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Characterization of the Anti-HLA Class I and II IgG Antibodies in Moroccan IVIg Using Regular Beads and Ibeads in Luminex Multiplex Single Antigen Immunoassay

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Abstract: Therapeutic Immunoglobulin Intravenous (IVIg), approved to treat a wide range of autoimmune and primary immunodeficiency diseases, contain mixture of polyreactive and polyclonal IgG purified from the pooled plasma of thousands of donors. The aim of this study is to characterize the profiles of anti- Human Leukocyte Antigen (HLA) class-I and class-II IgG antibodies in four lots of Moroccan IVIg preparations using Luminex Multiplex Single Antigen Bead Immunoassay and to compare it with the unique high frequency HLA types found in the Moroccan population. Anti-HLA class I IgG profiles were assessed using regular (Labscreen) Beads and iBeads. The regular beads are coated with all conformational and structural variants of HLA-I (HLA heavy chain (HC) with β2-microglobulin (β2m) with or without peptides, β2m-free HC with or without peptides or HC only), quite contrast to iBeads, which contained only native tissue-associated HLA HC with β2m and with or without peptides. The level of antibodies was measured as Mean Fluorescent Intensity (MFI). The reactivity of anti-HLA-I IgG antibodies to different alleles of HLA-I loci differed in their recognition of native HLA-I and other structural variants of the HLA-I. High MFI IgG antibodies in the IVIg corresponded with several high frequency HLA-I alleles (B*0801, B*5001, Cw*0602 and Cw*0702) and HLA-II haplotypes (DQA1*0201-DQB1*0201/DRB1*0301), which accounted for 50% of the total gene frequencies in the Moroccan population. HLA-I reactivity of the IVIg with iBeads confirms that the IgG reacting to normal tissue associated with peptide -associated or -free β2mHC. These findings caution the use of high dose IVIg for the carriers of the high frequency HLA types for it may cause tissue injury. The β2m-free-HC reactivity of IVIg indicates the potential of IVIg to bind to activated T and B cells that express these variants, to suppress antibody production. Such an immunomodulation by IVIg renders benefit for patients with autoimmune diseases and organ transplantation.

Keywords: Intravenous Immunoglobulin, HLA, Antibodies, Moroccan IVIg, Beads, Ibeads, MFI

1. Introduction

When Immunoglobulin (Ig) therapy was replaced by Intravenous Immunoglobulin (IVIg) with sera derived from multiethnic population from Morocco, the IVIg was not affordable for most patients, until the Blood Transfusion and Hematology National Center and Fractionation and Biotechnologies French Laboratory (LFB-France) industry subcontracted IVIg production using plasma from Moroccan

blood donors [1], which included both allo-immunized and non-alloimmunized males and females. As a result, the cost of IVIg therapy is reduced by about 66% [1]. Consequently Moroccan IVIg therapy is administered to primary deficiency, autoimmune and neurological diseases, including Guillain-Barre syndrome, for which it was found to be safer and effective alternative to standard therapies [2]. However, very little effort has been made to characterize the composition of Moroccan IVIg, pooled and purified from the plasma of Moroccan population, which has remarkable ethnic and genomic diversities, which is reflected in their Human Leukocyte Antigen (HLA) typing profiles. As of March 2017, it has been reported that the Human major histocompatibility complex includes highly polymorphic proteins of classical HLA class I (HLA-A [n = 2747], -B [n = 3465], and -C [n = 2450]) proteins and HLA-II (HLA-DRA [n = 7], -DRB [n = 1711], -DQA1 [n = 34], -DQB1 [n = 761], -DPA1 [n = 23] and -DPB1 [n =627] proteins [3]. Studies examining HLA-I types in Casablanca, the largest region of Morocco, reported that the most frequent types were HLA-A2 (21%), -A1 (11%), -A3 (10%), -B44 (11.4%), -B50 (9.9%), -B5 (8.5%) and -B35 (6.5%) [4]. Similarly, in the Amazigh ethnic group, the most frequent alleles were HLA-A*0201, A*0101; HLA-B*4403, B*4402, B*0801, B*5001, B*5002; HLA-Cw*0602, Cw*070101, Cw*070102, Cw*0702, Cw*0704, and Cw*0706, Cw*040101 [5]. Molecular typing of HLA-II in Amazigh group (Souss) revealed high frequencies for DRB1*0701, DRB1*0301, DQA1*0501, DQA1*0201, and DQB1*0201 [6]. Three haplotypes (DRB1*0701-DQA1*0201-DQB1*0201, DRB1*0301-DQA1*0501-DQB1*0201 and DRB1*11-DQA1*0501-DQB1*0301) are accounted for nearly 50% of the total gene frequencies. Many other studies on HLA typing of Moroccan population have confirmed the above mentioned high frequency types in the Moroccan population [7-10]. On the other hand, it is also well established that IgG antibodies against HLA-I and -II molecules do occur naturally as auto- or allo-antibodies in non-alloimmunized males [11 -20]. The origin of these antibodies in “so-called” healthy individuals is still far from clear. It remains to be seen whether the HLA antibodies in the Moroccan healthy blood donors reflect the HLA types of the population and whether they are auto-antibodies, which means, directed against self-antigens, as has been reported elsewhere [21, 22]. High level of anti-HLA antibodies are reported in several autoimmune diseases [22, 23]. Therefore, it is felt that the Moroccan IVIg may reflect the HLA profiles of the Moroccan population. Examination of the anti-HLA IgG antibody profiles of Moroccan IVIg preparations may validate the above assumption.

There is also yet another need to characterize HLA-I and -II antibodies in Moroccan IVIg, because IVIg may be administered to patients with Transfusion-related Acute Lung Injury (TRALI), while it is well established that the causal factor of TRALI is the presence of HLA antibodies in the patients. There are many reports on the occurrence of TRALI after IVIg administration [24-29]. Anti-HLA-II IgG observed in patients after plasma transfusion is implicated in TRALI [26]. The anti-HLA-II IgG binding to monocytes in patients with TRALI may induce activation of neutrophils that may penetrate the endothelium of lungs, causing destruction of the endothelial cells [24, 30]. Presence of HLA-II antibodies in allo-immunized females led to prevention of using blood from females for transfusion, Therefore, the avoidance of female blood has become routine as a preventive measure against TRALI in several countries [31, 32]. It was reported that this policy did indeed significantly reduce the incidence of TRALI both in large-scale surveillance studies and haemovigilance reports [32].

Furthermore, it is perplexing to note that IgG antibodies to allo–HLA proteins are common in sera after transfusions, organ transplantation, and autoimmune diseases [21-23, 33 - 35]. After all, Moroccan IVIg is prepared from plasma from over 40000 male and female healthy donors, but certainly with a history of infection, inflammation, injuries and unknown immune related diseases.

Therefore, the primary specific objective of this investigation is to characterize IgG reactivity against HLA-I and HLA-II in Moroccan IVIg. Such an investigation is necessary to determine whether the therapeutic application of IVIg should be preceded by HLA antibody screening. Commercially available IVIg preparations, depending on their polyreactive and their polyclonal antibody strength may contain variable amounts of IgG dimers in the range of 5-15% [36], although IgG dimer, unlike polymers, does not cause anaphylactic shock. Nevertheless IVIg preparations with high dimer content are less tolerated and can give rise to undesirable side effects such as fever, nausea and sometimes lowered blood pressure [37, 38]. Therefore, our second objective is to evaluate Moroccan IVIg formulation for dimer composition.

2. Materials and Methods

2.1. Source and Preparation of IVIg

In Morocco, IVIg is prepared from the whole blood of 40 000 male and female donors. The Moroccan IVIg is manufactured according to the Kistler-Nitschmann method, involving cryoprecipitation, multiple (11% 16% and 22%) ethanolic precipitations, viral inactivation, viral and prion filtration, acidic and enzymatic treatments, sterilization, and lyophilization. All four lots were received as lyophilized powder and were reconstituted with water for injection at a concentration of 50 mg/mL (or 5% proteins). All the IVIg preparations contained IgA (17mg/g of protein), traces of pepsin, sucrose and sodium. Most of the IVIg preparations from US and Europe are devoid of sucrose, due to adverse reports after IVIg administration to patients [38]. The source of IVIg is: (Immunoglobuliline Normale IV-LFB-CNTs (50 mg/ml) 2015, LFB Biomedicaments. Courtaboeuf Cedex, France). The details of the lots are as follows: Lot#1: 14L 00532; Lot#2: 14L 00534; Lot#3: 14L 01611; Lot#4: 14L 01617.

2.2. Determination of Monomer/Dimer Ration with Fast Protein Liquid Chromatography (FPLC)

The four lots of IVIg preparations (50mg/ml) were initially diluted with normal saline (0.9% NaCl) to 10 mg/mL, and examined within 24 hours, by FPLC using a superdex G-200 column (1.5x50 cm) pre-equilibrated with 50 mM sodium phosphate/150mM sodium chloride at pH 7.2, using Amersham Biosciences AKTA Purifier FPLC System (includes: Box 900; CU-950 System Interface; pH/C-900 Conductivity Detector; Amersham UV-900 UV/Monitor; Amersham P-900 Pump). The flow rate was maintained at 3ml/tube after 0.5ml of IVIg was loaded onto the column. The ratio of monomer/ dimer is determined as follows: [monomer/dimer] x 2. The percentage of monomers and dimers was determined as follows: monomer x 2 x100/ [dimer+ (monomer x 2)].

(1) Dimer % = (1/2)*(dimer integral)/ ((1/2)*dimer intergral+monomer integral); and

(2) Monomer % = (monomer integral)/ (monomer integral + (1/2)*dimer integral).

2.3. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)

Molecular sizes of the protein fractions in the four lots of IVIg were assessed to ascertain the presence of monomeric IgG fractions, their degradation, if any, following a standard protocol [39] using MALDI-TOF MS. The IVIg preparations (10 mg/mL) were analyzed using a microflex LT Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) instrument (Bruker Daltonik GmbH, Bremen, Germany). The spectra were recorded in the linear positive mode at a laser frequency of 5.0 Hz within a mass ranging from 10 to 100 kDa and 100 kDa to 200 kDa. Parameter settings for microflex instrument were ion source 1 at 20 kV, ion source 2 at 18.0 kV, lens at 9.5 kV, pulsed ion extraction of 50 nS and no gating. Mass spectrometry samples were prepared following the protocol [40]. Briefly, 0.5 µL of sample was loaded onto a spot on the MALDI-TOF steel target plate, and 0.5 µL of the calibration standard (ProteoMass Apomyoglobin MALDI-MS Standard; Sigma-Aldrich) was loaded onto a separate spot.

2.4. Immunoassay with Single Antigen Beads SABs

HLA-I and -II IgG reactivity were analyzed using Luminex Multiplex Single Antigen Bead immunoassay. The data obtained with a dual-laser flow-cytometry Luminex-xMAP® (http://www.luminexcorp.com/) (LABScanTM100; One Lambda, Canoga Park, CA) [41]. The single recombinant HLA-Ia and HLA-II (rHLA-Ia & rHLA-II) antigens in LS1A04-Lot 008 (for HLA-Ia) containing 97 HLA-I antigens (31 HLA-A, 50 HLA-B and 16 HLA-Cw) and in LS.2 A01009 (Lot 9) (for HLA-II) containing 91 HLA-II antigens (29 HLA-DRB1, 7 HLA-DRB3, 4, 5, 29 HLA-DQ, 26 HLA-DP). The SAB assay includes built-in control beads, coated with human IgG (positive control) or albumin (human or bovine) (negative control).

For HLA class I, two kinds of beads were used. They are regular Labscreen beads and iBeads [41]. The beads supplied by the manufacturer may have 2 categories of HLA proteins attached to the beads: HLA heavy-chain polypeptide only and heavy-chain polypeptides in association with Beta-2 microglobulin (β2m). Realizing the heterogeneity of proteins, the manufacturer recently developed iBeads (provided as Felix beads for in-house experimental use), in which regular HLA-Ia antigen-coated microbeads are subjected to proprietary enzymatic treatment to remove or reduce the amount of heavy chains (also referred to as "denatured antigens" by the manufacturers) [42, 43].

Briefly, the four lots of IVIg were titrated from 50 to 0.8mg/mL (diluted in 1X PBS, pH 7.2), and 20 µL of sample were incubated with 2µL of beads for 30 minutes at room temperature and on a shaker. The beads were then washed three times with LabScreen® wash buffer. The HLA-I and -II reactivity were monitored by incubating 50 µL (at 5µg/mL) of PE-conjugated Goat anti-human IgG (Fab') for 30 minutes. The beads were washed three times, and then resuspended in 1X PBS before acquisition. For each sample analysis, at least 100 beads were counted. The IgG reactivity against each HLA-I and -II antigens were recorded as Trimmed Mean Fluorescence Intensity (MFI), and the MFI values are normalized against the negative control (bead #1) and the negative control (1X PBS). The MFI cutoff used was 1,000 for a positive reaction.

2.5. Statistical Analysis

All data were analyzed using statistical software package for PC (version 13; Dell, Inc. Round Rock, Texas). Analyzed groups were tested for normal distribution using Shapiro-Walk W testing. Data sets with normal distributions were analyzed by multifactorial ANOVA to identify overall condition effects. Significant differences were determined by post hoc comparisons of means using Tukey's honest significant difference test. Significance was set at a confidence level of 95%. Data are presented as mean ± SEM.

3. Results

3.1. MALDI-TOF MS Profile of the Four IVIg Lots

The MALDI-TOF MS spectra analysis of the four different lots of IVIg revealed the presence of 6 main protein peaks (Figure 1). The major peak of ~149 kDa is corresponding to the molecular weight of IgG (~150 kDa). Prominent prevalence of the mass of 149 kDa suggests that the IgGs in Moroccan IVIg are intact with no obvious degradation.

Four lots (Lots 1-4) of Moroccan IVIg were mixed and crystalized with α-Cyano-4- hydroxycinnamic acid (4-HCCA) in formic acid: water: isopropanol (3:1:2) in a 1:9 ratio. The expected mass of Moroccan IgG antibody is ~150 kDa, the observed species is ~149 kDa. Each lot of Moroccan IVIg was collected in two spectrums ranging from 10 kDa to

100 kDa and 100 kDa to 200 kDa (shown as one spectrum).

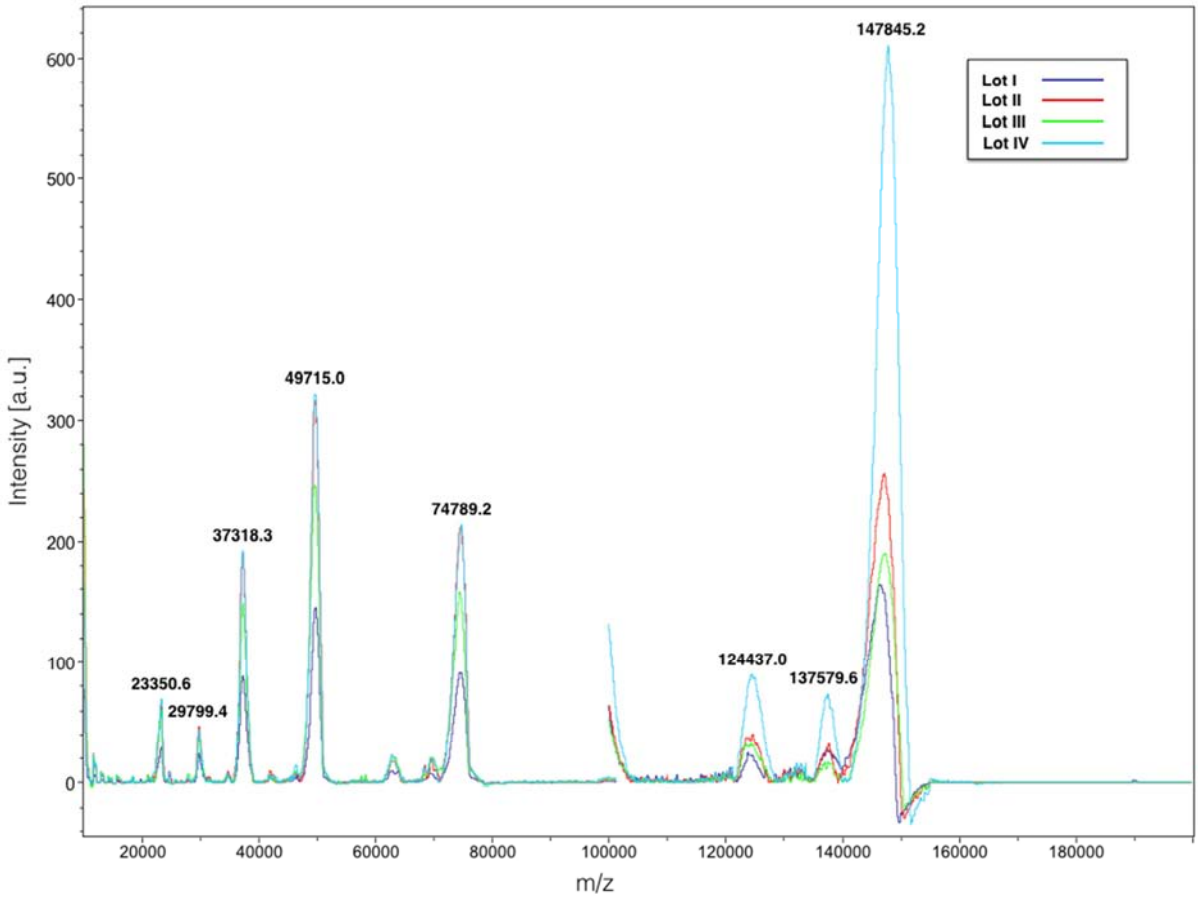

Figure 1. *MALDI-TOF MS spectra of the four Moroccan IVIg lots.*

3.2. FPLC Analysis of the Four IVIg Lots

The FPLC analysis of the four different lots of IVIg showed that all IVIg preparations contain monomeric, dimeric and polymeric IgG (Figure 2, Table 1). The highest peak corresponds to monomeric IgG, the second highest corresponds to dimeric IgG, and the lowest is indicative of polymeric IgG. Table 1 shows the percentage of monomer and dimer of IgG in all IVIg lots tested. IVIg lot# 4 being the highest dimer percentage (11 %), followed by IVIg lot # 3 (9.9 %), IVIg lot# 1 (6.4 %), and IVIg lot# 2 (5.8 %). The amount of polymeric IgG is negligible in all IVIg lots tested.

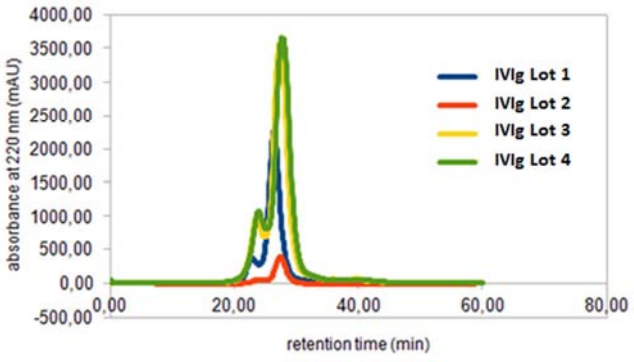

Figure 2. *FPLC chromatogram of the four Moroccan IVIg lots #1; 2; 3; 4.*

The highest peak corresponds to monomeric IgG, the second highest corresponds to dimeric IgG, and the lowest to polymeric IgG.

Table 1. *Fast protein liquid chromatography (FPLC) analysis.*

IVIg Lots	Dimer	Monomer	Monomer/Dimer ratio	Monomer (%)	Dimer (%)
	Peak Area	Peak Area			
# 1	704.45	5111.28	14.51	93.6	6.4
# 2	113.06	925	16.36	94.2	5.8
# 3	2130.7	9737.48	9.14	90.1	9.9
# 4	2427.08	9831.51	8.1	89	11

Percentage of monomer and dimer of the four Moroccan IVIg.

3.3. Anti-albumin Reactivity of the Four IVIg Lots

All the IVIg lots reacted with the negative control beads coated with albumin (Figure 3), indicating that the Moroccan IVIg preparations contain naturally occurring anti-albumin antibodies, almost a similar inference can be derived from a previous report monitoring anti-HLA antibodies of Moroccan sera [44]. Among the four IVIg lots, lot #1 showed the highest anti-albumin reactivity, followed by lot # 2, then lot # 3, and finally lot # 4 showed the least reactivity.

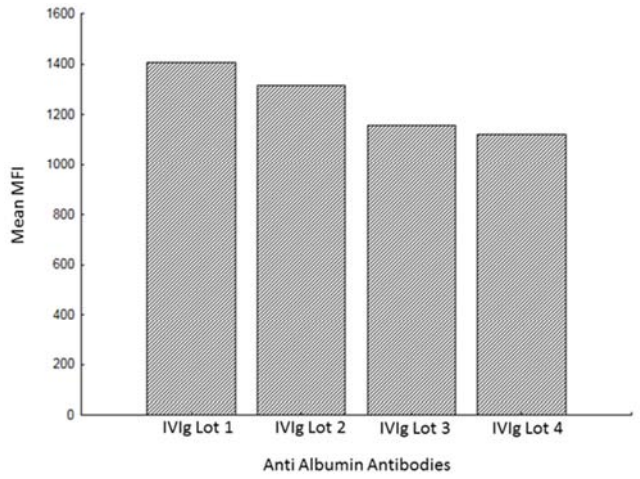

Figure 3. *Mean MFI values of Anti-albumin at a concentration of 50mg/mL of the four Moroccan IVIg lots.*

All the four IVIg lots reacted with the negative control beads coated with albumin, lot#1 showed the highest reactivity.

3.4. Anti-HLA-I and Anti-HLA-II IgG Reactivity of the Four IVIg Lots Using Regular Labscreen Beads

All IVIg lots recognize most of the HLA class-Ia alleles and HLA-II molecules. All preparations reacted with all beads coated with HLA-I and HLA-II alleles. However, for HLA-A and HLA-B alleles, there were no alleles reactive to any of the IVIg lots at 1:32 dilution. In contrast, HLA-DRB345 and HLA-DP alleles showed reactivity even at highest dilution 1/64 (Table 2).

Table 2. *Number of HLA-Ia and HLA-II alleles reactive to the four Moroccan IVIg.*

IVIg Lots	Dilutions	Number of HLA-Ia and HLA-II alleles reactive to IVIg						
		A	B	Cw	DRB1	DRB345	DQ	DP
	[1/64]	0	0	0	0	1	0	1
	[1/32]	0	0	0	7	2	0	10
	[1/16]	0	0	7	11	5	1	15
# 1	[1/8]	2	3	15	22	5	10	21
	[1/4]	24	38	16	28	7	29	26
	[1/2]	29	48	16	29	7	29	26
	Neat	31	50	16	29	7	29	26
	[1/64]	0	0	0	0	1	0	0
	[1/32]	0	0	0	1	1	0	4
	[1/16]	0	0	3	4	2	0	6
# 2	[1/8]	1	1	13	14	5	2	16
	[1/4]	6	20	16	26	7	20	22
	[1/2]	25	44	16	29	7	29	26
	Neat	31	50	16	29	7	29	26
	[1/64]	0	0	0	0	1	0	1
	[1/32]	0	0	1	5	2	0	8
	[1/16]	0	0	8	8	3	0	10
# 3	[1/8]	1	4	14	20	5	6	19
	[1/4]	16	32	16	26	7	20	23
	[1/2]	28	47	16	29	7	29	26
	Neat	31	50	16	29	7	29	26
	[1/64]	0	0	0	0	1	0	1
# 4	[1/32]	0	0	1	1	1	0	3
	[1/16]	0	0	8	9	5	0	14
	[1/8]	2	4	15	23	5	12	21
	[1/4]	16	32	16	26	7	20	23
	[1/2]	30	49	16	29	7	29	26
	Neat	31	50	16	29	7	29	26

All IVIg lots reacted to the panel of the 97 HLA-I (31 HLA-A, 50 HLA-B and 16 HLA-Cw) alleles coated on the regular Labscreen beads using Luminex single-antigen bead (SAB) assays. Figure 4 shows the mean MFI of the undiluted (neat) preparations of each IVIg lot (at 50mg/mL concentration) against the HLA-A, -B and -Cw loci. The combined allelic mean MFI of pooled HLA-A reactivity of lot #1, 2, 3, and 4 was 1865 ± 710, 1946 ± 893, 1560 ± 655, and 1566 ± 585, respectively. The mean combined allelic mean MFI of pooled HLA-B reactivity of lot #1, 2, 3, and 4 was 2090 ± 619, 2228 ± 693, 1786 ± 579, and 1706 ± 524, respectively. The combined allelic mean MFI of pooled HLA-Cw reactivity of lot #1, 2, 3, and 4 was 3656 ± 1116, 4189 ± 1467, 3099 ± 920, and 2982 ± 957, respectively. All IVIg preparations showed stronger reactivity to HLA-Cw alleles, followed by HLA-B alleles and then HLA-A alleles.

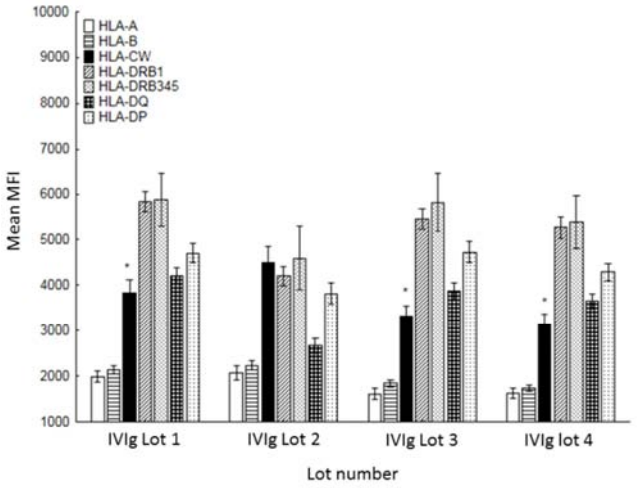

Figure 4. *Comparison of the normalized mean fluorescence intensity (MFI) of human leucocyte antigen (HLA) class II immunoglobulin (Ig)G antibodies of the four IVIg lots (at 50mg/mL concentration) against HLA-A (n=31), HLA-B (n=50), HLA-Cw (n=16), HLA-DRB1 (n=29), HLA-DRB345 (n=7), HLA-DQ (n=29), and HLA-DP (n=26) alleles in the HLA-I Single Antigen Bead panel.*

Data represent mean ± SEM. A two-way ANOVA showed a statistically significant interaction between antigens and lots ($F(18, 724) = 5.98$, $p<.00001$). There was a significant effect of antigens ($F(6, 724) = 234.49$, $p<.0001$) and lots ($F(3,724) = 11.54$, $p<0.0001$). Tukey post Hoc tests reactivity of HLA-A and B was significantly lower than all other antigens tested (*$P<.001$).

Table 3 provides a detailed profile of HLA-I reactivity of different lots of IVIg as MFI of individual alleles of each HLA-I loci. It may be noted that the most prevalent anti-HLA-A IgG antibodies in all the Moroccan IVIg lots, as assessed by the MFI strength at neat and 1/2 dilution, are

A*3401 and A*8001 (indicated by bold italics) and the least expressed anti-HLA-A IgGs are against A*0301 and A*7401. Similarly, the most prevalent anti-HLA-B IgG antibodies in all IVIg lots are B*1512 and B*8201. Among anti-HLA-Cw IgG antibodies, MFI of Cw* 0702 and Cw*1702 are the most predominant in all lots of the IVIg preparations.

Table 3. *HLA-A, HLA-B, and HLA-Cw allelic reactivity of the four lots of Moroccan IVIg determined using regular Labscreen Beads.*

HLA-A	**IVIg Lot 1**		**IVIg Lot 2**		**IVIg Lot 3**		**IVIg Lot 4**		**HLA-B**	**IVIg Lot 1**		**IVIg Lot 2**		**IVIg Lot 3**		**IVIg Lot 4**	
Dilution	(1/2)	Neat	(1/2)	Neat	(1/2)	Neat	(1/2)	Neat	Dilution	(1/2)	Neat	(1/2)	Neat	(1/2)	Neat	(1/2)	Neat
A*0101	952	1865	547	1772	683	1389	984	1543	B*0702	1855	2933	1390	3414	1714	2701	1899	2468
A*0201	862	1651	615	1662	778	1472	878	1398	B*0801	1954	3191	1383	3370	1700	2909	2053	2710
A*0203	797	1683	553	1527	671	1324	871	1325	B*1301	1251	2447	912	2615	1161	2022	1377	1951
A*0206	917	1654	691	1946	874	1492	967	1451	B*1302	938	1911	727	2033	886	1719	964	1578
A*0301		734		737		576		579	B*1401	1487	2593	1219	2646	1488	2157	1592	1934
A*1101	1264	2013	808	2346	972	1585	1337	1685	B*1402	1456	2484	1154	2690	1377	2136	1609	2031
A*1102	735	1590		1456	576	1183	753	1395	B*1501		863		801		818		620
A*2301	679	1368		1323	543	1112	764	1157	B*1502	1152	1917	915	2208	1126	1745	1198	1584
A*2402	1361	2127	884	2381	1004	1768	1390	1948	B*1503		1166		1167		912	631	818
A*2403	1441	2271	847	2002	1131	1750	1578	1846	B*1510	957	2018	675	1887	895	1583	1071	1539
A*2501	1016	1793	790	1699	904	1615	975	1449	B*1511	1047	1881	724	1755	835	1428	1192	1564
A*2601	1272	2363	810	2556	1017	1885	1178	1835	B*1512	2341	2972	1747	3017	2210	2742	2327	2557
A*2901	1196	2384	890	2469	1072	1860	1322	1858	B*1513	794	1463	569	1424	652	1111	925	1141
A*2902	1522	2859	1141	3365	1366	2431	1650	2316	B*1516	1576	2331	1209	2750	1415	2015	1597	1987
A*3001	892	1708	542	1555	634	1219	910	1320	B*1801	710	1473	501	1706	640	1285	745	1169
A*3002	1003	1832	783	1878	969	1560	1049	1566	B*2705	802	1768	659	1999	861	1612	907	1405
A*3101	926	1798	623	1974	800	1519	1001	1397	B*2708	1366	2552	1240	2713	1525	2275	1437	2022
A*3201	586	1442		1287	508	1061	630	1158	B*3501	787	1507	591	1509	720	1208	894	1134
A*3301	1166	2287	1007	2600	1208	1931	1376	1909	B*3701	1047	1774	700	2693	849	1503	1269	1595
A*3303	1099	1989	964	2399	1205	1809	1171	1604	B*3801	783	1512	742	1752	894	1429	945	1309
A*3401	1915	2818	1217	2696	1525	2140	1938	2292	B*3901	812	1456	748	1924	956	1573	885	1292
A*3402	633	1354	526	1331	603	1183	738	1098	B*4001	1055	2177	747	2248	971	1841	1134	1781
A*3601	814	1618	581	1424	657	1124	922	1251	B*4002	1108	2215	900	2596	1249	2088	1208	1890
A*4301	1235	2339	828	2335	1092	1718	1403	1959	B*4006	1568	2590	1081	2637	1314	2022	1631	2018
A*6601	1447	2543	890	2677	1112	2000	1438	1954	B*4101	808	1733	592	1793	759	1519	882	1494
A*6602	1142	2409	817	2493	1031	1867	1282	1997	B*4201	1166	2378	854	2456	1100	1982	1360	1952
A*6801	538	1158		1148		959	607	896	B*4402	1800	2993	1514	3195	1809	2837	2120	2703
A*6802	1091	2275	798	2629	955	1785	1219	1819	B*4403	1430	2514	1254	2908	1545	2466	1544	2110
A*6901	1141	2009	778	1886	928	1547	1348	1755	B*4501	1410	2278	1126	2431	1440	2156	1483	1774
A*7401		1039		955		731	532	846	B*4601	1547	2317	1302	2743	1596	2179	1592	1774
A*8001	3381	4716	2902	5608	3532	4362	3457	3883	B*4701	1021	1946	817	2115	1021	1663	1141	1675
HLA-Cw									B*4801	1366	2546	1026	2586	1335	2331	1574	2068
Cw*0102	2569	3608	2052	4142	2391	3199	2699	3011	B*4901	613	1397		1191	587	1157	713	1119
Cw*0202	2787	3961	1778	4345	2333	2989	2810	3356	B*5001	601	1188		1170	524	1037	607	1006
Cw*0302	1960	2861	1590	3160	1854	2716	2036	2252	B*5101	1089	1969	761	1709	940	1492	1279	1578
Cw*0303	1963	2973	1554	3176	1903	2609	2145	2404	B*5102	1139	1888	649	1513	861	1317	1175	1502
Cw*0304	2224	3149	1705	3414	2046	2832	2436	2570	B*5201	1042	1699	606	1649	752	1292	1112	1398
Cw*0401	2879	3949	1834	4359	2364	3124	3076	3162	B*5301	1638	2372	1410	2570	1594	2102	1831	2030
Cw*0501	2751	3704	2131	4470	2537	3295	2778	2953	B*5401	1434	2310	1091	2425	1295	1890	1536	1840
Cw*0602	3350	4069	2093	5215	2647	3305	3499	3402	B*5501	644	1263		1240		975	685	967
Cw*0702	4316	5789	3328	8082	4154	5333	4627	4740	B*5601	665	1356		1227	565	1125	741	1092
Cw*0801	1846	2904	1529	3436	1919	2752	1949	2325	B*5701	1164	2162	813	1934	1046	1826	1203	1737
Cw*1203	2374	3364	2108	4081	2502	3147	2452	2642	B*5703	1545	2758	1080	2378	1395	2025	1742	2148
Cw*1402	2953	3921	2033	4236	2463	3073	3060	3296	B*5801	1285	1909	855	1792	1141	1604	1332	1584
Cw*1502	1795	2874	1580	3625	1889	2692	1960	2326	B*5901	1114	1894	773	1790	911	1320	1248	1543
Cw*1601	1882	2646	1426	2912	1721	2202	2009	2041	B*6701	1549	2751	1373	3168	1717	2639	1680	2263
Cw*1701	5157	6618	3288	6424	4126	5245	4984	5530	B*7301	1502	2606	1370	3053	1776	2506	1541	1972
Cw*1802	3924	5008	3263	6884	3876	4594	4031	4062	B*7801	1193	1981	732	1997	867	1606	1265	1619
									B*8101	1858	3214	1354	3371	1716	2747	1873	2579
									B*8201	2690	4006	1865	3748	2280	3225	2739	3267

IVIg was diluted from neat to 1/64 and MFI was measured at all dilutions. Data is restricted to Neat and (1/2) dilution. The two most prevailing antibodies against each of the HLA-A, HLA-B, and HLA-Cw loci are shown in Bold and Italics, suggesting the most prevalent anti-HLA IgG in IVIg pooled from the Moroccan male and female donors.

In contrast to MFI of anti-HLA-I IgG antibodies, the MFI of anti-HLA-II IgG antibodies remained high even at higher dilutions. All IVIg lots reacted to the panel of 91 HLA-II (29 HLA-DRB1, 3 HLA-DRB3, 2 HLA-DRB4, 2 HLA-DRB5, 29 HLA-DQ and 26 HLA-DP) alleles in the Single Antigen Beads assay. Figure 4 shows that the combined allelic mean MFI of the neat preparations of each IVIg lot (at a concentration of 50mg/mL) against the HLA-DRB1, -

DRB345, -DQ, and -DP loci are higher than that of anti-HLA class I. The HLA-DRB1 reactivity of lot #1, 2, 3, and 4 was 6108 ± 1215, 4050 ± 1089, 5345 ± 1166, and 5254 ± 1238, respectively. The HLA-DRB345 reactivity of lot #1, 2, 3, and 4 was 5330 ± 1525, 3997 ± 1851, 5090 ± 1681, and 4859 ± 1528, respectively. The HLA-DQ reactivity of lot #1, 2, 3, and 4 was 4047±976, 2634±915, 3951 ± 1066, and 3473 ± 922, respectively. The HLA-DP reactivity of lot #1, 2, 3, and 4 was 4655 ± 1439, 3681 ± 1369, 4609 ± 1508, and 4277 ± 1335, respectively. Essentially, all IVIg preparations showed stronger reactivity to HLA-DRB1 alleles, followed by HLA-DRB345 alleles, HLA-DP and then HLA-DQ alleles.

Table 4 provides a detailed profile of HLA-II reactivity of different lots of IVIg as MFI of individual allele of each HLA-II loci. The most prevalent anti-HLA-DR IgG antibodies in all Moroccan IVIg lots, as assessed by the MFI strength at neat and at all dilutions (data shown only for 1/2 and 1/4 dilutions), are DRB3*03:03 and DRB1*03:02 (indicated by Bold italics). The lot # 2 is the least expressed MFI for anti-HLA-DRB IgGs. Similarly, the most prevalent anti-HLA-DQ IgG antibodies in all the IVIg lots are DQB1*03:01\DQA1*03:01 and DQB1*06:02\DQA1*01:02, while DQB1*02:01\DQA1*02:01 remains the least expressed in all lots of IVIg. Among anti-HLA-DP IgG antibodies, MFI of DPB1*19:01\DPA1*01:03, DPB1*23:01\DPA1*02:01 and DPB1*28:01\ DPA1*02:01 are the most predominant in all lots of the IVIg preparations.

Table 4. *HLA-DR, HLA-DQ, and HLA-DP allelic reactivity of the four lots of Moroccan IVIg. IVIg was diluted from neat to 1/64 and MFI was measured at all dilutions.*

DR Alleles	**IVIg Lot 1**			**IVIg Lot 2**			**IVIg Lot 3**			**IVIg Lot 4**		
Dilution	**(1/4)**	**(1/2)**	**Neat**	**(1/4)**	**(1/2)**	**Neat**	**(1/4)**	**(1/2)**	**Neat**	**(1/4)**	**(1/2)**	**Neat**
DRB1*01:01		1198	4361		933	2757		1167	3995	593	1114	4157
DRB1*01:02	877	1839	4595	790	1440	3533	820	2068	4777	964	1827	4404
DRB1*01:03	507	1200	4176		1068	2747		1375	3566	599	1187	3572
DRB1*03:01	1899	3681	7923	1653	3126	5874	1775	3941	7492	2210	3616	7256
*DRB1*03:02*	3226	5409	9762	3090	4552	7925	3046	5945	9425	3736	5457	9458
DRB1*04:01	1212	2351	6200	1061	1981	4221	1101	2743	5492	1438	2373	5683
DRB1*04:02	1447	2956	6440	1218	2289	4349	1277	3154	5992	1716	2920	6039
DRB1*04:03	1621	2919	6840	1408	2467	4637	1429	3085	6289	1911	2895	6106
DRB1*04:04	1072	2275	6442	910	1917	4474	981	2393	5925	1296	2075	6108
DRB1*04:05	1377	2828	6169	1060	2170	4050	1179	2846	5332	1704	2694	5335
DRB1*07:01	620	1349	4784	543	1194	3273	625	1510	4529	717	1246	4030
DRB1*08:01	1618	2624	6803	1375	2319	5334	1352	2886	6399	1886	2663	5788
DRB1*09:01	1850	2435	5465	1924	2358	5184	1549	2798	5912	1865	2422	5254
DRB1*09:02	846	1641	4623	712	1376	3177	772	1826	4424	959	1602	4058
DRB1*10:01	1489	2816	6108	1259	2433	4633	1337	2786	5432	1654	2643	5720
DRB1*11:01	1130	2279	5766	915	1796	3697	929	2338	5249	1407	2260	4842
DRB1*11:04	1945	3351	7045	1608	2513	5071	1626	3444	6466	2225	3234	6185
DRB1*12:01	962	1796	5242	819	1644	3566	863	1981	5101	1147	1741	4574
DRB1*12:02	1177	2243	6271	1008	1834	4315	1022	2415	5345	1479	2194	5497
DRB1*13:01	1258	2673	6122	1110	2020	3951	1168	2666	5766	1499	2472	5400
DRB1*13:03	725	1714	5359	611	1479	3669	657	1936	5049	889	1659	4700
DRB1*14:01	1163	2372	6168	1023	1802	3960	1032	2464	5369	1488	2310	5182
DRB1*14:02	1922	3058	6348	1648	2427	4830	1506	3146	6229	2215	2999	6269
DRB1*14:54	1636	2809	6238	1435	2403	5072	1417	3106	5970	1945	2892	6127
DRB1*15:01	507	1029	3921		917	2669		1156	3786	571	916	3369
DRB1*15:02	707	1321	4557	600	1239	2944	604	1509	4103	836	1341	3660
DRB1*15:03	1044	2005	4983	804	1549	3854	856	2017	4767	1123	1841	4477
DRB1*16:01	1229	1912	5455	1070	1614	4232	1051	2189	5267	1228	1907	4858
DRB1*16:02	974	1789	5251	942	1720	3751	975	2072	4912	1131	1721	4773
DRB3*01:01	1511	2000	4806	1216	1500	3997	975	1973	5034	1546	2036	4399
DRB3*02:02	1275	2129	4826	1104	1774	3484	1060	2296	4590	1385	2080	4359
*DRB3*03:01*	5180	6075	8922	4947	5075	8629	4129	6499	9440	5189	5974	8562
DRB4*01:01	1586	2805	6676	1458	2169	4907	1390	2925	6219	1900	2647	5996
DRB4*01:03	810	1661	5330	609	1236	3477	628	1544	5090	899	1571	4859
DRB5*01:01	1517	2837	5981	1290	2410	4107	1302	2988	5436	1930	3057	5233
DRB5*02:02	806	1746	4656	726	1510	3542	792	2082	4886	919	1576	4280
DQ alleles												
DQB1*02:01\DQA1*02:01		990	2843		821	1755		1041	2495		918	2447
DQB1*02:01\DQA1*03:01	732	1649	3742	617	1360	2522	643	1632	3107	900	1539	3180
DQB1*02:01\DQA1*04:01		1116	3161		857	2075		1114	2989	546	1089	2750
DQB1*02:01\DQA1*05:01		863	2873		703	1566		853	2646	515	752	2450
DQB1*02:02\DQA1*02:01		1080	2937		886	2039		1157	2546	559	1085	2699
DQB1*03:01\DQA1*02:01	786	1732	4424	707	1586	3061	727	1960	4390	980	1661	3884
*DQB1*03:01\DQA1*03:01*	1656	3038	6368	1148	2332	3924	1260	2971	5973	2047	3135	5273
DQB1*03:01\DQA1*05:03	655	1625	4820	618	1476	2855	758	1964	4557	793	1451	4090
DQB1*03:01\DQA1*05:05	652	1572	4690	633	1550	2873	769	1949	4655	831	1421	3901

DR Alleles	**IVIg Lot 1**			**IVIg Lot 2**			**IVIg Lot 3**			**IVIg Lot 4**		
Dilution	**(1/4)**	**(1/2)**	**Neat**	**(1/4)**	**(1/2)**	**Neat**	**(1/4)**	**(1/2)**	**Neat**	**(1/4)**	**(1/2)**	**Neat**
DQB1*03:01\DQA1*06:01		977	3285		968	1931	480	1154	3373		913	2502
DQB1*03:02\DQA1*01:01	1221	2211	4733	956	1730	3078	942	2261	4260	1536	2362	4353
DQB1*03:02\DQA1*02:01	999	1969	4645	852	1654	3328	867	2256	4561	1314	2069	4317
DQB1*03:02\DQA1*03:01	1131	2448	5166	1040	2040	4090	1056	2585	5276	1392	2403	4504
DQB1*03:02\DQA1*03:02	606	1288	3684	593	1377	2364	694	1779	3951	814	1373	3196
DQB1*03:03\DQA1*02:01	548	1155	3571	536	1205	2207	589	1667	3473	736	1155	2880
DQB1*03:03\DQA1*03:01	1047	2161	4969	926	1848	3436	948	2370	4727	1316	2201	4318
DQB1*03:03\DQA1*03:02	542	1196	3373	515	1255	2147	580	1535	3673	640	1179	2983
DQB1*04:01\DQA1*02:01	1057	2369	4701	857	1636	3248	882	2420	4218	1373	2280	4184
DQB1*04:01\DQA1*03:03	514	1068	2777		843	1554		1025	2322	719	1054	2368
DQB1*04:02\DQA1*02:01	853	1924	4382	663	1426	2955	713	2102	4129	1081	1838	3913
DQB1*04:02\DQA1*04:01	753	1666	4047	546	1279	2634	589	1693	3622	942	1589	3473
DQB1*05:01\DQA1*01:01	718	1389	3836	508	1006	1640	515	1180	2680	979	1502	2995
DQB1*05:02\DQA1*01:02	1128	2424	5212	833	1815	3395	858	2320	4934	1391	2266	4653
DQB1*06:01\DQA1*01:03	558	1173	3725		825	1506		1028	2804	824	1252	2902
DQB1*06:02\DQA1*01:01	1175	2262	5345	966	1695	4119	976	2142	5216	1427	2164	4805
*DQB1*06:02\DQA1*01:02*	1320	2268	5403	1025	1826	4303	1041	2455	5086	1674	2455	4733
DQB1*06:03\DQA1*01:03	594	1295	3872		764	1471		1005	2613	854	1300	3377
DQB1*06:04\DQA1*01:02	1339	2637	6029	1111	2057	4006	1168	2749	5414	1664	2688	5670
DQB1*06:09\DQA1*01:02	512	1132	3378		719	1298		906	2390	818	1166	2830
DP alleles												
DPB1*01:01\DPA1*01:03	1409	2348	4911	1252	1992	3824	1234	2718	4920	1692	2383	4495
DPB1*01:01\DPA1*02:01	1686	2834	5989	1414	2269	4299	1455	2792	5709	2021	2689	5049
DPB1*02:01\DPA1*01:03	1926	3162	5797	1538	2630	4193	1546	3056	5387	2369	3250	4694
DPB1*03:01\DPA1*01:03	1183	2292	5208	1130	1903	3780	1051	2481	5052	1350	2234	4636
DPB1*03:01\DPA1*01:05	1062	2059	4729	892	1639	3747	920	2134	4570	1242	2109	4371
DPB1*03:01\DPA1*02:01	1002	1578	4259	1005	1462	3473	989	1929	4416	1166	1584	3770
DPB1*04:01\DPA1*01:03		798	2612		696	2120		885	2753		709	2389
DPB1*04:02\DPA1*01:03	913	1637	4023	794	1475	3843	795	1817	4649	1047	1587	3941
DPB1*05:01\DPA1*02:01	1280	1938	3963	1088	1583	3297	899	1883	4077	1538	1991	3644
DPB1*06:01\DPA1*02:01	1608	2601	5447	1476	2309	4938	1433	3127	5920	1714	2535	5127
DPB1*09:01\DPA1*02:01		912	3192		768	2656		1014	3185		792	2904
DPB1*10:01\DPA1*02:01	786	1469	3779	650	1182	2945	654	1598	3437	919	1444	3490
DPB1*11:01\DPA1*01:03	1997	2556	5071	1776	1989	5229	1617	2856	5970	1780	2384	4814
DPB1*11:01\DPA1*01:05	1675	3283	5968	1502	2508	4182	1448	3261	5224	2049	3267	5434
DPB1*13:01\DPA1*01:05	1458	2143	4396	1400	1816	3337	1239	2416	4467	1745	2282	4300
DPB1*13:01\DPA1*02:01	1134	1885	4177	890	1318	3193	824	1697	4075	1514	1951	3710
DPB1*14:01\DPA1*02:01	1188	1984	4069	969	1595	3203	997	1988	4183	1437	1978	3882
DPB1*15:01\DPA1*02:01	1844	2738	5923	1635	2370	5007	1469	2844	5738	2066	2672	5405
DPB1*17:01\DPA1*02:01	1142	2310	4582	949	1792	3393	950	2136	4717	1348	2171	4215
DPB1*18:01\DPA1*02:01		968	3556		716	2350		865	3098		804	3146
DPB1*18:01\DPA1*01:04	635	1205	3308	551	1049	2393	545	1201	3068	636	1132	3066
DPB1*18:01\DPA1*01:05	557	1216	3290		980	2264	508	1088	3101	596	1113	2983
*DPB1*19:01\DPA1*01:03*	2573	3499	6735	2310	3063	5238	2019	3703	6712	3021	3678	6040
*DPB1*23:01\DPA1*02:01*	3187	3530	6809	3354	2839	7380	2257	3881	7784	3276	3619	6136
DPB1*28:01\DPA1*01:05	1123	2116	4810	1000	1785	3615	1043	2275	4540	1280	1971	4253
*DPB1*28:01\DPA1*02:01*	2403	3241	5811	2466	2915	5370	2131	3702	6279	2679	3375	5629

Data is restricted to Neat, 1/2 and 1/4 dilutions. Two or three most prevailing antibodies against each of the HLA-DR, HLA-DQ, and HLA-DP loci are shown in Bold and Italics, suggesting the most prevalent anti-HLA IgG in IVIg pooled from the Moroccan male and female donors.

3.5. Unique Profile of Anti-HLA-I IgG in IVIg on iBeads

HLA-Ia alleles on regular Labscreen single antigen beads may occur as intact or native trimeric HLA (HLA heavy chain and β2m with peptide), as well as monomeric form such as heavy chain only, and dimeric form such as peptide-free heavy chain with β2m or β2m-free heavy chain with peptide [43]. Whereas on iBeads, they may occur mostly as trimeric form HLA-I (peptide-associated heavy chain with β2m) or as dimeric form (peptide-free heavy chain with β2m) [43].

An MFI of an allele with iBeads that is higher than that of the regular bead would indicate that the HLA reactivity in question is toward intact or native trimeric HLA. Percentage of increase refers to the same [43]. In other words, an MFI of an allele with iBeads that is lower than that of the regular bead would indicate that the affinity of the antibody is towards other HLA-I variants such as the β2m-free HC of HLA with or without peptides. Percentage of decrease refers to the same.

In this investigation, the reactivity lot 3 of Moroccan IVIg (Table 5) to intact HLA-I (regular beads) and to heavy-chain HLA (iBeads) are compared. The percentage difference between regular beads and iBeads was calculated for every allele. The observations were restricted to the undiluted (neat) IVIg. The two most prevailing antibodies against the HLA-A, -B, and -Cw coated on iBeads are shown in (Bold

Italics) in the Table 5. The density of the peptide associated or peptide-free HLA heavy chains with β 2m coated on HLA-A; -B; -Cw antigen-coated iBeads in HLA-I Labscreen iBeads is 50%; 54%; 0% respectively.

Alternately, the density of β 2m-free HLA heavy chains coated on HLA-A; -B; -Cw antigen-coated beads in HLA-I Labscreen beads is 19%; 46%; 100% respectively. Therefore, not all antibodies bound to the Labscreen beads can recognize the trimeric HLA -I molecule, occurring naturally on tissues. The IgG reactivity against alleles (e.g., A*0301; A* 2901; A* 3402; A* 7401; B*1501; B*1503; B*1512; B*4501; B*4901; B*5001; B*5501) is mainly due to anti-HLA-I IgG binding to "intact" or "native" trimeric HLA (HLA heavy chain and β2m with peptide).

Table 5. *HLA-A, HLA-B, and HLA-Cw allelic reactivity of the lot 3 of Moroccan IVIg.*

MFI of the undiluted IVIg (neat) Lot 3							
HLA-A				**HLA-B**			
Alleles	**Regular**	**iBeads**	**% HC+ β2M**	**Alleles**	**Regular**	**iBeads**	**% HC+ β2M**
A*0101	**1389**	**687**	**49**	B*0702	**2701**	**1735**	**64**
A*0201	**1472**	**730**	**50**	B*0801	**2909**	**1017**	**35**
A*0203	**1324**	**901**	**68**	B*1301	**2022**	**540**	**27**
A*0206	**1492**	**1093**	**73**	B*1302	**1719**	**1324**	**77**
A*0301	**576**	**666**	**115**	B*1401	**2157**	**829**	**38**
A*1101	**1585**	**769**	**48**	B*1402	**2136**	**882**	**41**
A*1102	**1183**	**994**	**84**	B*1501	**818**	**1055**	**129**
A*2301	**1112**	**946**	**85**	B*1502	**1745**	**930**	**53**
A*2402	**1768**	**1036**	**59**	B*1503	**912**	**1086**	**119**
A*2403	**1750**	**1031**	**59**	B*1510	**1583**	**1003**	**63**
A*2501	**1615**	**1301**	**81**	B*1511	**1428**	**673**	**47**
A*2601	**1885**	**1263**	**67**	*B*1512*	**2742**	**3143**	**115**
*A*2901*	**1860**	**2244**	**121**	B*1513	**1111**	**579**	**52**
A*2902	**2431**	**1942**	**80**	B*1516	**2015**	**822**	**41**
A*3001	**1219**	**882**	**72**	B*1801	**1285**	**1064**	**83**
A*3002	**1560**	**767**	**49**	B*2705	**1612**	**860**	**53**
A*3101	**1519**	**878**	**58**	B*2708	**2275**	**1296**	**57**
A*3201	**1061**	**1052**	**99**	B*3501	**1208**	**960**	**79**
A*3301	**1931**	**939**	**49**	B*3701	**1503**	**751**	**50**
A*3303	**1809**	**843**	**47**	B*3801	**1429**	**652**	**46**
A*3401	**2140**	**1264**	**59**	B*3901	**1573**	**1069**	**68**
A*3402	**1183**	**1354**	**115**	B*4001	**1841**	**759**	**41**
A*3601	**1124**	**960**	**85**	B*4002	**2088**	**1292**	**62**
A*4301	**1718**	**1364**	**79**	B*4006	**2022**	**787**	**39**
A*6601	**2000**	**1500**	**75**	B*4101	**1519**	**728**	**48**
A*6602	**1867**	**1261**	**68**	B*4201	**1982**	**1124**	**57**
A*6801	**959**	**820**	**86**	B*4402	**2837**	**1025**	**36**
A*6802	**1785**	**1133**	**63**	B*4403	**2466**	**786**	**32**
A*6901	**1547**	**771**	**50**	*B*4501*	**2156**	**2466**	**114**
A*7401	**731**	**863**	**118**	B*4601	**2179**	**1081**	**50**
A*8001	**4362**	**1374**	**31**	B*4701	**1663**	**646**	**39**
HLA-Cw				B*4801	**2331**		**0**
Cw*0102	**3199**	**832**	**26**	B*4901	**1157**	**1304**	**113**
Cw*0202	**2989**	**608**	**20**	B*5001	**1037**	**1227**	**118**
Cw*0302	**2716**	**1326**	**49**	B*5101	**1492**	**986**	**66**
Cw*0303	**2609**	**1011**	**39**	B*5102	**1317**	**1015**	**77**
Cw*0304	**2832**	**911**	**32**	B*5201	**1292**	**1011**	**78**
Cw*0401	**3124**	**740**	**24**	B*5301	**2102**	**702**	**33**
Cw*0501	**3295**	**688**	**21**	B*5401	**1890**	**951**	**50**
Cw*0602	**3305**	**729**	**22**	B*5501	**975**	**1213**	**124**
Cw*0702	**5333**	**1117**	**21**	B*5601	**1125**	**632**	**56**
Cw*0801	**2752**	**1353**	**49**	B*5701	**1826**	**888**	**49**
Cw*1203	**3147**	**952**	**30**	B*5703	**2025**	**755**	**37**
Cw*1402	**3073**	**1218**	**40**	B*5801	**1604**	**560**	**35**
Cw*1502	**2692**	**904**	**34**	B*5901	**1320**	**598**	**45**
Cw*1601	**2202**	**812**	**37**	B*6701	**2639**	**1441**	**55**
Cw*1701	**5245**	**974**	**19**	B*7301	**2506**	**1695**	**68**
Cw*1802	**4594**	**725**	**16**	B*7801	**1606**	**1143**	**71**
				B*8101	**2747**	**747**	**27**
				B*8201	**3225**	**2139**	**66**

MFI obtained with regular Labscreen Beads and iBeads were compared for every allele of HLA-I locus. The data is restricted to undiluted (neat) IVIg. The percentage difference between regular beads and iBeads is shown for every allele in

each locus. The two most prevailing antibodies, against each of the HLA-A, HLA-B, and HLA-Cw coated on iBeads, are shown in Bold and Italics.

4. Discussion

Four lots Moroccan IVIg preparations purified from pooled plasma of males and females of different ethnic groups and origins from different parts of the country were tested for reactivity to HLA-I, HLA-II and albumin using Luminex single antigen beads and for the antibody dimerization. In contrast to measuring MFI of serum anti-HLA IgG, the MFI of anti-HLA IgG reactivity of IVIg is not affected by the presence of IgM or other classes of antibodies. The MFI values of IVIg indeed reflect HLA reactivity of IgG antibodies that binds to HLA coated beads.

Although it is known that the classical HLA-I and HLA-II molecules are highly polymorphic and represented by 2747 HLA-A, 3465 HLA-B, 2450 HLA-C, 7 HLA-DRA, 1711 HLA-DRB, 34 HLA- DQA1, 761 HLA-DQB1, 23 HLA-DPA1 and 627 HLA-DPB1 proteins [3], the number of HLA coated beads available for monitoring antibodies are far less (97 HLA-A, B and Cw and 91 HLA-DRA/DRB, DQA/DQB and DPA/DPB), thus imposing a limitation on characterizing the HLA antibodies prevailing in any population. In spite of the limitation, antibodies against high frequency alleles found in Moroccan population were observed.

The Moroccan IVIg preparations contain IgG antibodies against several high frequency HLA-I alleles found in the Moroccan population (A*0101, A*0201, B*0801, B*4403, B*4402, B*5001, Cw*0401, Cw*0602 and Cw*0702) [5]. The density of the antibodies as assessed by the levels of MFI for B*0801, B*5001, Cw*0602 and Cw*0702 are high parallel with their high frequency distribution. Similarly, the Moroccan IVIg had IgG antibodies against several high frequency HLA-II alleles found in the Moroccan population [6], which include DRB1*0701, DRB1*0301, DQA1*0501, DQA1*0201, and DQB1*0201 and haplotypes DQA1*0201-DQB1*0201/DRB1*0301, which nearly account for 50% of the total gene frequencies found in Moroccan Souss cohort. These findings caution administering high dose IVIg for the carriers of the HLA types, because they may experience adverse effects such as TRALI. This investigation emphasizes the need to carry out HLA typing of any patient who receives Moroccan IVIg and if Moroccan IVIg is administered to these typed patients, a critical patient care is required at least for a week after administering high dose IVIg.

The HLA molecules on the tissues appear as different conformational variants. The most common configuration on normal tissues is considered to be an HLA trimer, which consists of a heavy chain (HC) (40-45 kDa) non-covalently associated with β2-microglobulin (β2m) (12 kDa) and an 8-10 amino acid long peptide that are bound in the HC groove. Frequently, the native HLA may also exist, devoid of the peptide, as a HC with β2m. Indeed, using Flow cytometric Cross match analyses, with epitope specific monoclonal antibodies, confirmed the prevalence of peptide-associated β2m–associated HLA HC (pepA-β2aHC), on resting T-cells [43]. In addition to the above structural variants, a pool of β2m-free HLA as β2-free HC (β2fHC) was observed in proliferating human lymphoid cells [45], and in activated human T and B cells [46, 47]. Binding of IVIg to activated T and B cells may bring about immunomodulation such as suppression of antibody production, which may be beneficial for patients with autoimmune diseases and organ transplants [48].

Jucaud and co-investigators [43] have carefully compared the conformational variants on the Labscreen regular Beads and iBeads, to confirm the striking differences between the two beads. They have confirmed that the two beads differ as follows:

(1) The presence and the heterogeneity of density of peptide-associated-β2m-associated HLA-heavy-chain (pepA-β2aHC), peptide-free-β2aHC (pepF-β2aHC), and β2-free-HC (β2fHC) on the regular Labscreen Single antigen beads.

(2) In contrast, iBeads harbored a high density of pepA-β2aHC and low density of pepF-β2aHC, but devoid of β2fHC.

High prevalence of IgG antibodies for 81% of HLA-A and 54% HLA-B alleles on iBeads confirms the presence of IVIg antibodies reacting to normal tissue associated pepA-β2aHC and pepF-β2aHC variants. Such antibodies that bind to HLA trimers found on normal tissues are at potential risk for inflammatory diseases, such as TRALI.

However, IVIg reactivity to β2fHC, which frequently found on activated T and B cells, and are responsible of increased production of antibodies in autoimmune diseases, may significantly suppress activated T and B cells involved, and thus alleviates autoimmune diseases. The dual functionality of HLA antibodies in IVIg emphasizes the need to modify the profiles of HLA antibodies to be directed against the HLA variants (β2fHC) found on activated immune cells and may possibly have a beneficial influence on the patients with autoimmune diseases. Possibly the antibody profile can be modified by adsorbing out IgG antibodies directed against pepA or pepF-β2aHC using bead preparations like that of iBeads. Anticipating such benefits of IVIg, the US Federal Food and Drug Administration has recommended IVIg [51] for; (1) Primary Immune Deficiencies (PID), (2) Idiopathic Thrombocytopenic Purpura (ITP), (3) Chronic Lymphocytic Leukemia (CLL), (4) Kawasaki Disease, (5) Bone Marrow Transplantation (BMT). Again comparing the characteristics of IVIg from different manufacturers (Alpha, Baxter, Bayer, Centeon, Novartis), FDA has noted that while all the preparations are well suited for PID but not for all other - above mentioned- disease conditions. The recent findings about HLA and IVIg has raised the necessity for screening HLA antibodies in different lots of Moroccan IVIg preparations and especially against the two kinds of HLA coated beads; one coated only with HLA-Trimer and the other one only with β2fHC. Such a critical evaluation and the selection of lots reacting mostly to β2fHC would minimize adverse reactions (of course, without sucrose) and promote the

utility value of IVIg for autoimmune diseases, and PID that are highly prevalent in Morocco. The dimer analysis emphasizes the need to examine the IgG subclasses in Moroccan IVIg preparations, since subclasses are different in their ability for dimerization [52-54].

5. Conclusion

HLA-I and HLA-II reactive high MFI IgG antibodies in the Moroccan IVIg corresponded with several high frequency HLA-I alleles (B*0801, B*5001, Cw*0602 and Cw*0702) and HLA-II haplotypes (DQA1*0201-DQB1*0201/DRB1*0301), which accounted for 50% of the total gene frequencies in the Moroccan population. Measuring anti-HLA-I IgG antibodies was performed using regular (Labscreen) Beads coated with all conformational and structural variants of HLA-I (pepA- and pepF- β2aHC, pepA- and pepF- β2fHC) and iBeads coated with native tissue-associated HLA-I trimers (pepA-β 2aHC > pepF- β2aHC). It was realized that the IVIg contains IgG antibodies against all of the structural variants. While HLA-A and HLA-B reactive antibodies in the four lots of IVIg were predominantly binding to native HLA-trimers, HLA-Cw reactive antibodies were mostly reactive to pepA and pepF - β2fHC. These findings caution use of high dose IVIg for the carriers of the high frequency HLA types for it may cause tissue injuries such as TRALI. The β2fHC reactivity of IVIg suggests the potential of IVIg to bind to activated T and B cells that overexpress β 2fHC, and to suppress antibody production.

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