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Oligoclonality, impaired class switch and B cell memory responses in WHIM syndrome

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Abstract

Heterozygous truncating mutations in CXCR4 have been identified as a cause of WHIM syndrome (warts, hypogammaglobulinemia, immunodeficiency and myelokathexis). The receptor truncations have been proposed to lead to altered lymphocyte trafficking. The purpose of the described studies was to characterize the B-cell repertoire in WHIM subjects. We confirmed profound B-cell lymphopenia and demonstrated oligoclonality of the circulating B-cell pool by HCDR3 spectratyping. The response to immunization was studied in one subject utilizing a bacteriophage ΦX174 immunization protocol. Spectratyping showed oligoclonality at baseline with normalization of the HCDR3 length distribution by five months after immunization with ΦX174 with eventual return to the baseline state. Isotype switching from phage specific neutralizing antibody of the IgM class to IgG was markedly reduced. Overall, these data suggest that impaired CXCR4 signaling in WHIM syndrome results in defective B-cell function and abnormal isotype switching, possibly through effects on germinal center trafficking of lymphocytes.

Keywords

WHIM syndrome; CXCR4; isotype switching; memory B-cells

Introduction

WHIM syndrome is a dominantly inherited condition characterized by warts, hypogammaglobulinemia, immunodeficiency and myelokathexis.[1] The genetic basis of WHIM syndrome is mutation of the chemokine receptor CXCR4.[2] All mutations described to date are heterozygous nonsense or frameshift lesions that result in truncations of the cytoplasmic tail of the receptor. The loss of distal phosphorylation sites has been proposed to impair the ability of the activated receptor to interact with β-arrestin and thus prolong receptor signaling.[3] Leukocytes from WHIM patients show impaired association of GRK6 and delayed recruitment of β-arrestin2 to CXCR4 leading to slower internalization of the receptor and prolonged signaling ERK1/2 signalling.[4;5] One functional consequence of dysregulated

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CXCR4 signaling is failure of mature neutrophils to exit the bone marrow (myelokathexis) resulting in peripheral neutropenia. Clinical complications of neutropenia in WHIM syndrome include recurrent superficial (cellulitis, cutaneous abscesses) and deep tissue infections (pneumonia, osteomyelitis, meningitis). Treatment with cytokines (G-CSF, GM-CSF) has been reported to decrease the number of infections in WHIM patients.[6] Hypogammaglobulinemia is a more variable feature with patients displaying only a moderate deficiency to normal levels. [7;8;9;10;11] This deficiency may be limited to serum IgG, or may involve only IgM or IgM/IgA.[1] Although the cardinal feature of WHIM is neutropenia, lymphopenia is also commonly present, although normal bone marrow lymphoid progenitor cells are present. [7;9;12;13;14] A memory response has been demonstrated in the setting of lymphopenia, however evidence suggests that the development and maintenance of memory B-cells is impaired.[13]

CXCR4 is expressed on all subsets of B-cells during B-cell ontogeny. Its ligand, CXCL12, is expressed in central and peripheral lymphoid organs, particularly in the bone marrow, high endothelial venules, lymph nodes, spleen and peritoneum, demonstrating the importance of CXCR4 in lymphocyte trafficking.[15;16;17] CXCR4/CXCL12 interactions are critical for the survival of the earliest committed B-cell progenitors[18] but the release mature B-lymphocytes from the bone marrow has been shown to be regulated by the downregulation of responsiveness to CXCL12.[19] These observations support the notion that lymphocyte trafficking in WHIM patients is altered due to enhanced CXCR4 signaling. Persistence of responsiveness to stromal CXCL12 is thought to be the primary mechanism underlying the sequestration of myeloid cells in the bone marrow characteristic of myelokathexis. While neutrophils, and in some cases eosinophils[9;20], show morphologic features of apoptosis, this has not been reported for B-lymphocytes.[21] A role for accelerated apoptosis cell in the profound lymphopenia of WHIM syndrome cannot be excluded, but in light of the demonstrated internalization defect on T-cells with mutant CXCR4 receptor [22;23], a similar effect on B-lymphocytes is likely.

There are few published data regarding peripheral lymphoid organ histology from WHIM patients but abnormal architecture with a depletion of B-cells was described in a biopsied lymph node.[11] Since CXCR4 is integral to B-cell development and affinity maturation, we hypothesized that the truncating mutations in WHIM syndrome lead to altered germinal center microenvironment resulting in impaired isotype switching and a reduced capacity to maintain memory B-cells. Utilizing B-cell spectratyping, we examined the third complementarity-determining region of the rearranged heavy chain variable region (HCDR3) genes, and demonstrated B-cell oligoclonality in five members of a pedigree affected with WHIM syndrome. To test the characteristics of the primary and secondary immune responses, immunization with the T-cell dependent neoantigen, bacteriophage ΦX174, was performed in one adult WHIM patient. Consistent with other reported data on immune challenges in WHIM patients, we observed a robust primary response but delayed and depressed isotype switch from IgM to IgG following secondary immunization. Of interest, the HCDR3 repertoire during this challenge showed a short-term normalization, with eventual return to the baseline oligoclonal state. These findings support the hypothesis of an altered germinal center microenvironment that leads to impaired secondary immune responses in patients with WHIM syndrome.

Methods

Study subjects

Patients with WHIM syndrome were collected from a single family with multiple affected individuals reported previously.[2] All members have confirmed WHIM-associated mutations (R332X truncating mutation) by DNA sequencing. Control samples for spectratype analysis were obtained from 4 normal healthy volunteers without inflammatory conditions.

DNA, RNA and cDNA preparation

For DNA extraction, peripheral blood was collected via venipuncture from study subjects and controls. For RNA extraction and cDNA synthesis, 80–100 mL of peripheral blood was collected from subject (II.5) prior to antigenic challenge and at 1, 5, 7, and 14 months after bacteriophage immunization. Samples were processed the same day of acquisition. Genomic DNA was isolated from mononuclear cells using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN) and total RNA was isolated using Purescript RNA Isolation Kit (Gentra Systems, Minneapolis, MN). Both of these isolation kits were used according to the manufacturer's directions. One µg of RNA was reverse transcribed to cDNA using a modified MMLV-reverse transcriptase with RNase H⁺ activity (iScript, Bio-Rad, Hercules, CA) according to the manufacturer's directions.

PCR reactions

To estimate the amount of immunoglobulin constant region transcripts in each sample to control for variations in B-cell numbers and transcriptional activity, cDNA was standardized using IgM constant region specific primers.[24] These reactions were carried out in 25µL using 25 pmol of each primer and were cycled with a MJ Mini Gradient Thermal Cycler (Bio-Rad, Hercules, CA) as follows: denaturation at 94°C for 30 s; annealing at 58°C for 35 s; and extension at 72°C for 60 s. After 35 cycles, extension was continued at 72°C for an additional 10 min. The PCR products were visualized on a 2% agarose gel. Relative amounts of PCR products were estimated by band intensity and adjusted accordingly in subsequent PCR reactions (see below).

For B-cell HCDR3 PCR, genomic DNA (1 µg) or standardized cDNA (above) was amplified using a 6-FAM-labeled sense consensus framework region 3 (FR3) primer in conjunction with an antisense J-segment (JH) consensus primer.[25] These reactions were carried out as described for the IgM amplifications with the exception that annealing was carried out at 52°C and cycles were 45 s.

To determine VH3 family usage of B-cells post-antigenic challenge, cDNA was amplified using a sense VH3 framework region 1 (VH3FR1) primer in conjunction with an antisense IgG constant region primer (Cγ). These reactions differed from the FR3-JH amplifications in that annealing was done 65°C.

Cloning and sequencing of VH3-IgG fragments

Products of the VH3FR1-IgG amplification were used to make a cDNA library using PCR2.1 TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Briefly, 4 µL of PCR reaction were incubated with the vector and ligase under standard conditions. Chemically competent cells were then transformed with the PCR2.1 vector and insert, plated on ampicillin/X-Gal plates and resultant white colonies then grown out for analysis. Colonies were amplified with M13 primers to determine proper insert size and then submitted for sequencing. Immunoglobulin sequences were aligned using IMGT/V-QUEST and VH, JH and DH gene identities were determined. [26]

HCDR3 length analysis

The distribution of immunoglobulin HCDR3 lengths was visualized using the ABI 3130 Genetic Analyzer. The B-cell specific HCDR3 PCR products were separated by capillary electrophoresis. The fluorescence intensity of each band was quantified and used as a surrogate measure of abundance of each given HCDR3 length. A histogram was constructed displaying the HCDR3 fragments and the number of peaks was counted as a measure of B-cell repertoire diversity.

Lymphocyte subsets, serum immunoglobulin levels and antibody

The numbers and proportions of peripheral blood T-, B- and NK lymphocyte subsets were quantified using fluorescence activated cell sorting.. B-lymphocyte memory, and isotype switched memory B cells were determined as previously described.[27] Serum antibodies to vaccinations were also examined, and compared to laboratory reference protective levels. Antibody levels were expressed as immune status ratio (ISR), international units (IU) or index values according to the guidelines of the referring laboratory.

Immunization with Φ X174

Subject (II.5) was immunized intravenously with the T-cell dependent neoantigen bacteriophage Φ X174 at a dose of 2×10^9 PFU/kg body weight and boosted with a second dose 1 month later under an IRB-approved protocol. Phage neutralizing antibody titers expressed as the geometric mean of the rate of phage inactivation (K_v) were determined four weeks post-primary and at two and four weeks post-secondary immunization as previously described.[28] The one-month booster immunization facilitates the development of a secondary immune response characterized by amplification and isotype switching from IgM to IgG and determined as described. [28]

Statistical analysis

To determine the distribution of HCDR3 lengths, length of HCDR3 was plotted against the area under the curve for an estimation of relative amounts of HCDR3 per given length. These were then converted to continuous data and a nonlinear regression analysis was applied to determine the normality of the Gaussian distribution expected[29]. This transformation enabled comparison between HCDR3 spectratypes at different timepoints. Nonlinear regression analysis on HCDR3 size and area under the curve was performed to determine the normality of the distribution. Control samples were obtained from 4 patients and an oligoclonal human Burkitt's lymphoma cell line (Ramos) to determine the validity of the assay.

VH3 family gene usage was expressed as frequency distributions and compared to previous reports.

Results

WHIM family patient characteristics

An extended family with WHIM syndrome manifesting all of the characteristics of this disease was utilized in these studies.[2;9] Figure 1 shows the affected subjects in this three-generation pedigree. Table 1 shows the immunologic characteristics. All subjects for whom information was available had had leucopenia. Absolute numbers of T- and B-cells available for four subjects (II.5, II.7 III.1 and III.3) were reduced. Modest hypogammaglobulinemia was documented in 3 subjects (II.5, II.7 and III.1), affecting IgG and/or IgM. Phenotypically, all patients had cutaneous warts, but to varying degrees. Female patients reported cervical warts as well, with a single patient undergoing vulvectomy for dysplastic changes. Infections indicative of neutrophil and B-cell dysfunction were also reported: cellulitis, abscesses, sinusitis, and periodontal disease.

Peripheral lymphocyte populations

Peripheral lymphocyte populations from four subjects (II.5, II.7, III.1 and III.3) were examined (Table 2) for absolute numbers of T-helper ($CD3^+4^+$), T-suppressor ($CD3^+8^+$), natural killer cells ($CD3^-16^+56^+$) and B-cells ($CD19^+$). In addition, proportions of total memory B-cells ($CD19^+27^+$) and isotype switched memory B-cells ($CD19^+27^+IgM^-D^-$) were also determined. Nearly all the lymphocyte subsets were below the normal range. T-helper (4/4 subjects) and

T-suppressor (3/4 subjects) absolute numbers were markedly reduced while natural killer cells were reduced for two subjects (II.5, II.7). The B-cell population was reduced for all subjects. In terms of percent of the total B cell population, (CD27⁺) memory B-cells approached control levels in both subjects (II.5 27.98% of total B-cells; III.1 34.50% of total B-cells). However, isotype switched memory B-cells (CD27⁺ IgM⁻D⁻) were significantly reduced in subject II.5 (4.17% of B-cells) and subject III.1 (6.85% of B-cells, normal adults 18.5–29.9%).

Antibody to previous immunizations

Table 3 shows the serum antibody levels to several childhood immunizations for Subject II.5. The subject maintained protective vaccination titers to all protein immunogens tested: measles 2.71 ISR (>0.90 ISR), varicella 1.59 Index value (>0.90 Index value), rubella >5.00 Index value (>0.91 Index value), mumps 3.84 ISR (>0.90 ISR), diphtheria toxoid 0.17 IU/mL (>0.01 IU/mL) and tetanus toxoid 2.76 IU/mL (>0.15 IU/mL). Interestingly, polysaccharide vaccination titers, *i.e.*, pneumococcal vaccine titers, were not maintained. For antibodies to 12 pneumococcal antigen serotypes tested 11 had non protective titers (>1.3 µg/mL). However, optimal assessment of these titers would require re-immunization. Isohemagglutinin titers (anti-A and anti-B) for subject II.5 were maintained.

De novo antibody production after immunization with ΦX174

The results of these studies are depicted in Figure 2. Subject II.5 was immunized with bacteriophage ΦX174 at baseline followed by a booster immunization at one month and antibody titers (Kv) were determined. Prior to immunization, the subject did not have detectable phage neutralizing antibody titers. Following primary immunization, the subject showed a normal total Kv at 4 weeks. As expected, the majority of this antibody was of the IgM isotype. Following secondary immunization, a brisk rise in antibody titer occurred, but only 3% was of the IgG class, as compared to 47% (95% CI 23.4%–91.6%) IgG at this time point for normal controls. At ten weeks, the IgM antibody disappeared as expected but with lower levels of IgG (Kv=25.4, 91% IgG).

B-cell HCDR3 spectratyping

In order to examine the clonality of the B-cell repertoire of VH and CH regions in genomic DNA we examined the HCDR3 region. Using a fluorescent VHFR3 consensus primer and a consensus JH primer, all HCDR3s were amplified without respect to VH or CH gene families. Representative HCDR3 spectratype profiles shown for each affected family member in the pedigree demonstrate oligoclonality as evident by the relatively sparse number of peaks amplified from each (Figure 1). The polyclonal distribution of the HCDR3 lengths for a healthy control subject is shown for comparison.

HCDR3 spectratyping of peripheral B-cells before and after antigenic challenge

Spectratype analysis of HCDR3 in subject II.5 using cDNA from peripheral leukocytes was performed. To control for differences in B-cell number and transcriptional activity, cDNA was normalized using the constant region of IgM prior to HCDR3 PCR. The results of these studies are presented in Figure 3. Control wild type samples gave a normal Gaussian distribution of HCDR3 lengths, as expected. A Burkitt lymphoma cell line (Ramos) was used as a control for oligoclonality. Goodness-of-fit determinations for nonlinear regression analyses of Gaussian distributions showed R² values ranging from 0.80–0.91. Prior to antigenic challenge, the HCDR3 spectratype patterns were comparable in the observed degree of oligoclonality and similar to the patterns seen in the family gDNA analyses, with a small number of prominent peaks present. Nonlinear regression analyses showed R² values ranging from 0.06 to 0.48. After antigenic challenge, the curve of HCDR3 lengths begins to normalize. At one month post-immunization, there is a general increase in peak height and oligoclonal peaks

approximating a normal distribution. The distribution normalized by five months post-immunization, with an R^2 in the normal control range (R^2 0.86). At 7 months, R^2 values declined out of the normal range with diminished intensity of peaks corresponding to the center of the length distribution and relatively higher peak intensities at the periphery of the distribution (Figure 3). This trend was accentuated in the 14-month sample.

Somatic hypermutation status in subject II.5

In order to examine the diversity of the immunoglobulin heavy chain gene usage on the gene level and to look for post-germinal center characteristics, we amplified VH3-IgG-containing transcripts from peripheral blood samples (Table 4). The heavy chain variable region VH3 gene family is the largest in terms of gene members and hence most common gene family used in heavy chain VDJ rearrangements. This amplification strategy was chosen since isotype switched transcripts likely represent non-naïve B-cells. Total RNA was isolated from peripheral blood leukocytes collected after antigenic challenge and cDNA was amplified using consensus primers for the VH3 family (VH3FR1) and the constant region of IgG (C γ). The resulting PCR reaction was then cloned and plated to create a cDNA library. Fifty-seven clones were analyzed. The results of VH3-IgG sequencing are given in Table 3.

Somatic hypermutation was found in 55 of 57 (96%) clones analyzed. The DH segment showed the highest rate of somatic hypermutation with 107 nucleotide changes (53%), followed by JH with 61 (30%) and VH with 33 (16%). For VH3 family usage, the most common gene segments were 3–23 (25%) > 3–21 (16%) > 3–33 or 3–9 (12%). The frequency of 3–23 as the most common gene used in the VH3 family is similar to previous reports[30;31;32;33;34;35]. For DH family usage, the most common gene segments observed were 6–19 (18%) > 3–22 (12%) > 2–15 (11%). Although the frequencies of DH usage differed slightly from a previous report [36], the order of D-gene segments usage was similar. JH segments showed the following frequencies: 4 (60%) > 6 (16%) > 3 (12%). The frequency of JH gene segment use was consistent with previous reports on the adult B-lymphocyte repertoire[36].

Discussion

In the current studies, we used bacteriophage Φ X174 immunization and HCDR3 spectratyping to investigate primary and secondary immunization responses in WHIM syndrome. The family investigated here displayed many aspects of B-cell immunodeficiency characteristic of WHIM syndrome as indicated by clinical (e.g sinusitis, pneumonia, recurrent otitis media) and immunologic (e.g. mild hypogammaglobulinemia, reduced number of memory cells) parameters (Table 1).

Both WHIM subjects who were immunophenotyped had a circulating B-cell pool that contained a paucity of isotype switched memory B cells (Table 2), as occurs in other immune defects such as hyper IgM syndrome[37], common variable immune deficiency [38], and X-linked lymphoproliferative syndrome [39]. This finding suggests a lack of appropriate transit of B-cells into or within the germinal center leading to impaired isotype switching and B-cell maturation. Abnormal isotype switch kinetics were noted following immunization of subject II.5 with phage Φ X174. Following a normal primary response (99% IgM and normal Kv at one month) abnormal isotype switching was observed following secondary immunization (3.3% IgG versus 47% for control) (Figure 2). However, by 10 weeks after the secondary immunization, Kv was 25.4 and serum antibody was predominantly IgG (91%), both parameters falling within the normal range. The ability to eventually mount a good immune response to the neoantigen was consistent with data showing normal (IgG) antibody titers to measles, mumps and rubella (Table 3).

The clinical response of WHIM patients to IVIG despite normal immunoglobulin levels [40] suggests that antibody production is ineffective in these patients. We confirmed that despite relatively minimal deficiencies in IgG and IgM, subject II.5 displayed marked oligoclonality by spectratyping analysis of peripheral blood samples done at the level of genomic DNA and cDNA (Figures 1 and 3), supporting the role of humoral immune deficiency in the susceptibility to infection, regardless of the abundance of circulating immunoglobulins. We also assessed the effect of a significant antigen exposure on the B-cell repertoire in subject II.5 before and after phage immunization. While it is not possible to determine the mechanism for the striking normalization of the repertoire observed following immunization with Φ X174, it is possible that this represents mobilization of B-lymphocytes (naïve and non-naïve) from the bone marrow following antigen challenge. However, this presumptive mobilization is ineffective in generating a normal secondary immune response as evident by the defect in the kinetics of isotype (Figure 2).

While the immunospectratyping data suggested an improved antibody repertoire, the delayed switch response to an antigenic challenge suggested persistent dysfunction in the germinal center microenvironment. To examine whether the mature B-lymphocyte population had post-germinal center characteristics (i.e., somatic hypermutation), we constructed a cDNA library of VH3-IgG transcripts. Following phage immunization, we found normal somatic hypermutation in the HCDR3 segments in the WHIM subject studied, a finding that is consistent with the presence of a small post-germinal center population. These data are also consistent with previous reports on WHIM syndrome regarding somatic hypermutation status. [13]

Hypogammaglobulinemia, reduced memory B-cell populations and oligoclonal antibody repertoire are all suggestive of perturbations of the germinal center environment in WHIM syndrome. Previous studies on inguinal lymph node biopsies following DT immunization in two WHIM subjects showed absent to poorly formed lymphoid follicles and reduced numbers of plasma cells.[11] Proper trafficking of B-cells is essential in order to populate germinal centers in the follicles of peripheral lymphoid organs such as the spleen, lymph nodes, Peyer's patches of the gut, and the tonsils. Naïve B-cells initially enter peripheral lymphoid organs and interact with T-cells and dendritic cells and proliferate in germinal centers upon antigenic activation. The germinal center dark and light zones provide a microenvironment for B-cell affinity maturation (Figure 4). B-cells proliferate and diversify their antigen receptors by somatic hypermutation in the former and selection of B-cells with high affinity antibodies, isotype switching and differentiation into memory cells and plasma cells occurs in the latter. [41] In addition to molecules involved in adhesion (e.g. E-selectin) and survival regulation (e.g. Bcl-2), long-lived memory plasma cells also require CXCR4 to enter survival niches. [41]

Trafficking of B-lymphocytes within peripheral lymphoid organs upon B-cell activation and differentiation is influenced by responsiveness to CXCL12.[42;43] CXCR4-expressing centroblasts are competent to signal in response to CXCL12 expressed by stromal cells in the dark zone.[44] Subsequent migration of proliferating centroblasts from the dark zone into the light zone for B-cell maturation is facilitated by the downregulation of CXCR4 and upregulation of CXCR5.[44] After affinity maturation and class switching in the germinal center, memory B-cell and plasmablasts regain CXCL12 responsiveness and downregulate chemotactic responsiveness to B-cell lymph node zones.[45;46] This chemotactic change is critical for the homing of antibody secreting cells to their niches (e.g. red pulp of the spleen, bone marrow). The critical role of CXCR4 in germinal center organization is further supported by studies in chimeric mice reconstituted with CXCR4^{-/-}. Fetal livers in these animals displayed normal numbers of germinal center B-lymphocytes but the germinal centers were disorganized, with loss of distinct dark zone and light zone segregation.[44] Tissue-specific

ablation of CXCR4 murine B-cells also resulted in mislocalization as engineered cells migrated prematurely from the bone marrow and localized in splenic follicles and in ectopic follicles in the lamina propria of the gut.[47] Interestingly, despite lacking CXCR4, these mice generated significant numbers of long-lived plasma cells.

To account for the body of data suggesting significant B-cell functional deficiencies in WHIM syndrome, we speculate that, as in the case of the neutrophil lineage, B-cells fail to emigrate appropriately to their proper developmental niche. By analogy to the situation in neutrophils, the local chemotactic milieu could override the effect of the mutant CXCR4 to permit appropriate migration, similar to the effects of epinephrine and glucocorticoids on bone marrow leukocyte release in WHIM patients.[6;11] At baseline, this process would likely be inefficient and lead to ineffective production of protective antibody. One potential model for such impaired trafficking is shown in Figure 4, with activated B-cells that to emigrate to follicles trapped in the dark zone where they may undergo apoptosis. It would be of interest to examine follicles in a WHIM animal model to determine the rate of B-cell apoptosis and the ability of the cells to transition between dark and light zones. This dark to light zone transition may be developmentally regulated as most WHIM patients maintain their titers to childhood immunizations. Subject II.5 showed protective titers to measles, mumps, rubella, diphtheria and tetanus (Table 3). In addition, subject III.1, the 9 year-old daughter of subject II.5, had normal levels of IgG and IgA (Table 1) as well as higher levels memory B-lymphocytes (Table 2). Her total memory B-lymphocyte population (CD27⁺CD19⁺) was within normal range when compared to controls. However, like her father II.5, there was a deficiency of isotype switched cells (Table 2).

In conclusion, the studies presented here confirm that significant abnormalities in peripheral antibody repertoire as assessed by immunospectratyping can occur in the setting of relatively normal immunoglobulin levels and demonstrate abnormal kinetics of isotype switching following neoantigen challenge. We suggest that activating and truncating mutations in WHIM syndrome may lead to altered B-lymphocyte trafficking, analogous to the myelokathexis defect in neutrophils. Such a defect might lead to an impaired capacity for isotype switching and B-cell maturation, potentially by disturbing the trafficking of B-cells out of the bone marrow and through the germinal center microenvironment.

Abbreviations

WHIM	Warts, hypogammaglobulinemia, immunodeficiency, myelokathexis
HCDR3	Heavy chain complementarity determining region 3
VH3FR1	Framework region 1 of the immunoglobulin heavy chain variable region family 3

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Figure 1.

CDR3 gDNA spectratyping of a three-generation WHIM pedigree. A) Updated pedigree of a previously reported WHIM family. B) Spectratyping of HCDR3 was performed using a fluorescently labeled consensus VH framework 3 (VHFR3) and a consensus JH primers. PCR products were then separated and visualized by capillary gel electrophoresis (ABI 3130 Genetic Analyzer).

Figure 2.

Titers following immunization with bacteriophage Φ X174. A subject with WHIM syndrome (II.5) was immunized with bacteriophage Φ X174 at time 0 months followed by a booster at 1 month and the rate of phage inactivation and immunoglobulin isotype were determined. Boxed numbers indicate %IgG at each timepoint.

Figure 3.

HCDR3 analysis pre- and post-immunization with bacteriophage Φ X174. Spectratyping data are shown for four control subjects without inflammatory conditions and an oligoclonal Ramos cell line (top) with the raw data displayed as HCDR3 length plotted against fluorescence intensity and transformation of data into continuous variables with HCDR3 length plotted against the area under the curve shown above raw data. Non-linear regression analyses of the normality of the distribution were performed to enable comparisons across timepoints. For the WHIM subject (bottom), baseline spectratyping data were collected prior to immunization with bacteriophage Φ X174. Follow-up samples were obtained at 1, 5, 7, and 14 months post-challenge. Note that the distribution of peaks shifts markedly from an oligoclonal pattern to a more normal distribution over the time interval studied.

Figure 4.

Summary slide postulating germinal center events in normals versus WHIM. Normal B-cells after antigenic stimulation enter the dark zone of the germinal center and proliferate and undergo somatic hypermutation. Cells then downregulate CXCR4 to transition to the light zone for isotype switching and differentiation. In this model, WHIM B-lymphocytes transition to the light zone in severely reduced numbers due to their inability to downregulate CXCR4.

Table 1

Laboratory values for WHIM subjects.

Subject	WBCx 10 ³ /uL (norm)	ABST cells/uL (norm)	ABSB cells/uL (norm)	Immunoglobulins mg/dL (norm)	Phenotype
I.1	NA	NA	NA	NA	Warts, vaginal warts, pneumonia, pansinusitis
II.4	1.1 (5–11)	NA	NA	NA	Warts, pneumonia, sinusitis, abscesses, periodontal disease, B-cell lymphoma with recurrence
II.5	11 (5–11)	287 (750–2500)	9 (75–375)	IgM 35(38–206)	Warts, pneumonia, pleurisy, cellulitis, otitis media, periodontal disease
II.7	2.2 (5–11)	701 (750–2500)	40 (75–375)	IgG 735(768–1728) IgA 186(99–396) Low	Warts, vaginal warts, pneumonia, sinusitis, otitis media, cellulitis, periodontal disease
III.1	0.7 (5–11)	427 (750–2500)	23 (75–375)	IgM 30(60–300) IgG 826(700–1500) IgA 224(60–400)	Warts, cellulitis, otitis media
III.3	1.2 (5–11)	437 (876–3394)	41 (200–1259)	IgM Normal IgG Low IgA Normal	Warts, cellulitis

Table 2

Analysis of T and B-cell populations in four subjects with WHIM syndrome.

Subject	CD3 ⁺ 4 ⁺ (cells/mm ³)	CD3 ⁺ 8 ⁺ (cells/mm ³)	CD 3 ⁺ 16 ⁺ 56 ⁺ (cells/mm ³)	CD19 ⁺ (cells/mm ³)	CD27 ⁺ IgD ⁺ B ⁺ cells (%)	CD27 ⁺ CD19 ⁺ (%)
I.1	NA	NA	NA	NA	NA	NA
II.4	NA	NA	NA	NA	NA	NA
II.5	173 (480–1700)	116 (180–1000)	72 (135–525)	9 (75–375)	4.17 (18.19–31.47)	27.98 (28.12–67.97)
II.7	331 (480–1700)	381 (180–1000)	98 (135–525)	40 (75–375)	NA	NA
III.1	275 (480–1700)	111 (180–1000)	249 (135–525)	23 (75–375)	6.85 (18.19–31.47)	34.50 (28.12–67.97)
III.3	219 (412–2095)	179 (236–995)	190 (135–525)	41 (200–1259)	NA	NA

Table 3

Serum antibody titers to childhood vaccinations in subject II.5.

	Value	Range
MeaslesAB (IgG)	2.71	0.00–0.90 ISR
Varicella-Zoster AB (IgG)	1.59	0.00–0.90 Index value
Rubella AB(IgG)	>5.0	<0.91 Index value
Mumps AB (IgG)	3.84	0.00–0.90 ISR
Diphtheria antitoxoid AB	0.17	>0.01 IU/mL
Tetanus antitoxoid	2.76	>0.15 IU/mL
Pneumo IgG (all subtypes)	<2.0µg/mL	>2.0µg/mL
<i>Isohemagglutinins: (blood Type O)</i>		
AntiA(IgM) 1:32	1:32	≥1:8
Anti A (IgG) 1:128	1:128	≥1:8
Anti B(IgM) 1:8	1:8	≥1:8
Anti B(IgG) 1:16	1:16	≥1:8

Table 4

Mutation status for VH, DH and JH family members in subject II.5.

	VH gene	Clones	DH gene	Clones	JH gene	Clones
	3-23	14(25%)	6-19	10(18%)	4	34 (60%)
	3-21	9(16%)	3-22	7(12%)	6	9(16%)
	3-33	7(12%)	2-15	6(11%)	3	3 (12%)
	3-9	7(12%)				
Mutations	33(16%)		107 (53%)		61 (30%)	