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IgA and IgG hypogammaglobulinemia in Waldenström's macroglobulinemia

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ABSTRACT

Background

Hypogammaglobulinemia is common in Waldenström's macroglobulinemia (WM), and has been attributed to disease-related suppression. The etiology for this finding remains unclear, and has been speculated to be on the basis of tumor-induced suppression.

Design and Methods

We evaluated the incidence of IgA and IgG hypogammaglobulinemia in 207 untreated WM patients and addressed the associated clinicopathological findings, and impact of therapy. We also sequenced 8 genes (*AICDA; BTK; CD40; CD154; NEMO, TACI, SH2D1A, UNG*) implicated in immunoglobulin deficiency in 19 WM patients with IgA and/or IgG hypogammaglobulinemia.

Results

At baseline 63.3%, 58.0% and 49.3% of the 207 patients had abnormally low serum levels of IgA, IgG, or both. No association between IgA and IgG hypogammaglobulinemia and disease burden, serum IgM levels, B2M, IPSS score, or incidence of recurrent infections was observed, though presence of adenopathy and/or splenomegaly was associated with a lower incidence of hypogammaglobulinemia. Lower IgA and IgG levels were associated with disease progression in watch and wait patients. IgA and/or IgG levels remained abnormally low despite response to treatment, including complete remissions. A missense mutation in the highly conserved catalytic site of UNG was observed in a patient with hypogammaglobulinemia warranting further study of this pathway in WM.

Conclusions

IgA and IgG hypogammaglobulinemia is common in WM which persists despite therapeutic intervention and response. IgA and IgG hypogammaglobulinemia do not predict for recurrent infection risk in WM patients, though lower levels of serum IgA and IgG are associated with disease progression in WM patients on watch and wait.

Key words: hypogammaglobulinemia, Waldenstrom's macroglobulinemia, serum IgM levels.

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Introduction

Waldenström's macroglobulinemia (WM) is a distinct B-cell lymphoproliferative disorder characterized primarily by bone marrow infiltration with lymphoplasmacytic cells, along with demonstration of an IgM monoclonal gammopathy.[1] This condition is considered to be lymphoplasmacytic lymphoma as defined by the REAL and WHO classification systems.[2,3] Up to 20% of patients with WM appear to have a first degree relative with WM or related B-cell disorder suggesting a possible genetic predisposition to this rare disease.[4]

Recurrent infections, particularly involving the respiratory tract are commonly observed among patients with WM, and may be related to the presence of IgA and IgG hypogammaglobulinemia.[5] The presence of hypogammaglobulinemia involving the *uninvolved* immunoglobulin has also been reported among other B-cell malignancies.[6,7] The etiology for this finding remains unclear, and has been speculated to be on the basis of tumor-induced immunoparesis and host-mediated homeostatic regulation of *uninvolved* immunoglobulin production.

Little is also known about the impact of IgA and IgG immunoglobulin levels on infection risk in WM patients, who often present with respiratory tract infections,4 as well as the evolution IgA and IgG levels over the course of disease and following therapy in WM. As such, we evaluated the incidence of IgA and IgG hypogammaglobulinemia in 207 untreated WM patients and addressed the associated clinicopathological findings as well as disease course on IgA and IgG hypogammaglobulinemia. Additionally, we evaluated the impact of therapy, as well as clinical remission status on IgA and IgG immunoglobulin levels on 93 serially treated patients with WM.

Lastly, as part of these studies, we sequenced 8 genes often implicated in immunoglobulin deficiency disorders such as common variable immunodeficiency disorder (CVID), Hyper IGM syndrome (HIGM), and X-linked agammaglobulinemia (XLA) in 19 patients with WM who demonstrated IgA and/or IgG deficiency.[8–12]

Design and Methods

To analyze the incidence of hypogammaglobulinemia in WM, serum immunoglobulin levels calculated by immunofixation were serially gathered from 207 previously untreated patients at their first clinic visit. This study was conducted in accordance with the Dana-Farber/Harvard Cancer Center institutional review board. All participants met the consensus panel definition for WM1 and were considered to have hypogammaglobulinemia if their uninvolved immunoglobulin levels were below the lower limit of our institutional normal range (i.e. <700 mg/dL and <70 mg/dL for IgG and IgA, respectively). As part of this analysis, we assessed the impact of patient age, sex, bone marrow disease infiltration with lymphoplasmacytic cells, adenopathy, splenomegaly, serum IgM levels as determined by immunonephelometry, complete blood counts, absolute lymphocyte count, β2-microglobulin, and prognostic score as assessed by the WM International Prognostic Scoring System[13] on presence or absence of IgA and IgG hypogammaglobulinemia. The impact of IgA and IgG hypogammaglobulinemia on the risk of recurring infections, defined as an infection occurring more than once in a year, was also assessed.

To delineate the impact of disease progression on uninvolved immunoglobulin levels over time, we followed the IgA and IgG levels in 102/207 patients who were placed in observation at their first visit, and who continued to have follow-up at our Institution. This included 60 patients who remained progression free, and 42 patients who eventually progressed during our follow-up period. To understand the impact of therapeutic intervention on uninvolved immunoglobulin levels, we also analyzed changes in IgA and IgG levels in a separate cohort of 93 patients who underwent treatment for WM and had immunoglobulin levels tested pre-therapy, post-therapy, and at least one month follow up after completion of all therapy. Outcomes for these patients were determined using consensus panel response criteria from the Third International Workshop on WM.[14]

Sequencing of CVID associated genes

DNA from peripheral blood mononuclear cells was obtained from 19 WM patients who demonstrated IgA and/or IgG hypogammaglobulinemia. Sequence analysis of the promoter, all exonic, and flanking intronic regions was performed for AICDA; BTK; CD40; CD154; NEMO, TACI, SH2D1A, UNG which are associated with the immunoglobulin deficiency disorders CVID, HIGM, and XLA. All sequencing studies were performed by Correlagen Diagnostics Inc. (Waltham, Massachusetts, USA) using CLIA approved diagnostic assays.

Biostatistics

Analysis of clinical data was performed by logistic regression analysis, ANOVA, and Spearman Rank Correlation analysis. All analyses were performed using SAS (SAS Institute, Cary NC, USA). Non-parametric testing was performed using a two-tailed Fisher's exact probability test (VassarStats). A p-value <0.05 was deemed to be significant in all studies.

Results

IgA and IgG hypogammaglobulinemia in WM

We first analyzed the prevalence of IgA and IgG hypogammaglobulinemia in newly diagnosed patients with WM. The median age of these patients was 60 (range 32–83 years) and the male to female ratio was 1.4. The median IgM was 2,910 (range 179–12,400 mg/dL) and the median bone marrow involvement with disease was 40% (range 5–95%). Of these patients, 131 (63.3%) and 120 (58.0%) patients

demonstrated decreased serum IgA and IgG levels respectively, while 102 (49.3%) of these patients were abnormally low for both. The median serum IgA and IgG levels for this cohort were 50 (range 7-597 mg/dL) and 635 (range 127–3,130 mg/dL), respectively. No correlation was found between bone marrow infiltration and uninvolved immunoglobulin levels (p=0.351 and p=0.141 for IgG and IgA respectively). Additionally, age, sex, serum IgM levels, white blood count, hematocrit, platelet count, absolute lymphocyte count, β2-microglobulin, or the WM International Prognostic Scoring System13 score had no impact on the odds ratio of having IgA or IgG, or both IgA or IgG hypogammaglobulinemia by logistic regression analysis (*data not shown*). Interestingly, a higher incidence of adenopathy was observed among those patients who presented with normal range IgA (13.16%) and IgG (11.63%), versus those patients presenting with IgA (5.04%) and IgG (4.65%) hypogammaglobulinemia (p=0.03; p=0.08, respectively). Similarly, the incidence of splenomegaly was higher in those patients who presented with normal range IgA (11.84%) and IgG (11.63%), versus those patients who presented with IgA (3.88%) and IgG (3.36%) hypogammaglobulinemia (p=0.02; p=0.03, respectively).

Impact of IgA and IgG hypogammaglobulinemia on infection risk in WM

We next assessed the impact of presenting with IgA and IgG hypogammaglobulinemia on recurrent infection risk for 205 of the 207 WM patients for whom this data was available. The presence of IgA, IgG or both IgA and IgG hypogammaglobulinemia, even at lower cutoffs (i.e. IgA <30; IgG <300 mg/dL) did not predict for the occurrence of recurring infections, which were nearly all respiratory in nature, mainly <grade 2, and consisted of sinus (n=53; 25.85%), bronchial (n=16; 7.80%), unspecified upper respiratory tract (n=14; 6.83%), and pneumonic (n=7; 3.41%) infections (Table 1).

Table 1. Impact of IgA and IgG hypogammaglobulinemia on recurring infection risk in patients with WM.

mg/dL	With recurring infections (n=75)	Without recurring infections (n=130)	p=
IgA <70	52	77	0.1492
IgA <30	21	25	0.1473
IgG <700	45	74	0.6714
IgG <300	10	11	0.2674
IgA <70, IgG <700	41	60	0.2401
IgA <30, IgG <300	9	7	0.0891
Median IgA	51 (range 9-266)	51 (range 7-597)	0.3536
Median IgG	658 (range 127-1450)	642 (range 175-3130)	0.7002

Table 2. Median baseline characteristics for 93 WM patients whose immunoglobulin changes were determined pre- and post-therapy.

Therapy	N=	Age (years)	IgM (mg/dL)	BM (%)	Prior Therapies
Rituximab	3	77	1,890	20	1
Fludarabine/Rituximab	19	58	3,730	40	0
CHOP/Rituximab	8	54	4,655	55	1
Thalidomide/Rituximab	14	62	3,500	35	0
Lenalidomide/Rituximab	10	66	3,625	55	0
Bortezomib	7	63	5,540	45	1
Bortezomib/Dex/Rituximab	15	69	4,830	45	0
Alemtuzumab	19	58	3,400	30	2
All Therapies	93	61	3,730	40	0

Evolution of IgA and IgG hypogammaglobulinemia in WM

We next evaluated those untreated patients who did not demonstrate disease progression during the course of our follow-up. With a median follow-up of 26 (range 4–106 months), IgA and IgG levels remained stable for most patients. Twenty-eight of 60 (47%) patients demonstrated decreased IgA levels at baseline, while 32/60 (53%) of these patients were abnormally low at the end of follow-up; similarly 29/60 (48%) of patients demonstrated subnormal IgG levels at baseline, which remained unchanged at last follow-up (p=0.47 and 1.0, respectively). We also evaluated those untreated patients who eventually demonstrated disease progression during the course of our follow-up. With a median follow-up of 13 (range 4-144 months), 29/42 (69%) patients demonstrated decreased IgA levels at baseline, while 31/42 (74%) of these patients were abnormally low at the end of follow-up (p=0.63). Similarly, 27/42 (64%) of patients demonstrated decreased IgG levels at baseline, and 30/42 (71%) remained low at last follow-up (p=0.48). While comparison of the presence of IgA and/or IgG hypogammaglobulinemia between those patients who remained progression free and those who progressed did not demonstrate any significant differences (data not shown), the median level of serum IgA (45 *vs.* 72 mg/dL) and IgG (558 versus 716 mg/dL) was lower for patients who demonstrated disease progression versus those patients who remained progression free during the follow-up period, though was only significant for IgG (p=0.018).

Impact of therapy and response on IgA and IgG levels

To understand the impact of WM directed therapeutic intervention on uninvolved immunoglobulin levels, we analyzed changes in IgA and IgG levels in a cohort of 93 patients who underwent treatment for WM and whose baseline characteristics are depicted in Table 2. The median age for these patients was 61 (range 43 – 86 years), and the median pre-therapy IgM level and bone marrow disease burden were 3,730 (range 458 – 12,400 mg/dL) and 40% (range 5–95%), respectively. Therapies for these patients included rituximab (n=3); fludarabine and rituximab (n=19); cyclophosphamide, adriamycin, vincristine, prednisone and rituximab

(CHOP-R) (n=8); thalidomide and rituximab (n=14); lenalidomide and rituximab (n=5), bortezomib (n=7), combination bortezomib, dexamethasone, and rituximab (n=15), and alemtuzumab (n=19). The median number of prior therapies for patients in this analysis was 0 (range 0 to 4), and 61% of patients were previously untreated.

At baseline, 73 (78.5%), 63 (67.7%) and 59 (63.4%) of the patients in this cohort demonstrated decreased levels of IgA, IgG or both. The median baseline IgA and IgG levels for these patients were 37 (range 6 – 597 mg/dL) and 488 (range 50–2,800 mg/dL), respectively. With a median follow-up of 12 (range 1–61 months), 72 (77.4%), 64 (68.8%) and 58 (62.4%) demonstrated decreased levels of IgA, IgG or both (p=0.86, 0.88, and 0.88, respectively). No significant recovery in the median IgA and IgG levels was observed with any therapy during the course of follow-up (Table 3), including in those patients who had who had follow-up in excess of 1 (n=46), 2 (n=25), and 3 (n=8) or more years post-therapy (p=NS). Median serum IgM levels during this period declined from 3,730 (range 458-12,400 mg/dL) to 1,320 (23-5,650 mg/dL) at best response.

Lastly, we analyzed the impact of response quality on recovery of IgA and/or IgG immunoglobulin levels in this cohort. Eighty-one (87%) of the 93 patients demonstrated at least a minor response. Categorical responses for these patients were as follows: 9 complete (CR), 49 partial (PR; > 50% decrease in IgM), and 23 minor (MR; > 25% decrease in IgM) responses. No significant recovery in IgA and IgG levels was observed in any therapeutic response category during the course of follow-up, including among patients who attained a complete response (Table 4).

Sequencing of CVID associated genes

Sequence analysis of the promoter, all exonic, and flanking intronic regions for each gene was performed in 19 patients with WM who exhibited at baseline IgA (n=5), or both IgA and IgG (n=14) hypogammaglobulinemia. Sequencing studies demonstrated no novel variants in the promoter, flanking introns, and exons of *AICDA, BTK, CD40, CD154, TACI*, and *SH2D1A* in all 19 patients. For NEMO, we observed an intronic variation at position c.1056-6T>C in 2 patients, and a hemizygous missense mutation at c.337G>A resulting in a change from aspartic acid to asparagine at amino acid position 113 in one other patient. Lastly, a heterozygous missense mutation at c.425A>T resulting in a change of aspartic acid to valine at amino acid position 142 was observed in UNG for one patient who had both IgA and IgG deficiency.

Discussion

In these studies, we demonstrate that hypogammaglobulinemia of the *uninvolved* immunoglobulins (IgA, IgG) is a common finding in patients with WM. The incidence of IgA, IgG, or both IgA and IgG hypogammaglobulinemia in this series of 207 untreated WM

Table 3. Changes in IgA and IgG levels following treatment for 93 patients with WM. Post-therapy values reflect each patient's best immunoglobulin level during follow-up.

		Serum IgA					Serum IgG				
		Pre-Therapy		Post-Therapy			Pre-Therapy		Post-Therapy		
	N	Median	<70 mg/dL	Median	<70 mg/dl	p-value	Median	<700 mg/dL	Median	<700 mg/dL	p value
Rituximab	3	29	3/3 (100%)	45	2/3 (67%)	1.00	448	3/3 (100%)	369	2/3 (67%)	1.00
Fludarabine/Rituximab	17	56	12/17 (71%)	45	11/17 (65%)	0.2399	658	10/17 (59%)	551	12/17 (71%)	0.7207
CHOP/Rituximab	8	80	3/8 (38%)	47	7/8 (88%)	0.1534	759	4/8 (50%)	535	6/8 (75%)	0.6084
Thalidomide/Rituximab	14	26	13/14 (93%)	20	13/14 (93%)	1.00	424	11/14 (79%)	489	12/14 (86%)	1.00
Lenalidomide/Rituximab	10	40	9/10 (90%)	41	8/10 (80%)	1.00	585	6/10 (60%)	587	6/10 (60%)	1.00
Bortezomib	7	34	5/7 (71%)	36	5/7 (71%)	1.00	401	5/7 (71%)	580	4/7 (57%)	1.00
Bortezomib/Dexamethasone/ Rituximab	15	45	10/15 (67%)	25	13/15 (87%)	0.3898	474	9/15 (60%)	499	11/15 (73%)	0.6999
Alemtuzumab	19	30	18/19 (95%)	31	16/19 (84%)	0.6039	401	15/19 (79%)	583	11/19 (58%)	0.2953
All Therapies	93	37	73/93 (78%)	33	75/93 (81%)	0.8559	488	63/93 (68%)	511	54/93 (69%)	1.00

Table 4. Changes in IgA and IgG levels according to response category for 93 patients with WM. Post-therapy values reflect each patient's best immunoglobulin level during follow-up.

	Serum IgA					Serum IgG				
	Pre-Therapy		Post-Therapy			Pre-Therapy		Post-Therapy		
	Median	<70 mg/dL	Median	<70 mg/dL	p value	Median	<700 mg/dL	Median	<700 mg/dL	p value
Complete Response	99	4/9 (44%)	75	5/9 (56%)	1.00	857	2/9 (22%)	504	5/9 (56%)	0.33484
Partial Response	45	33/49 (67%)	35	32/49 (65%)	1.00	556	40/49 (82%)	572	32/49 (65%)	0.10827
Minor Response	31	18/23 (78%)	28	18/23 (78%)	0.6854	362	21/23 (91%)	410	20/23 (87%)	1.00
Stable Disease/Non-Responder	19	8/12 (67%)	14	9/12 (75%)	0.06854	407	10/12 (83.3%)	323	11/12 (92%)	0.6087

patients was 63.3%, 58.0% and 49.3%, which is similar to the incidence reported among patients with chronic lymphocytic leukemia,[6,7] but higher than that reported in other indolent lymphomas wherein the incidence of IgA and IgG hypogammaglobulinemia ranges from 10-20%.7 The incidence of IgA and/or IgG hypogammaglobulinemia observed in this study also appears higher in comparison to those observed in individuals with IgM monoclonal gammopathy of unknown significance (MGUS), though there has been great variation in the reported incidence (8-35%).[15,16] The inclusion of patients having up to 10% bone marrow disease involvement, who are now considered to have WM based on consensus diagnostic criteria, may account for the higher incidence of IgA and/or IgG hypogammaglobulinemia observed in Kyle et al17 series in which up to 35% of IgM MGUS patients had IgA and/or IgG hypogammaglobulinemia.

Importantly, in our series of 207 untreated WM patients, was the observation that the presence of IgA and/or IgG hypogammaglobulinemia was unrelated to age, sex, serum IgM levels, degree of bone marrow disease involvement, myelosuppression, absolute lymphocyte count, or prognostic indices including b2-microglobulin levels, and the WM IPSS score. Therefore the presence of IgA and IgG hypogammaglobulinemia is unlikely to be a consequence of disease burden per se. Surprising, a lower incidence of IgA and IgG hypogammaglobulinemia was observed in the presence of adenopathy and splenomegaly, both features which are seen in less than 20% of WM patients. This finding may signify that the pathogenesis for WM patients exhibiting adenopathy/and or splenomegaly may reflect a disease process more in keeping with other indolent non-Hodgkin's lymphomas in whom IgA and/or IgG hypogammaglobulinemia is less common as previously discussed.

The presence of IgA and/or IgG hypogammaglobulinemia was reported to predict for evolution of IgM MGUS to WM in the Kyle et al series.[16] While the presence of IgA and IgG hypogammaglobulinemia per se was not associated with disease progression, WM patients in this series who were initially placed on watch and wait and who ultimately progressed demonstrated lower median IgA and IgG levels. Striking was also the observation that the presence of IgA and/or IgG hypogammaglobulinemia did not predict for recurrent infections. Comparison of the median IgA and IgG levels for patients with and without recurrent infections, as well as the presence of IgA and/or IgG hypogammaglobulinemia using our institutional cutoffs did not show any significant differences. The use of lower IgA (<30 mg/dL) and IgG (IgG <300 mg/dL) cutoffs also did not demonstrate any differences in the incidence of recurrent infections. These results suggest that other immunological and possibly even anatomical differences may contribute to the risk of recurrent infections, particularly for respiratory tract infections. The routine use of intravenous immunoglobulin replacement for WM patients with low IgA and IgG levels should therefore be considered prudently, since low IgA and IgG levels per se do not imply increased infection risk for WM patients, and should be considered in a case by case basis taking into account a patient's infection history as has been advocated in patients with other B-cell malignancies.[17]

An important finding in this study was the impact of therapy on IgA and/or IgG levels in WM patients. Despite effective treatment, including the attainment of complete remissions in some patients, no substantial recovery of IgA and/or IgG levels was observed, which was inclusive of patients who had post-therapy follow-up exceeding 3 years. While most of the 93 patients in this series received a rituximab based therapy, which could have further aggravated IgA and IgG hypogammaglobulinemia through sustained B-cell depletion, lack of IgA and IgG recovery was also observed in this study as well as in another studies in patients who received and responded to bortezomib.[18] These findings suggest that the IgA and IgG hypogammaglobulinemia in WM may represent either a constitutional defect in plasma cell or immunoglobulin production, including heavy chain switching, or that other cellular elements may contribute to the continued presence of humoral immunodeficiency. As such, patients should not be treated with the intent of restoring their IgA and IgG levels.[19]

As part of these studies, we performed extensive sequence analysis of the 8 most often observed genes involved in immunoglobulin deficiency disorders such as common variable immunodeficiency disorder (CVID), Hyper IGM syndrome (HIGM), and X-linked agammaglobulinemia (XLA).[8-12] These genes included *AICDA; BTK; CD40; CD154; NEMO, TACI, SH2D1A*, and *UNG*. The only novel variant of interest demonstrated by these studies was an amino acid substitution at position 142 of *UNG* that is present in the highly conserved catalytic domain of uracil-DNA glycosylase.[20] Uracil-DNA glycosylase is essential to the generation of somatic hypermutation by generating strand breaks that lead to immunoglobulin heavy chain class switching.[10,21] Individuals deficient in uracil-DNA glycosylase exhibit a hyper-IgM syndrome, with increased IgM levels and decreased IgA and IgG levels. Moreover, mice deficient in UNG develop B-cell lymphomas late in life suggesting a tumor suppressor role for uracil-DNA glycosylase.[22,23] To our knowledge, however, this is the first report of a patient with a B-cell lymphoma in whom a mutation in UNG has been identified. Further validation of these findings, as well as investigation of UNG, other uracil DNA glycosylases, as well as other less characterized mutations involved in humoral immunodeficiencies such as ICOS, ICOS ligand, and CD19 are therefore warranted in clarifying the underlying etiology for IgA and IgG hypogammaglobulinemia in WM.

In summary, the results of these studies suggest that most patients with WM demonstrate IgA and IgG hypogammaglobulinemia which persists despite therapeutic intervention and response. Moreover, IgA and IgG hypogammaglobulinemia did not predict for recurrent infection risk in WM patients, though lower

levels of serum IgA and IgG were associated with disease progression in WM patients placed on watch and wait. These studies highlight the importance for further investigations into the IgA and IgG hypogammaglobulinemia of WM, as well as the signaling pathways involved in B-cell differentiation and immunoglobulin heavy chain class switching in the pathogenesis of WM.

Authorship and Disclosures

SPT and JYS designed the experiments and prepared the manuscript. RJM, LL, CH, CJP, MCL collected patient data and supported clinical data base tht made these studies possible. BTC, HT, PG, XL, YZ, GY, JS, MM collected and processed samples, and were involved in performing and analyzing data from sequencing analyses. SPT and PS were involved in patient care contributing to these studies.

The authors reported no potential conflicts of interest.

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