Published in final edited form as:
J Allergy Clin Immunol. 2014 March ; 133(3): 896–899.e4. doi:10.1016/j.jaci.2013.08.050.

Molecular and phenotypic abnormalities of B lymphocytes in patients with Wiskott-Aldrich syndrome

Karen L. Simon, MS, **Stacie Anderson, BS**, **Elizabeth K. Garabedian, RN, MSLS**, **Daniele Moratto, PhD***, **Robert A. Sokolic, MD**, and **Fabio Candotti, MD**
Genetics and Molecular Biology Branch, National Human Genome Research Institute, national Institutes of Health, Bethesda, MD, 20833, USA

Keywords

Wiskott-Aldrich syndrome; B-lymphocytes; humoral immunity; antibody; autoimmunity

To the Editor

Wiskott-Aldrich Syndrome (WAS) is an inherited immunodeficiency characterized by recurrent infections, thrombocytopenia, eczema, and high risk of lymphoid malignancy and autoimmune diseases. Mutations in the *WAS* gene are responsible for the disease and result in defective expression of the WAS protein (WASp) (1). Autoimmune complications are frequent in WAS and their pathophysiological bases remain unclear. Defective regulatory T-cell function and reduced CD4+ T-cell apoptosis have been associated with the development of autoimmunity in WAS patients and mouse models of the disease (2). Impaired B-cell tolerance has also been observed in humans with WAS (3) and detailed studies in mice have correlated the generation of anti-nuclear and tissue-specific autoantibodies with intrinsic defects of WASp-deficient B lymphocytes (4, 5). Previous clinical reports on the composition of the B-cell compartment in WAS patients have described diminished B-cell numbers and phenotype abnormalities, including reduced frequency of CD21/CD35-expressing B-cells and CD27+ memory B-cells (6). To further investigate possible defects of B cell differentiation in WAS and their association with autoimmunity, we set out to perform phenotypical and molecular studies of B lymphocyte subpopulations in a cohort of 18 WAS patients.

Under procedures approved by the Institutional Review Board of the National Human Genome Research Institute, peripheral blood samples were obtained from nine adult and nine pediatric subjects with WAS (Supplementary Table 1), and matched healthy controls. CD19+CD21loCD38+ transitional, CD19+CD21hiCD27− naïve, and CD19+CD21loCD27+ memory B-cells, as well as CD19hiCD21lo B lymphocytes were enumerated and isolated by cell sorting.

Four pediatric WAS patients showed total B-cell counts below the normal age range, whereas all adult WAS subjects showed normal total B-cell numbers (Supplementary Table 1). No significant differences were observed between the percentages of transitional, naïve, or memory B-cells detected in pediatric WAS patients and controls. Adult WAS subjects, however, showed a significant reduction of memory B-cell populations. This reduction was

Correspondence to: Fabio Candotti, MD, Head, Disorders of Immunity Section, Genetics and Molecular Biology Branch, National Human Genome Research Institute, National Institutes of Health, 49 Convent Dr., Blg. 49, Rm 3A04, Bethesda, MD 20892-4442, Tel: (301) 435 2944 - (301) 402 2347, Fax: (301) 480 3678, fabio@nhgri.nih.gov.
*Current address:

more pronounced in WAS adult patients with autoimmunity. Comparison of memory B-cell population prevalence in adult WAS patients with (n=6) and without autoimmunity (n=3) did not indicate significant differences (p=0.82, data not shown). Both pediatric and adult WAS subjects showed significantly increased percentages of CD19hiCD21lo B lymphocytes (Table 1A–B).

Analysis of somatic hypermutation (SHM) was performed on transcripts of the human immunoglobulin heavy chain (IGH) VH3 and VH4 gene families isolated from sorted B-cell subsets. For memory B-cells, comparison to germline IGH sequence demonstrated significantly reduced mutation frequency of Cγ transcripts from VH3 and VH4 genes in WAS adults and from VH3 genes in pediatric WAS patients compared to controls (Table 2A–B).

As noted in patients with Common Variable Immunodeficiency (7), the frequency of SHM in CD19hiCD21lo B-lymphocytes was similar to that of naïve B-cells both in WAS patients and controls (data not shown).

Analysis of the RGYW, WRCY, AGCT, WA, TW and TA hypermutable motifs in the IGH sequences did not evidence significant differences between mutations detected in WAS patients and controls. We also did not detect significant differences in the numbers of G/C transversions, G>A and C>T transitions in sequences isolated from WAS patients and controls.

We compared the IGH variable region gene repertoire in WAS patients and controls by analysis of Cμ and Cγ VH3 and VH4 gene expression. Transitional B-cells from adult and pediatric patients each showed significantly increased usage of two out of the 17 Cμ VH gene families analyzed. Interestingly, the overrepresented families included mostly uncommonly used VH genes (i.e. VH3-15, VH3-33, VH4-61) and families frequently found in autoantibodies (i.e. VH4-34, VH4-61) (8). Naïve B-cells also showed increased usage of two uncommon Cμ VH families in adult WAS patients (VH3-33, VH4-31) and of the VH4-61 gene family in children with WAS. Memory B-cells from adult WAS patients showed significantly increased usage of three Cγ VH gene families (among which were the uncommon VH3-15 and VH4-31 genes).

CD19hiCD21lo B-lymphocytes from adult WAS patients showed statistically increased usage of three Cμ VH gene families. Among these were the VH3-23 and VH4-34 genes frequently found in anti-DNA human autoantibodies. The commonly used VH4-59 gene was overrepresented in CD19hiCD21lo B-cells from both adult and pediatric WAS patients (Supplementary Figure 1; Supplementary Table 2). No clear differences were observed in the VH3 and VH4 gene family usage in B-cells from adult WAS patients with autoimmunity compared to the whole adult WAS cohort (data not shown).

Defects of humoral immunity in WAS are yet to be completely defined. The findings in our series of patients confirm that CD27+ memory B-cells can be reduced in this disease, which can contribute to the immunodeficiency of WAS, although we did not make such observation in patients of younger age. The significant reduction of SHM frequency points to maturation defects in WASp-deficient developing B-cells and may result in reduced antibody diversification. The mechanism underlying these abnormalities is unknown, however, the absence of preferential mutation patterns within hypermutable motifs does not support a specific role of WASp in the mutational machinery.

Both adult and pediatric WAS patients showed significantly increased CD19hiCD21lo B-lymphocyte populations. These cells have been characterized as a naïve-like, partially auto-reactive B-cell subset unable to enter the germinal center reaction (7, 9). While their

developmental pathway remains to be determined, it is tempting to speculate a relationship between their expansion, the splenic germinal center defects, and the low levels of CD27+ memory B-cells observed in WAS. Expansions of CD19hiCD21lo B-cells have been associated with chronic inflammation and autoimmunity (10, 11). However, whether these cells have any pathogenic role in WAS remains unclear and deserves further investigation. Most B-cell populations isolated from WAS patients showed increased usage of uncommon VH gene segments, as well as VH families frequently encoding autoantibodies. As for the other B-cell phenotypic and molecular abnormalities observed in our studies, the biased VH gene usage was more pronounced in adult WAS patients than in pediatric subjects, thus suggesting an age-dependent deterioration of B-cell differentiation and homeostasis in WAS that may predispose affected patients to increased susceptibility to infection and onset of autoimmune complications.

Methods

Cell Sorting and RNA extraction

Upon informed consent, peripheral blood was obtained from WAS patients and healthy controls. Lymphocytes were separated from peripheral blood using standard protocols and sorted into four subpopulations using the following antibodies: CD19 anti-human PerCP-Cy5.5, CD21 anti-human APC, CD38 anti-human PE, and CD27 anti-human FITC (BD Biosciences, San Jose, California). RNA was isolated from sorted cells using the Picopure RNA Isolation kit (Molecular Devices, Sunnyvale, CA). On average, RNA was isolated from 8,300 CD19+CD21loCD38+ transitional B-cells, 153,000 CD19+CD21hiCD27− naïve B-cells, 117,000 CD19+CD21loCD27+ memory B-cells, and 36,000 CD19hiCD21lo B lymphocytes

Immunoglobulin variable region gene analysis

cDNA was synthesized from RNA using oligo d(T) and the SuperScript III First Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). PCR amplification was performed as described (12). Amplicons were cloned into the TOPO-TA vector (Invitrogen, Carlsbad, CA) and used to transform DH5α cells. A minimum of 10 colonies were picked and PCR was performed using M13 Forward and reverse primers under the following conditions: 95C 5′ [95C 30″, 55C 45″, 72C 45″] × 30 cycles, followed by 72C 5″. Inserts from multiple independent clones were sequenced and mutations were identified by comparison to the germline sequence using Joinsolver (http://joinsolver.niaid.nih.gov/).

Statistical Analysis

Comparisons of VH gene family usages were performed using the Fisher's exact test. The student's t test was used for analyses of sequence homology data after arcsine transformation of percentage values.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Ms. Nisha Basappa for excellent technical help in the generation of data presented in this manuscript.

Funding source: This work was supported by the Intramural Research Program of the National Human Genome Research Institute, National Institutes of Health.

References

1. Albert MH, Notarangelo LD, Ochs HD. Clinical spectrum, pathophysiology and treatment of the Wiskott-Aldrich syndrome. Curr Opin Hematol. 2011; 18(1):42–8.
2. Catucci M, Castiello MC, Pala F, Bosticardo M, Villa A. Autoimmunity in wiskott-Aldrich syndrome: an unsolved enigma. Front Immunol. 2012; 3(209):1–14. [PubMed: 22679445]
3. Dupuis-Girod S, Medioni J, Haddad E, Quartier P, Cavazzana-Calvo M, Le Deist F, et al. Autoimmunity in Wiskott-Aldrich syndrome: risk factors, clinical features, and outcome in a single-center cohort of 55 patients. Pediatrics. 2003 May; 111(5 Pt 1):e622–7. [PubMed: 12728121]
4. Becker-Herman S, Meyer-Bahlburg A, Schwartz MA, Jackson SW, Hudkins KL, Liu C, et al. WASp-deficient B cells play a critical, cell-intrinsic role in triggering autoimmunity. J Exp Med. 2011 Sep 26; 208(10):2033–42. [PubMed: 21875954]
5. Recher M, Burns SO, de la Fuente MA, Volpi S, Dahlberg C, Walter JE, et al. B cell-intrinsic deficiency of the Wiskott-Aldrich syndrome protein (WASp) causes severe abnormalities of the peripheral B-cell compartment in mice. Blood. 2012 Mar 22; 119(12):2819–28. [PubMed: 22302739]
6. Park JY, Shcherbina A, Rosen FS, Prodeus AP, Remold-O'Donnell E. Phenotypic perturbation of B cells in the Wiskott-Aldrich syndrome. Clin Exp Immunol. 2005 Feb; 139(2):297–305. [PubMed: 15654828]
7. Rakhmanov M, Keller B, Gutenberger S, Foerster C, Hoenig M, Driessen G, et al. Circulating CD21low B cells in common variable immunodeficiency resemble tissue homing, innate-like B cells. Proc Natl Acad Sci U S A. 2009 Aug 11; 106(32):13451–6. [PubMed: 19666505]
8. Dorner T, Lipsky PE. Molecular basis of immunoglobulin variable region gene usage in systemic autoimmunity. Clin Exp Med. 2005 Mar; 4(4):159–69. [PubMed: 15750762]
9. Vlkova M, Fronkova E, Kanderova V, Janda A, Ruzickova S, Litzman J, et al. Characterization of lymphocyte subsets in patients with common variable immunodeficiency reveals subsets of naive human B cells marked by CD24 expression. J Immunol. 2010 Dec 1; 185(11):6431–8. [PubMed: 21041728]
10. Warnatz K, Denz A, Drager R, Braun M, Groth C, Wolff-Vorbeck G, et al. Severe deficiency of switched memory B cells (CD27(+)IgM(−)IgD(−)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood. 2002 Mar 1; 99(5): 1544–51. [PubMed: 11861266]
11. Wehr C, Eibel H, Masilamani M, Illges H, Schlesier M, Peter HH, et al. A new CD21low B cell population in the peripheral blood of patients with SLE. Clin Immunol.
12. Wang X, Stollar BD. Human immunoglobulin variable region gene analysis by single cell RT-PCR. J Immunol Methods. 2000 Oct 20; 244(1–2):217–25. [PubMed: 11033034]

Table 1A

Comparison of B-cell subsets between WAS patients and healthy controls.

		WAS patients	Controls	p-value
ADULTS Patients: n=9 Controls: n=9	CD21loCD38+ (%)	5.62±3.1	2.29±1.7	NS
	CD21hiCD27− (%)	59.32±14.8	51.40±11.8	NS
	CD21hiCD27+ (%)	15.09±9.3	25.73±10.6	0.0331
	CD19hiCD21lo (%)	16.87±10.3	8.43±5.8	0.0329
CHILDREN Patients: n=9 Controls: n=3	CD21loCD38+ (%)	5.70±3.2	4.53±3.1	NS
	CD21hiCD27− (%)	52.40±9.8	43.33±37.7	NS
	CD21hiCD27+ (%)	13.25±7.8	18.67±13.3	NS
	CD19hiCD21lo (%)	17.59±9.6	4.17±1.4	0.0373

Table 1B

Comparison of B-cell subsets between WAS adult patients with autoimmunity and healthy controls.

		WAS patients	Controls	p-value
Patients: n=6 Controls: n=9	CD21loCD38+ (%)	3.08±1.4	2.29±1.7	NS
	CD21hiCD27− (%)	63.43±13.8	51.40±11.8	NS
	CD21hiCD27+ (%)	11.03±5.9	25.73±10.6	0.0058
	CD19hiCD21lo (%)	16.43±10.3	8.43±5.8	0.0424

Table 2A

Comparison of somatic hypermutation frequency in WAS patients and controls

	Transcript	Homology (%)			Mutation frequency (%)			Unmutated sequences (%)		
		WAS	Ctrl	p-value	WAS	Ctrl	p-value	WAS	Ctrl	p-value
Adult CD21hiCD27+	Cγ VH3	94.35 ± 2.78	93.21 ± 2.16	0.0009	4.94 ± 2.87	6.31 ± 2.75	0.0002	13.98 ± 22.37	4.01 ± 8.68	NS
	Cγ VH4	94.09 ± 2.85	92.77 ± 2.66	0.0009	5.26 ± 2.18	6.82 ± 2.55	0.0001	20.67 ± 22.36	0.617 ± 1.85	0.0149
Children CD21hiCD27+	Cγ VH3	95.65 ± 2.97	94.00 ± 1.53	0.0007	3.51 ± 2.01	4.90 ± 1.78	0.0011	37.01 ± 28.89	0.00	0.0303
	Cγ VH4	94.63 ± 2.97	94.83 ± 2.37	NS	4.47 ± 2.69	4.76 ± 2.22	NS	24.57 ± 15.10	6.06 ± 10.49	0.0190

Table 2B

Comparison of somatic hypermutation frequency in WAS adult patients with autoimmunity and controls

	Transcript	Homology (%)			Mutation frequency (%)			Unmutated sequences (%)		
		WAS	Ctrl	p-value	WAS	Ctrl	p-value	WAS	Ctrl	p-value
CD21hiCD27+	Cγ VH3	93.97 ± 2.51	93.21 ± 2.16	0.0342	5.26 ± 2.48	6.31 ± 2.75	0.0099	4.84 ± 8.26	4.01 ± 8.68	NS
	Cγ VH4	94.28 ± 2.95	92.77 ± 2.66	0.0006	5.01 ± 2.24	6.82 ± 2.55	0.0001	26.01 ± 24.03	0.617 ± 1.85	0.0052

WAS: Wiskott-Aldrich syndrome patients (adults, n=9; children, n=9); Ctrl: Healthy control subjects (adults, n=9; children, n=3).