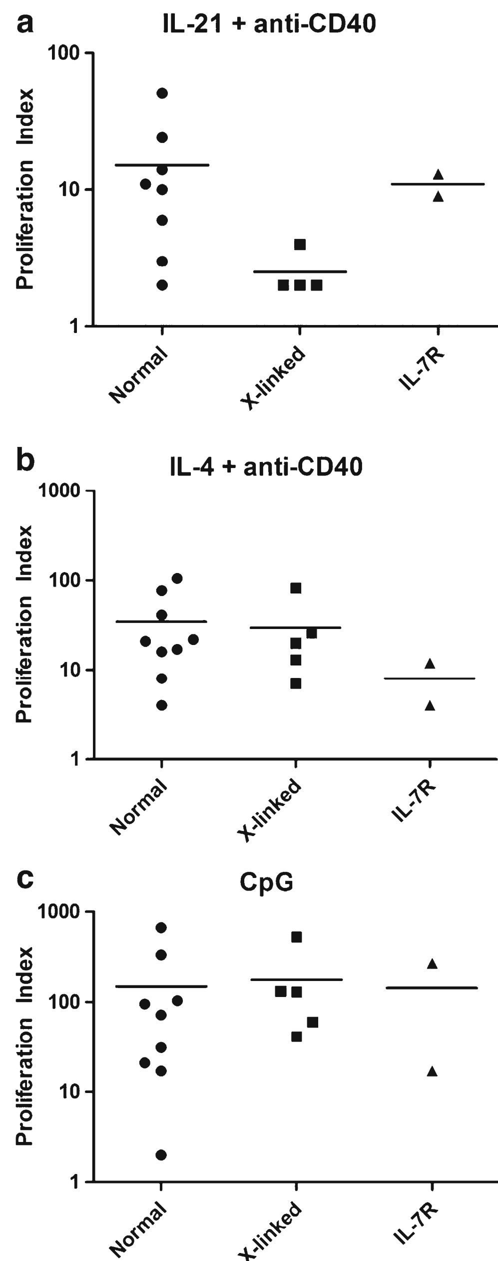

Fig. 5 B cell proliferative responses in vitro. Each *point* represents the proliferation index of cultures of B cells isolated from X-SCID patients [n=4 in (**a**), n=5 in (**b**),(**c**)], from IL7Rα-Def SCID [n=2 in (**a**),(**b**),(**c**)] patients and normal controls [n=8 in (**a**), n=9 in (**b**),(**c**)] stimulated with IL-21+ anti-CD40 (**a**), with IL-4+ anti CD40 (**b**), or with CpG (**c**) The *horizontal bars* represent the mean value of each series

B cells fail to develop [49]. The finding of good B cell function in the ADA-Def SCIDs with donor T cells but only host B cells implies that the purine salvage pathway may not be as crucial for B cell function as it is for T cell function. The poor B cell function seen in Jak3-Def and X-linked SCIDs post-transplantation despite the presence of phenotypically normal B cells is most likely due to failure of signaling through their defective cytokine receptors on the host B cells and not a result of inadequate T cell help [30].

Table IV Analysis of the BCR repertoire by spectratyping

Source of RNA samples	Number of peaks / VH family						BCR Spectra Type[a]	TCR Spectra Type[b]
	VH1	VH*2*	VH3	VH4	VH5	VH6		
Normal PBMC range	17–20	13–19	18–20	19–20	15–17	15–17	100	64–100
EBV-transf. PBMC	5	6	7	7	0	1	0	–
Namalwa B cells	0	0	0	1	0	0	0	–
OCI-Ly8 B cells	0	0	1	0	0	0	0	–
Yurkat T cells	0	0	0	0	0	0	0	0
X-linked PBMC (days post-transplantation):								
X-1 (64)	19	15	18	19	18	15	100	0
X-3 (1984)	0	13	13	17	0	0	17	100
X-4 (140)	19	17	18	20	16	17	100	43
X-6 (4,263)	0	15	15	16	0	0	17	0
X-7 (839)	17	16	17	18	16	15	83	41
Jak3-Def PBMC (days post-transplantation):								
J-1 (2,408)	18	18	18	18	15	15	100	64
J-2 (3,676)	20	19	17	19	16	15	83	50
J-3 (1,084)	20	14	18	21	14	17	83	50
IL7Rα-Def PBMC (days post-transplantation):								
7-4 (544)	20	17	20	20	18	16	100	96

[a] BCR spectratype data are presented as % VH families with normal distribution of size peaks

[b] TCR spectratype data are presented as % Vβ families with normal distribution of size peaks (Sarzotti et al., JI)

Although 48.8 % of our patients are currently receiving IG replacement, the percentage receiving it varies based on molecular defect. Only 6 % of the IL-7Rα-Def patients are currently receiving IG, while 83 % of RAG-Def patients and 66 % of X-SCID patients require replacement. It has been suggested that this high IG requirement is due to lack of donor B cell chimerism because they did not receive pre-transplant conditioning. However, we do see donor B cell engraftment in approximately one third of our X-linked patients despite the lack of conditioning and those patients do not require IG replacement [1, 2]. Indeed, the percentage with B cell chimerism was higher in X-SCIDs than in any other molecular type. What determines whether or not B cell chimerism will develop is unknown. This was not due to over representation of the HLA-identical transplants among any molecular type. For example, among the 58 X-linked patients, there were only 5 who had an HLA-identical donor and among the 17 with IL7Rα−Def, there was only 1. Moreover, only 10 of the 17 patients who received HLA-identical transplants had donor B cells (Table II). It is possible that, in patients with specific genetic variants of SCID, there is a competitive inhibition of engraftment of normal donor-derived hematopoietic progenitors by defective host progenitors for occupancy of one or more of the specialized niches in the thymus or bone marrow [50, 51]. In murine models, such resistance to the development of normal precursors within the thymus has been demonstrated in RAG-2 $^{-/-}$ and TCR B $^{-/-}$ but not $\gamma c^{-/-}$ or IL7Rα $^{-/-}$ forms of SCID [52]. Age at transplant is not a factor in B cell chimerism or B cell function and neither is race or sex [3]. Genetic analyses of the cells from γc-Def and Jak3-Def patients without B cell function have revealed all T cells to be genetically donor, whereas the B and antigen-presenting cells remain those of the recipient [2]. A majority of our patients received rigorously T cell depleted bone marrow transplants. Some have advocated using pre-transplant conditioning followed by unfractionated matched unrelated donor cord blood, adult marrow or mobilized peripheral blood stem cells and post-transplantation immunosuppression to mitigate GVHD in an effort to achieve donor B cell reconstitution. However, Stephan et al. [53] and Haddad et al. [21] reported that 35 % and 45 % of survivors, respectively, required immunoglobulin substitution despite conditioning. Thus, conditioning does not always result in B-cell function, and the associated risks may not justify the chance to achieve normal B cell function. Whether unfractionated cord blood or marrow has any advantage over T-depleted marrow in this regard is unknown but the unfractionated unrelated donor products are much more likely to carry significant risks of severe GVHD. It was hoped that gene therapy for X-linked SCID could ensure that B cell function would be normal. However, two recent follow-up reports both revealed that the only lineage corrected long term by gene therapy was T cells [54, 55].

We performed in vitro studies in order to understand whether B cell function in X-linked SCIDs could be detected in vitro under the right type and amount of stimulation provided experimentally. Results from the in vitro stimulation of CD19+ selected B cells with stimuli (IL-21 + anti-CD40) that require the γc receptor for cytokines, revealed that B cells from X-linked SCIDs proliferated very poorly in comparison to IL7Rα-Def or normal B cells. These results are in keeping with those from a recent report showing that IL-21 is the primary common γ chain-binding cytokine required for human B-cell differentiation in vivo [30]. When X-linked B cells were stimulated with IL-4 + anti-CD40 or with CpG, which bypass the γc receptor, they proliferated as efficiently as IL7Rα−Def or normal B cells. These findings support the hypothesis that poor host B cell function post-transplantation is due to the underlying SCID-causing molecular defect, and that B cells from these patients have the potential to develop B cell function under appropriate stimulation conditions. When the γc receptor is normal, as is the case in the IL7Rα-Def SCIDs, B cells can receive sufficient signals from the successfully engrafted T cells to develop function.

It was also possible that B cells in SCIDs who do not develop B cell function have defective expression of receptors (BAFF-R) for essential maturation signals, such as BAFF. We tested this hypothesis by performing flow cytometric analysis of 27 patients of different SCID molecular types, some of whom developed B cell function (IL7Rα-Def) and some of whom did not (Jak3-Def and X-linked) and determined that BAFF-R is expressed on 80 % of CD19 + cells in >93 % of the samples tested over time post-transplantation. Only four samples (3 IL7Rα-Def and 1 Jak3-Def) had low BAFF-R expression at early time points, a result obtained also when testing B cells from a normal newborn. Although the mechanism is unclear at this time, the low receptor expression normalized with time. Thus, the constitutive expression of BAFF-R in B cells from all molecular types of SCID does not correlate with the development of B cell function.

Finally, we tested the hypothesis that the lack of B cell function in some SCID molecular types reflects a limited utilization of Ig genes similar to that taking place during ontogeny. Fumoux et al. [56] showed that, in adults with hematologic malignancies who received BMTs, the expression of the VH repertoire was very different from that of age matched controls: the expression of the VH 3 family was decreased two- to threefold, while other families were over-expressed. Minegishi et al. [57] found in studying the repertoire of IgH chain gene in three X-SCID patients that the JH3 segment was preferentially utilized in the CDR3 and that somatic mutation was absent in all of the JH3 segments. We studied the expression of the B cell repertoire in 5 X-linked, 3 Jak-3Def and 1 IL7Rα-Def SCIDs to understand the extent of genetic diversity of B cells in these patients and the influence that re-populating donor T cells have on the clonal expansion of B cells. The spectratype results showed a normal or quasi-normal distribution of CDR3 size peaks of the VH families of SCID patients' PBMC indicating that the majority of these patients (7/9) do not appear to have a biased B cell repertoire expression. Finally, the expression of a polyclonal B cell repertoire in BMT treated SCIDs appears to be independent of the expression of a normal T cell repertoire.

Conclusions

These results showed that B cell chimerism was not required for normal B cell function in several molecular types of SCID and that the most important factor determining whether B cell function develops is the underlying molecular defect. In those molecular defects where host B cell function does not develop, donor B cell chimerism is necessary for the development of B cell function.

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References

1. Buckley RH. Transplantation of hematopoietic stem cells in human severe combined immunodeficiency: longterm outcomes. Immunol Res. 2011;49(1–3):25–43.
2. Buckley RH, Schiff SE, Schiff RI, Markert L, Williams LW, Roberts JL, et al. Hematopoietic stem cell transplantation for the treatment of severe combined immunodeficiency. N Engl J Med. 1999;340:508–16.
3. Myers LA, Patel DD, Puck JM, Buckley RH. Hematopoietic stem cell transplantation for severe combined immunodeficiency in the neonatal period leads to superior thymic output and improved survival. Blood. 2002;99(3):872–8.
4. Puck JM, Deschenes SM, Porter JC, Dutra AS, Brown CJ, Willard HF, et al. The interleukin-2 receptor gamma chain maps to Xq13.1 and is mutated in X-linked severe combined immunodeficiency, SCIDX1. Hum Mol Genet. 1993;2:1099–104.
5. Noguchi M, Yi H, Rosenblatt HM, Filipovich AH, Adelstein S, Modi WS, et al. Interleukin-2 receptor gamma chain mutation results in X-linked severe combined immunodeficiency in humans. Cell. 1993;73:147–57.
6. Hirschhorn R. Immunodeficiency diseases due to deficiency of adenosine deaminase. In: Ochs HD, Smith CIE, Puck JM, editors. Primary Immunodeficiency Diseases: A Molecular and Genetic

Approach. 1st ed. New York and Oxford: Oxford University Press; 1999. p. 121–39.

7. Roberts JL, Lengi A, Brown SM, Chen M, Zhou Y-J, O'Shea JJ, et al. Janus Kinase 3 (JAK3) deficiency: clinical, immunologic and molecular analyses of 10 patients and outcomes of stem cell transplantation. Blood. 2004;103:209–18.
8. Puel A, Ziegler SF, Buckley RH, Leonard WJ. Defective IL7R expression in T(−)B(+)NK(+) severe combined immunodeficiency. Nat Genet. 1998;20(4):394–7.
9. Schwarz K, Gauss GH, Ludwig L, Pannicke U, Li Z, Lindner D, et al. RAG mutations in human B cell-negative SCID. Science. 1996;274:97–9.
10. Roberts JL, Buckley RH, Luo B, Pei J, Lapidus A, Peri S, et al. CD45-deficient severe combined immunodeficiency caused by uniparental disomy. Proc Natl Acad Sci U S A. 2012;109:10456–61.
11. Moshous D, Li L, Chasseval R, Philippe N, Jabado N, Cowan MJ, et al. A new gene involved in DNA double-strand break repair and V(D)J recombination is located on human chromosome 10p. Hum Mol Genet. 2000;9(4):583–8.
12. Buck D, Moshous D, de Chasseval R, Ma Y, Le Deist F, Cavazzana-Calvo M, et al. Severe combined immunodeficiency and microcephaly in siblings with hypomorphic mutations in DNA ligase IV. Eur J Immunol. 2006;36(1):224–35.
13. van der Burg M, Ijspeert H, Verkaik NS, Turul T, Wiegant WW, Morotomi-Yano K, et al. A DNA-PKcs mutation in a radiosensitive T-B- SCID patient inhibits Artemis activation and nonhomologous end-joining. J Clin Invest. 2009;119(1):91–8.
14. Dadi HK, Simon AJ, Roifman CM. Effect of CD3delta deficiency on maturation of alpha/beta and gamma/delta T-cell lineages in severe combined immunodeficiency. N Engl J Med. 2003;349(19):1821–8.
15. de Saint Basile G, Geissmann F, Flori E, Uring-Lambert B, Soudais C, Cavazzana-Calvo M, et al. Severe combined immunodeficiency caused by deficiency in either the delta or the epsilon subunit of CD3. J Clin Invest. 2004;114(10):1512–7.
16. Roberts JL, Lauritsen JHP, Cooney M, Parrott RE, Sajaroff EO, Win CM, et al. T-B+ NK+ severe combined immunodeficiency caused by complete deficiency of the CD3 zeta subunit of the T cell antigen receptor complex. Blood. 2007;109:3198–206.
17. Buckley RH, Schiff SE, Sampson HA, Schiff RI, Markert ML, Knutsen AP, et al. Development of immunity in human severe primary T cell deficiency following haploidentical bone marrow stem cell transplantation. J Immunol. 1986;136:2398–407.
18. Moen RC, Horowitz SD, Sondel PM, Borcherding WR, Trigg ME, Billing R, et al. Immunologic reconstitution after haploidentical bone marrow transplantation for immune deficiency disorders: treatment of bone marrow cells with monoclonal antibody CT-2 and complement. Blood. 1987;70:664–9.
19. Dror Y, Gallagher R, Wara DW, Colombe BW, Merino A, Benkerrou M, et al. Immune reconstitution in severe combined immunodeficiency disease after lectin-treated, T cell depleted haplocompatible bone marrow transplantation. Blood. 1993;81:2021–30.
20. Sarzotti-Kelsoe M, Win CM, Parrott RE, Cooney M, Moser BK, Roberts JL, et al. Thymic output, T-cell diversity, and T-cell function in long-term human SCID chimeras. Blood. 2009;114(7):1445–53.
21. Haddad E, Deist FL, Aucouturier P, Cavazzana-Calvo M, Blanche S, Basile GD, et al. Long-term chimerism and B-cell function after bone marrow transplantation in patients with severe combined immunodeficiency with B cells: a single-center study of 22 patients. Blood. 1999;94(8):2923–30.
22. van Leeuwen JE, van Tol MJ, Joosten AM, Schellekens PT, van den Bergh RL, Waaijer JL, et al. Relationship between patterns of engraftment in peripheral blood and immune reconstitution after allogeneic bone marrow transplantation for (severe) combined immunodeficiency. Blood. 1994;84:3936–47.
23. O'Marcaigh AS, DeSantes K, Hu D, Pabst H, Horn B, Li L, et al. Bone marrow transplantation for T-B- severe combined immunodeficiency disease in Athabascan-speaking native Americans. Bone Marrow Transplant. 2001;27(7):703–9.
24. Mazzolari E, Forino C, Guerci S, Imberti L, Lanfranchi A, Porta F, et al. Long-term immune reconstitution and clinical outcome after stem cell transplantation for severe T-cell immunodeficiency. J Allergy Clin Immunol. 2007;120(4):892–9.
25. Neven B, Leroy S, Decaluwe H, Le Deist F, Picard C, Moshous D, et al. Long-term outcome after hematopoietic stem cell transplantation of a single-center cohort of 90 patients with severe combined immunodeficiency. Blood. 2009;113(17):4114–24.
26. Patel NC, Chinen J, Rosenblatt HM, Hanson IC, Brown BS, Paul ME, et al. Long-term outcomes of nonconditioned patients with severe combined immunodeficiency transplanted with HLA-identical or haploidentical bone marrow depleted of T cells with anti-CD6 mAb. J Allergy Clin Immunol. 2008;122(6):1185–93.
27. Patel NC, Chinen J, Rosenblatt HM, Hanson IC, Krance RA, Paul ME, et al. Outcomes of patients with severe combined immunodeficiency treated with hematopoietic stem cell transplantation with and without preconditioning. J Allergy Clin Immunol. 2009;124(5):1062–9.
28. Slatter MA, Brigham K, Dickinson AM, Harvey HL, Barge D, Jackson A, et al. Long-term immune reconstitution after anti-CD52-treated or anti-CD34-treated hematopoietic stem cell transplantation for severe T-lymphocyte immunodeficiency. J Allergy Clin Immunol. 2007;121:361–7.
29. Dvorak CC, Hung GY, Horn B, Dunn E, Oon CY, Cowan MJ. Megadose CD34(+) cell grafts improve recovery of T cell engraftment but not B cell immunity in patients with severe combined immunodeficiency disease undergoing haplocompatible nonmyeloablative transplantation. Biol Blood Marrow Transplant. 2008;14(10):1125–33.
30. Recher M, Berglund LJ, Avery DT, Cowan MJ, Gennery AR, Smart J, et al. IL-21 is the primary common gamma chain-binding cytokine required for human B-cell differentiation in vivo. Blood. 2011;118(26):6824–35.
31. Buckley RH. B cell function in severe combined immunodeficiency after stem cell or gene therapy: A review. J Allergy Clin Immunol. 2010;125:790–7.
32. Buckley RH. Transplantation of hematopoietic stem cells in human severe combined immunodeficiency: longterm outcomes. Immunol Res. 2011;49:25–43.
33. Moshous D, Callebaut I, de Chasseval R, Corneo B, Cavazzana-Calvo M, Le Deist F, et al. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. Cell. 2001;105(2):177–86.
34. Buckley RH, Dees SC, O'Fallon WM. Serum immunoglobulins I. levels in normal children and in uncomplicated childhood allergy. Pediatrics. 1968;41:600–11.
35. Buckley RH, Dees SC. Serum immunoglobulins. III. Abnormalities associated with chronic urticaria in children. J Allergy. 1967;40:294–303.
36. Ochs HD, Davis SD, Wedgwood RJ. Immunologic responses to bacteriophage phi-X 174 in immunodeficiency diseases. J Clin Invest. 1971;50(12):2559–68.
37. Van Den Berg H, Vossen JM, van den Bergh RL, Bayer J, van Tol MJD. Detection of Y chromosome by in situ hybridization in combination with membrane antigens by two-color immunofluorescence. Lab Investig. 1994;64:623–8.
38. Kruetzmann S, Rosado MM, Weber H, Germing U, Tournilhac O, Peter HH, et al. Human immunoglobulin M memory B cells controlling Streptococcus pneumoniae infections are generated in the spleen. J Exp Med. 2003;197(7):939–45.
39. Smith JG, Liu X, Kaufhold RM, Clair J, Caulfield MJ. Development and validation of a gamma interferon ELISPOT assay for

quantitation of cellular immune responses to varicella-zoster virus. Clin Diagn Lab Immunol. 2001;8(5):871–9.

40. Campbell MJ, Zelenetz AD, Levy S, Levy R. Use of family specific leader region primers for PCR amplification of the human heavy chain variable region gene repertoire. Mol Immunol. 1992;29(2):193–203.
41. Shuttleworth J, Morser J, Burke DC. Expression of interferon-alpha and interferon-beta genes in human lymphoblastoid (Namalwa) cells. Eur J Biochem. 1983;133(2):399–404.
42. Tweeddale M, Lim B, Jamal N, Minden M, Messner HA. J Cell Biochem. 1985;117(Suppl 9A). Abstract.
43. Moser BK, Stevens GR, Watts CL. The Two-sample T test versus Satterthwaite's approximate F test. Commun Stat Theory Methods. 1989;18:3963–75.
44. Bonferroni CE. Il Calcolo Delle Assicurazioni su Gruppi di Teste. Studi in Onore del Professore Salvatore Ortu Carboni. Rome, Italy: 1935. p. 13–60.
45. Foy TM, Aruffo A, Bajorath J, Buhlmann JE, Noelle RJ. Immune regulation by CD40 and its ligand GP39. Annu Rev Immunol. 1996;14:591–617.
46. Taylor N, Candotti F, Smith S, Oakes SA, Jahn T, Isakov J, et al. Interleukin-4 signaling in B lymphocytes from patients with X-linked severe combined immunodeficiency. J Biol Chem. 1997;272(11):7314–9.
47. Habib T, Senadheera S, Weinberg K, Kaushansky K. The common gamma chain (gamma c) is a required signaling component of the IL-21 receptor and supports IL-21-induced cell proliferation via JAK3. Biochemistry. 2002;41(27):8725–31.
48. Sarzotti M, Patel DD, Li X, Ozaki DA, Cao S, Langdon S, et al. T cell repertoire development in humans with SCID after nonablative allogeneic marrow transplantation. J Immunol. 2003;170(5):2711–8.
49. Peschon J, Morrissey PJ, Grabstein KH, Ramsdell FJ, Marakowsky E, Gliniak BC, et al. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J Exp Med. 1995;180:1955–60.
50. Bhattacharya D, Rossi DJ, Bryder D, Weissman IL. Purified hematopoietic stem cell engraftment of rare niches corrects severe lymphoid deficiencies without host conditioning. J Exp Med. 2006;203(1):73–85.
51. Czechowicz A, Kraft D, Weissman IL, Bhattacharya D. Efficient transplantation via antibody-based clearance of hematopoietic stem cell niches. Science. 2007;318(5854):1296–9.
52. Prockop SE, Petrie HT. Functional assessment of alphaEbeta7/E-cadherin interactions in the steady state postnatal thymus. Clin Dev Immunol. 2004;11(2):135–41.
53. Stephan JL, Vlekova V, Le Deist F, Blanche S, Donadieu J, de Saint-Basile G, et al. Severe combined immunodeficiency: a retrospective single-center study of clinical presentation and outcome in 117 cases. J Pediatr. 1993;123:564–72.
54. Hacein-Bey-Abina S, Hauer J, Lim A, Picard C, Wang GP, Berry CC, et al. Efficacy of gene therapy for X-linked severe combined immunodeficiency. N Engl J Med. 2010;363(4):355–64.
55. Gaspar HB, Cooray S, Gilmour KC, Parsley KL, Adams S, Howe SJ, et al. Long-term persistence of a polyclonal T cell repertoire after gene therapy for x-linked severe combined immunodeficiency. Sci Transl Med. 2011;3(97):97ra79.
56. Fumoux F, Guigou V, Blaise D, Maraninchi D, Fougereau M, Schiff C. Reconstitution of human immunoglobulin VH repertoire after bone marrow transplantation mimics B cell ontogeny. Blood. 1993;81:3153–7.
57. Minegishi Y, Okawa H, Sugamura K, Yata J. Preferential utilization of the immature JH segment and absence of somatic mutation in the CDR3 junction of the IgH chain gene in three X-linked severe combined immunodeficiency patients. Int Immunol. 1994;6:1709–15.